REAGENS Kft.

ReaCell I, II, III
Screening Cells for Antibody Detection

INSTRUCTIONS FOR USE

Red Blood Cell Suspension: 3±0,5%

IVD CE 1011

REF 41100, 41120

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 direct agglutination and IAT. 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 screening cell panel of 3 is 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 50µl. The current antigen chart of the screening 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 screening cell suspension is ready to use.
Do not use the reagent if the supernatant is haemolytic.
Shake gently the vials in order to homogenize the suspension before use.

Storage and transporting conditions:
These screening 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
- isotonic saline solution, pH=7,2 (REF 15015)
- ReaSol diluent (REF 11114)
- LISS (AGGI-LISS, REF 13110)
- Coombs serum (AHG)
- laboratory cell washer/centrifuge

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

Procedures:
Manual tube technique, Coombs method:
1. Mark 4 tubes with number 1 to 4 for each blood sample: I, II, III and one for autocontrol. Write the identification number of the blood sample on the tube to be tested.

One further tube for each blood sample is for autocontrol. **Preparation of autocontrol:** - wash the RBC of the sample to be tested in isotonic saline solution (pH=7,2) twice
- prepare an appr. 3% suspension with the ReaSol diluent (e.g. add 30 µl washed RBC residue into 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 screening cell suspension into the adequate tubes.
4. Shake gently the contents of the tubes and incubate them at 37 °C for 60 minutes or use 100 µl of AGGI-LISS to reduce the incubation time to 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 is observed. Record the result.
7. Shake gently the tubes, over a white background, and observe the agglutination with a desk lamp magnifier. Record the result. Mark the strength of agglutination with crosses.
8. Evaluate the agglutination scheme with the help of the antigen chart.

Indirect Method
9. Wash the tubes three times with isotonic saline solution.
10. Add 100-100 µl anti-human-globulin into each tube.
11. Shake gently and centrifuge for 20 seconds at 1000 g or for 60 seconds at 150 g
12. After resuspending read the results, possibly on a white background, with a desk lamp with magnifier and record the result. Record the strength of agglutination with crosses.

Evaluate the agglutination scheme with the 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 screening cells to 50 µl serum. In this case 50 µl anti-human-globulin 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 with cross strength and printing of the test will be made by the automat.
4. Evaluate the agglutination scheme with the help of the antigen chart.

LISS can be used for both methods, but the instructions for use should be followed strictly provided by the manufacturer and make the necessary steps accordingly.

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

Evaluation of results:
If agglutination or haemolysis is visible in any of the tubes during the screening test procedure (step 7, 8), or even while reading the agglutination (step 12) the antibody-screening is positive, continue testing to identify the antibody with a kit of panel cells.
If there is no way to identify the antibodies, blood sample should be forwarded to a special laboratory, where identification can be performed.
*Autocontrol: red blood cells of the sample are tested against it's own plasma.

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
- the RBC were centrifuged inadequately
- the over resuspendation 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 resuspendation
- rarely the tested serum or plasma may contain antibodies which are against of some of the used resuspendation 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 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.

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 41100	3 x 5 ml
REF 41120	3 x 10 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)

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REAGENS Kft.

ReaCell I, II, III
Screening Cells for Antibody Detection

INSTRUCTIONS FOR USE

Red Blood Cell Suspension: 0,8±0,1%
for Micromethod

IVD CE 1011

REF 41150, 41180

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 proposed for the detection of IgG (Rh-, Kell-, Duffy-, Kidd-, etc.) irregular RBC antibodies with IAT (indirect Coombs test) and IgM (mainly MN system) antibodies detection with DAT (direct Coombs test) on gel cards.

Composition:
The screening cell panel of 3 is produced from human RBCs of known group "0" and known antigen composed of individual donor's blood and is supplied in vials with droppers. Donors are selected based on their antigen strength. Dropper volume is 50µl.
The current antigen chart of the screening 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 contains: 1 mmol/l chloramphenicol and 0,4 mmol/l neomycin-sulphate

The screening cell suspension is ready to use.
Do not use the reagent if the supernatant is haemolytic.
Before use shake gently the vials in order to homogenize the suspension.

