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Cross-Matched Test Check Using Gel Method (Column Agglutination Test)

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ABSTRACT

Crossmatch test is a test between the donor's blood and the patient's blood that is principally done in vitro and must be done before blood transfusion. The crossmatch test is performed to determine whether there is a reaction between the donor's blood and the patient's blood to ensure the compatibility of the blood to be transfused to the patient.1 Cross-match test consists of major crossmatch and minor crossmatch. The crossmatch test is necessary to prevent transfusion reactions by ensuring that the patient does not have antibodies that are reactive to antigens on donor erythrocytes and benefit the patient. Test principles are IgG antibodies contained in serum/plasma, when reacted with antigens on the surface of erythrocytes through controlled centrifugation in microtubes containing dextran-acrylamide gel and Coombs serum, agglutination will be formed. Agglutination is also formed due to the presence of anti-IgG (antibodies against IgG) that has been planted on the surface of the gel with IgG serum/donor plasma. If the major matching cross-test mixes the donor erythrocyte suspension with the patient's serum/plasma, the surface of the donor erythrocytes containing the same antigens as the antibodies in the patient's serum/plasma will react. If the minor concordance cross-test mixes the patient's erythrocyte suspension with the donor's serum/plasma, the surface of the patient's erythrocytes containing the same antigens as the antibodies in the donor's serum/plasma will react to form agglutination. The degree of agglutination in the gel method is graded from negative, 1+ to 4+ and mixed-field. Erythrocytes on the surface of the gel are agglutinated erythrocytes, while erythrocytes that settle to the bottom of the gel are non-agglutinated erythrocytes.

Keywords: cross-matched; gel method; column agglutination test

BACKGROUND

A crossmatch test is a test between the donor's blood and the patient's blood that is principally done in vitro and must be done before blood transfusion. The crossmatch test is performed to determine whether there is a reaction between the donor's blood and the patient's blood to ensure the compatibility of the blood to be transfused to the patient.[1] Cross-match test consists of major crossmatch and minor crossmatch. The crossmatch test is necessary to prevent transfusion reactions by ensuring that the patient does not have antibodies that are reactive to antigens on donor erythrocytes and benefit the patient.[2]

The main purpose of cross-match testing is to prevent life-threatening transfusion reactions and mild or moderate transfusion reactions that can interfere with patient comfort.[3] Blood transfusion reactions are reactions that can occur during blood transfusion or sometime after blood transfusion, consisting of fast-type reactions and slow-type reactions. Another important goal is to maximize the in vivo life span of the transfused blood cells.[3] Based on the type of patient and donor blood components being reacted, the cross-matched test has two objectives: to detect the presence of antibodies in the patient's serum (including anti-A & anti-B) that can destroy the transfused erythrocytes and to detect antibodies in the donor's serum that will enter the patient's body.[3,4]

Cross-matched testing can be done serologically and electronically or computerized. Cross-matched test examination can be done by tube test or by column agglutination test or better known as gel test.[3] In line with the times, the cross-matched test examination with the tube method has begun to be replaced by the gel method. The gel method has many advantages over the tube method including being more practical, easy and fast and the reaction results are stable and can be stored.[2,3] The cross-matched test examination with the tube method that is often carried out has several limitations including the need for a long time which is more than one hour so that the implementation of transfusion is delayed, the results are very subjective (depending on the skill of the officer), the need for washing cells three times and the visual reaction results cannot be documented.[5]

In this tutorial, we will discuss the examination of cross-matched tests using the Matrix™ AHG (Coombs) Gel Cards tool with the gel column agglutination test method.

AIM

The purpose of this test is to explain the cross-matched gel test (*colum agglutination test)* using Matrix™ AHG (Coombs) *Gel Cards* to determine the blood group compatibility between patients and donors.

METHOD

A. Pre-analytic[6,7]

1. Patient preparation

a. No special preparation of the patient is required before sampling

2. Sample preparation

a. Prepare patient and donor blood samples that have the same blood type.
b. Prepare a 1% suspension of patient and donor erythrocytes by mixing 10 ml of Liss Diluent with 10 µL of patient and donor erythrocytes.
c. Prepare two 12x75 m tubes and label them.
d. The first tube 10 µL of donor erythrocytes and add 1 ml of LISS Diluent.
e. Second tube 10 µL of patient erythrocytes and add 1 ml of LISS Diluent.

