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Interpretation Guide

ID-Micro Typing System™

Rx ONLY

Ortho Clinical Diagnostics

Micro Typing Systems, Inc.

Customer Technical Services

Contact OCD Customer Technical Services (CTS) at 1-800-421-3311.

Proprietary Notice

Acknowledgments

ID-Micro Typing System™ Interpretation Guide

Contents

Introduction

Ortho-Clinical Diagnostics, Inc. (OCD) prepared the ID-Micro Typing System™ (ID-MTS™) Interpretation Guide to provide assistance with ID-MTS™ Gel Test training and to provide a tool for the maintenance of technologists' competency levels. This guide provides illustrations of typical gel test agglutination reactions and a troubleshooting section. If you have specific questions not addressed in this guide, contact OCD Customer Technical Services (1-800-421-3311).

Instructions for Use

Additional information is available in the Instructions for Use for each of the ID-MTS™ gel cards.

- Instructions for Use can be downloaded at www.orthoclinical.com.
- Instructions for Use are available through the OCD Fax-on-Demand system: dial 1-888-329-1020 and follow the voice prompts.
- Paper copies of Instructions for Use can be ordered by contacting Customer Technical Services at 1-800-421-3311.

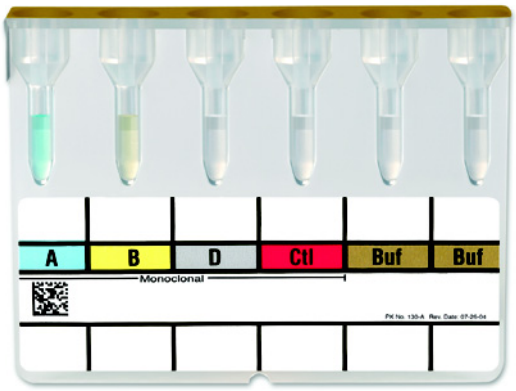
Overview of the ID-MTS™ Gel Test

Since its introduction in North America in 1995, the ID-MTS™ Gel Test has widely replaced traditional tube technology for a broad range of blood bank applications. The ID-MTS™ Gel Test provides standardized and simplified procedures. The gel test, licensed in North America as the ID-Micro Typing System™, utilizes dextran acrylamide gel particles that are pre-dispensed into specially designed microtubes manufactured in plastic cards. The gel card measures 2 x 3 inches, approximately the size of a credit card, and contains 6 microtubes. Each microtube consists of an upper reaction chamber that is wider than the microtube and a long narrow section that contains the pre-dispensed gel and reagents. The top of the gel card is sealed with a foil strip to prevent spillage or drying of the microtube contents.

In the ID-MTS™ Gel Test, measured volumes of red blood cells and plasma/serum are first added to the reaction chamber of the microtube. The reaction chamber provides an area for red blood cell sensitization during the incubation of any indirect antiglobulin test. With the exception of the MTS™ Buffered Gel Card and the MTS™ Monoclonal Control Card, specific antibody is incorporated into the gel. The centrifugation step allows sufficient time for the contact of the red blood cells with the antisera and gel particles, resulting in the separation of agglutinated red blood cells from unagglutinated red blood cells. During this controlled centrifugation, the gel matrix functions as a molecular sieve to trap agglutinated red blood cells.

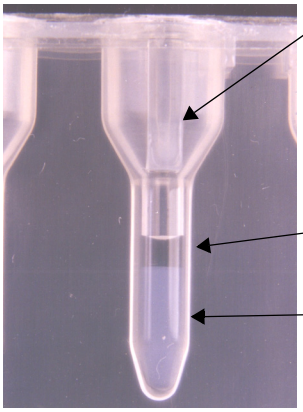
Agglutinated red blood cells become trapped in the gel at various levels within the microtube, depending on the size of the agglutinates. Free unagglutinated red blood cells pass through the gel and form a button of red blood cells on the bottom of the microtube.