Storage and transporting conditions:
These screening 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 (10µl, 25 µl, 50 µl)
- ReaSol diluent (REF 11114), or any other solutions recommended by the card manufacturer
- isotonic saline solution, pH=7,2 (REF 15015)

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 original technical inserts must be read with attention before use.)
Direct method
1. Neutral card shall be used. The identification number of the patient and the screening cell's number is to be marked on them.
2. Remove the film and put 50-50 µl of each screening 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 card for 15 minutes at 37°C.
5. Centrifuge them for 10 minutes.
6. Read and record the reactions on the antigen chart accordingly. The strength of agglutination shall be marked with crosses.
7. Evaluate the agglutination scheme with the help of the antigen chart.

Indirect method
1. LISS/Coombs card shall be used. The identification number of the patient is to be marked on them.
2. Remove the film and add 50-50 µl of each screening cell suspension into the adequate column of the gel card. Primarily add the RBCs to gel chamber.

The 4th column of the gel card is for autocontrol.
Preparation of autocontrol: - wash the RBC of the sample to be tested in isotonic saline solution (pH=7,2) twice
- prepare an appr. 1% suspension with the ReaSol diluent (e.g. add 10 µl washed RBC residue into 1 ml ReaSol diluent).
3. Add 25-25 µl of the serum/plasma to be tested into each gel column.
4. Incubate the card for 15 minutes at 37°C.
5. Centrifuge them for 10 minutes.
6. Read and record the reactions on the antigen chart accordingly. Mark the strength of agglutination with crosses.
7. 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 17th Edition, Chapter 16th Autologous control (autocontrol).

Evaluation of results:
If agglutination or haemolysis is visible during the screening in any of the columns of a gel card, the antibody-screening is positive, continue testing to identify the antibody with panel cells. If there is no way to identify the antibodies, blood sample should be forwarded to a special laboratory, where identification can be performed.
*Autocontrol: red blood cells of the sample are tested against it's own plasma.

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
- the RBC were centrifuged inadequately
- the over resuspendation 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 resuspendation
- rarely the tested serum or plasma may contain antibodies which are against of some of the used resuspendation 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 result of the autocontrol the tested serum/plasma may contain antibodies, which needs further investigation.
- 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.
- With gel column method further LISS usage is not allowed.

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 41150	3 x 5 ml
REF 41180	3 x 10 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)

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REAGENS Kft.

REF 41200, 41220

ReaCell IP, IIP, IIIP
Screening Cells for Antibody Detection
Papainized
Red Blood Cell Suspension: 3±0,5%

INSTRUCTIONS FOR USE

IVD CE 1011

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 screening cells, since enzyme pre-treated cells may cause aspecific reactions.

Composition:
The screening cell panel of 3 is 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 screening cells is always included in the packaging.
The suspension solution ReaSol diluent (REF 11114) is a stabilizing isotonic saline solution, which preserves the reactivity of antigens and prevents their haemolysis.
Preservative solution contains: 1 mmol/l chloramphenicol, and 0,4 mmol/l neomycin-sulphate
The screening cell suspension is ready to use.
Do not use the reagent if the supernatant is haemolytic.
Shake gently the vials in order to homogenize the suspension before use.

Storage and transporting conditions:
These screening 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. (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 (100 µl)
- water bath, 37°C
- laboratory centrifuge
- isotonic saline solution, pH=7,2 (REF 15015)
- ReaSol 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)
- ReaSol diluent (REF 11114)
- Coombs serum (AHG)
- Papain

Procedures:
Manual tube technique:
1. Number 4 tubes for each blood sample (IP, IIP, IIIP). Write the identification number of the blood sample to be tested on the tube.

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 screening cell suspension into the adequate tubes.
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, for haemolysis presence. Record the result on the antigen chart.
7. Shaking gently the tube, above a white background, and with a desk lamp magnifier, observe, if there is agglutination. Record the result. Mark the strength of agglutination with crosses.
8. Evaluate the agglutination scheme with the help of the antigen chart.

Indirect method
9. Wash the test tubes content for 3 times with isotonic saline solution
10. Add into each test tube 100-100 µl Coombs serum (AHG)
11. Shake gently the test tubes and centrifuge them for 20 seconds with 1000g or for 60 seconds with 150g
12. After resuspendation read the result possibly above a white background using a desk lamp with magnifier. Record the results on the antigen chart. Mark the strength of agglutination with crosses.
13. Evaluate the result of agglutination or haemolysis 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 results and printing of the test will be made by the automat.
Evaluate the agglutination scheme with the help of the antigen chart.
After result evaluation if it is necessary continue the testing with direct Coombs method.