3. Instruments and materials

The tools and materials needed to perform a crossmatch check with the gel method include:

a. Micropipette volumes of 10 µL, 25 µL and 50 µL
b. Test tube size 12x75 mm
c. Sample tube rack
d. LISS ID rack/Coombs test card
e. Matrix[tm] Gel AHG (Coombs) Test Card consists of 6 *microtubes*
f. *Card centrifuge* Matrix CC-2400
g. *Card warmer* Matrix CW-2400
h. Reagents: Matrix™ Diluent-2 LISS (*Low Ionic Strength Solution)*
i. Centrifuge
j. Blood samples and blood bags

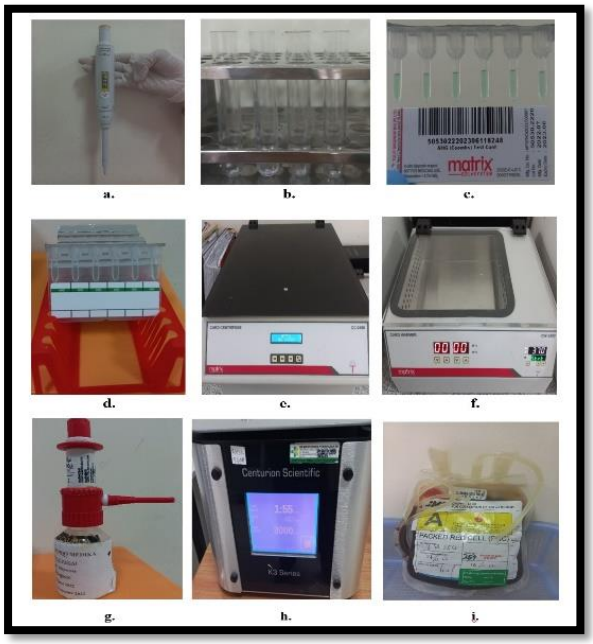

FIGURE 1: Instruments Cross Match Using Gel Method. A. Micropipette, B. Test tube and tube rack, C. Matrixtm Gel AHG (Coombs) Test Card, D. LISS ID rack/ Coombs test card, E. Matrix CC-2400 centrifuge card, F. Card warmer Matrix CW-2400, G. Matrix™ Diluent-2 LISS, H. Centrifuge, I. Blood bag sample (Source: Personal documentation).

B. Analytic

1. Test principle[6,8]

IgG antibodies contained in serum/plasma, when reacted with antigens on the surface of erythrocytes through controlled centrifugation in *microtubes* containing *dextran-acrylamide* gel and Coombs serum, agglutination will be formed. Agglutination is also formed due to the presence of anti-IgG (antibodies against IgG) that has been planted on the surface of the gel with IgG serum/donor plasma. If the major matching cross-test mixes the donor erythrocyte suspension with the patient's serum/plasma, the surface of the donor erythrocytes containing the same antigens as the antibodies in the patient's serum/plasma will react. If the minor concordance cross-test mixes the patient's erythrocyte suspension with the donor's serum/plasma, the surface of the patient's erythrocytes containing the same antigens as the antibodies in the donor's serum/plasma will react to form agglutination.

In a positive reaction, the agglutinates are trapped in the gel to varying degrees based on the degree of agglutination, whereas in a negative reaction, the antibodies do not attach to the erythrocytes and the erythrocytes freely pass through the gel, and granules to the bottom of the *microtube*.[6,8]

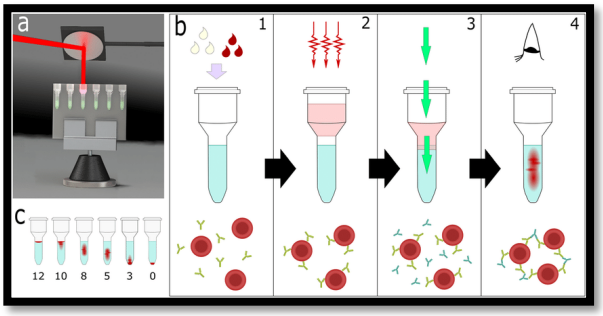

FIGURE 2: Principle of the gel method *cross-match* test. [8]

2. The procedure[7,8]

Label the *gel card* with the patient's identity (patient name, age and medical record number) and mark the *microtube* major (My), minor (Mn) and autocontrol (AK).

a. The sample was inserted into the *microtube* in *an* inclined position. Plasma erythrocyte suspension *was* put right into the *reaction chamber* in the *microtube.*
b. 50 µL of 1% erythrocyte suspension from tube 1 of the donor was taken and placed into a "My" major *microtube* and 25 µL of patient plasma was added.
c. A 1% erythrocyte suspension from tube 2 of the patient was taken 50 µL then put into a minor *microtube* "Mn" and add 25 µL of donor plasma.
d. Erythrocyte suspension from tube 2 of the patient was taken 50 µL then put into the autocontrol *microtube* "AK" and add 25 µL of patient plasma.
e. *Gel cards* were incubated at 37° C for 15 minutes.
f. *Gel cards* were centrifuged for 10 minutes at 1500 rpm.
g. Read and record the results of the reaction that occurs.

C. Post-analytic[7,8]

1. Interpretation

The degree of agglutination in the gel method *is* graded from negative, 1+ to 4+ and *mixed-field.* Erythrocytes on the surface of the gel are agglutinated erythrocytes, while erythrocytes that settle to the bottom of the gel are non-agglutinated erythrocytes.

- Negative reaction: indicates a match between the donor's blood and the recipient's.
- Positive reaction: indicates the incompatibility of the donor's blood with the recipient, due to the presence of antibodies directed against antigens on the donor's erythrocytes.

Degree of agglutination

+4: Solid band-shaped erythrocyte agglutination at the top of the gel column. There is no sediment at the bottom of the microtube.

+3: Erythrocyte agglutinates predominate at the top of the gel column with few agglutinates below the thick band. Most of the agglutinates are located in the upper half of the gel column.

+2: Erythrocyte agglutinates are dispersed along the gel column with a few agglutinates at the bottom of the *microtube.* Agglutinates are distributed in the top and bottom half of the gel column.

+1: Erythrocyte agglutinates predominate at the bottom half of the gel column with some at the bottom of the *microtube.*

Negative: Formation of a clear erythrocyte precipitate at the bottom of the *microtube.* The gel above the erythrocyte precipitate is clear and free of agglutinates.

Mixed-field reaction: erythrocyte agglutination layer at the top of the gel accompanied by cell sediment at the bottom of the *microtube*.

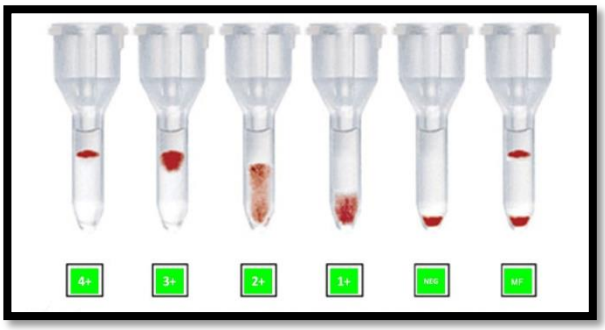

FIGURE 3: The degree of agglutination in the *crossmatch* test by gel method[9].

Table 1. Conclusion of the *Crossmatch* Test Examination Results[9]

No.	Major	Minor	AC/DCT	Conclusion
1.	-	-	-	Blood can be channeled
2.	+	-	-	Check once again the patient's blood type, whether it is the same as the donor, if the blood type is the same, it means that there are irregular antibodies in the patient's serum. → Replace donor blood
3.	-	+	-	Replace Donor Blood
4.	-	+	+	If positive degree in minor ≤ positive degree in AC/DCT→ Blood may be dispensed → INFORM CONCENT
5.	+	+	+	Recheck the blood type of patients and donors, compare the positive degree of AC with minor Major Positive→ Change blood donor

2. Limitations [6,7,8]

(1) *Matrix™ gel cards* that show air bubbles in the gel or at the top of the *microtubes* should be centrifuged before use. Centrifugation can be done for 2 cycles to remove air bubbles, if air bubbles do not disappear *Matrix™ gel cards* then they cannot be used.
(2) *Matrix™ gel cards* that show signs of drying (no or reduced volume of *reagent buffer* under the gel column) cannot be used.
(3) Damaged aluminum foil on *Matrix™ gel cards* cannot be used.
(4) The fibrin residue in the erythrocyte suspension may trap non-agglutinated cells, resulting in a fine pink line at the top of the gel

While most of the cells are at the bottom of the *microtube* after centrifugation.

(5) Strict adherence to recommended procedures and equipment is essential.
(6) The use of suspension solutions other than Matrix™ Diluent-2 LISS may affect the reaction.
(7) Contamination of reagents can affect false positive or false negative results.

3. Advantages[9]

(1) Standardized and measurable.
(2) Simple, fast and easy to use.
(3) More sensitive than the tube method.
(4) Requires only small amounts of antisera and erythrocytes, ideal for neonates and children.
(5) The results of the reaction are easy to see (macroscopically) so the use of a microscope is no longer needed.
(6) Objective result.
(7) Reaction results are stable for up to 48 hours, so they can be stored and printed.
(8) No wash phases.
(9) Reducing waste in the laboratory.

CONFLICT OF INTEREST STATEMENT

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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