Coombs (Antiglobulin) testing on ACT-24:
Enter the parameters of the test in the computer and start F2 program. For testing 25-25 µl test cell serum addition of 50 µl AHG is needed. The ACT performs all the Coombs test procedures and evaluates and prints the results. Evaluate the result of agglutination or haemolysis with the help of the antigen chart.

Positive and negative and washing control must be used for every testing (see AABB Technical Manual 17th Edition, Chapter 16th Autologous control in antibody identification).

Evaluation of results:
If agglutination or haemolysis is observed in any of the tubes during the screening test procedure, the antibody-screening is positive the antibody identification has to be attempted with a kit of panel cells.
If there is no way to identify the antibodies, blood sample should be forwarded to a special laboratory, where identification can be performed.

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
- the RBC were centrifuged inadequately
- the over resuspendation 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 resuspendation
- rarely the tested serum or plasma may contain antibodies which are against of some of the used resuspendation 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.
- Enzym 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 41200 3 x 5 ml
REF 41220 3 x 10 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)

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REAGENS Kft.

REF 41250, 41270

ReaCell IP, IIP, IIIP
Screening Cells for Antibody Detection
Papainized
Red Blood Cell Suspension: 0,8±0,1%
for Micromethods

INSTRUCTIONS FOR USE

IVD CE 1011

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 convenient 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 screening cells, since enzyme pre-treated cells may cause aspecific reactions in that case.

Composition:
The screening cell panel of 3 is 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 screening cells is always included in the packaging.
The suspension solution ReaSol diluent (REF 11114) is a stabilizing isotonic saline solution, which preserves the reactivity of antigens and prevents their haemolysis.
Preservative solution contains: 1 mmol/l chloramphenicol, and 0,4 mmol/l neomycin-sulphate
The screening cell suspension is ready to use.
Do not use if the supernatant is haemolytic.
Before use shake gently the vials in order to homogenize the suspension.

Storage and transporting conditions:
These screening 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. (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:
Microplate method:
- microplates
- pipettes (50 µl)
- isotonic saline solution, pH=7,2 (REF 15015)
- ReaSol diluent (REF 11114)
- Papain
- incubator of 37 °C
- microplate centrifuge
- mirror reader / automate reader
- microplate shaker

Gel column method:
- Neutral/Coombs cards
- pipettes (25 µl, 50 µl)
- isotonic saline solution, pH=7,2 (REF 15015)
- ReaSol diluent (REF 11114)
- 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 samples to be tested into 3-3 wells.
3. Add 50-50 µl of each screening cell suspension in the adequate wells.
4. Shake the contents of the microplates at maximum grade for 1-2 minutes.
5. Incubate the microplates for 15 minutes at 37°C.
6. Centrifuge them at 1000 rpm for 1 minute. Shake the plates at maximum grade for a few seconds, then at low grade for 2-3 minutes.
7. Read the results in mirror reader; record the result; mark the strength of agglutination with crosses on the antigen chart. The test can be evaluated in automate reader, too
8. Evaluate the result of agglutination or haemolysis 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. The identification number of the patient is to be marked on them.
2. Remove the film and add 50-50 µl of each screening 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 card for 15 minutes at 37°C.
5. Centrifuge them for 10 minutes.
6. Read and record the reactions. Mark the strength of agglutination with crosses.
7. Evaluate the result of agglutination or haemolysis with the help of the antigen chart.

Indirect Method
8. Note: In case of 0,8% papainized screening cells the DAT can be carried out on gel column using Coombs card.

Evaluation of results:
If positive reaction is observed during the screening in any of the gel columns, the antibody-screening result is positive, in this case attempt to identify antibodies with a kit of panel cells. If there is no way to identify the antibodies, blood sample should be forwarded to a special laboratory, where identification can be performed.

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
- the RBC were centrifuged inadequately
- the over resuspendation 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 resuspendation
- rarely the tested serum or plasma may contain antibodies which are against of some of the used resuspendation 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 need 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 41250 3 x 5 ml
REF 41270 3 x 10 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)

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