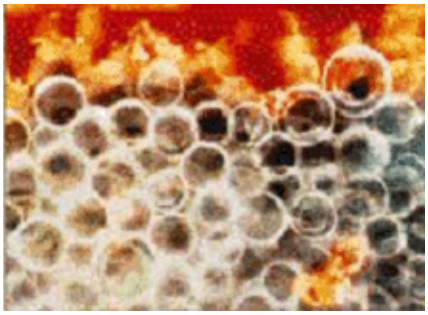
Gel Card with 6 Microtubes



Microtube Enlarged

Each microtube has a gel layer and a liquid layer above the gel.





Dextran Acrylamide Gel Particles with Agglutinated Cells

Reading Gel Test Reactions

Read macroscopically the front and back of each microtube for agglutination and/or hemolysis. Record the reactions. If either side of the microtube is positive, the reaction is to be considered positive. Agglutination and/or hemolysis of the red blood cells is a positive test result. If hemolysis is observed, consult your facility's approved procedures.

Interpreting Gel Test Reactions

Because agglutination reactions are stabilized within the gel matrix, the ID-MTS™ Gel Test provides a definitive end point. The strength of the agglutination reaction determines the extent of migration of the agglutinated red blood cells in the gel. The agglutination reactions may be graded in a similar fashion as observed in traditional tube tests using a scale of 0 to 4+. The grading system for agglutination is based upon the position of the agglutinated red blood cells within the microtube.

0 Negative (page 7)	▪ Unagglutinated red blood cells form a well-defined button at the bottom of the microtube.
1+ Reaction (page 8)	▪ Agglutinated red blood cells are observed predominantly in the lower half of the gel microtube. Unagglutinated red blood cells form a button in the bottom of the microtube.
2+ Reaction (page 9)	▪ Agglutinated red blood cells are dispersed throughout the length of the gel microtube. A few unagglutinated red blood cells may be observed in the bottom of the microtube.
3+ Reaction (page 10)	▪ The majority of agglutinated red blood cells are trapped in the upper half of the gel microtube.
4+ Reaction (page 11)	▪ Solid band of red cell agglutinates on top of the gel. A few agglutinates may filter into the gel but remain near the predominant band.
Mixed Field (page 12)	▪ Agglutinated red blood cells at the top of the gel or dispersed throughout the gel microtube accompanied by a button of negative red blood cells in the bottom of the microtube. See note below.

Note: Caution must be taken in interpreting a reaction as mixed field. Additional patient history and testing will be necessary for resolution. However, not all mixed cell situations have a sufficient minor population to be detected.

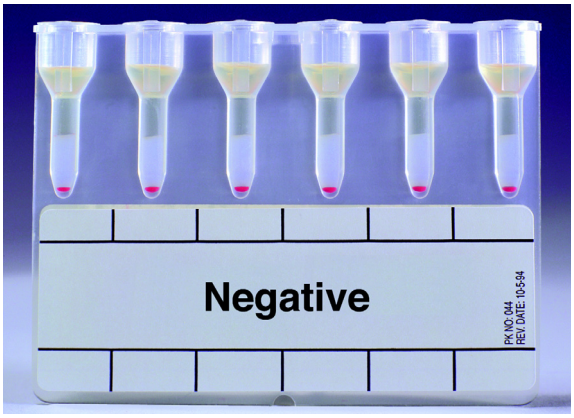
CAUTION: Clots, particulates or other artifacts may cause some red blood cells to be entrapped at the top of the gel. This may cause an anomalous result in a negative test.

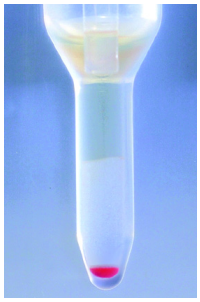
Range of Reactions in the ID-MTS™ Gel Test

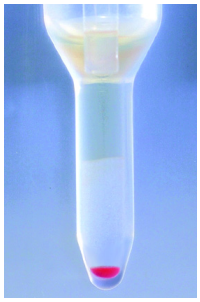
Negative Reactions

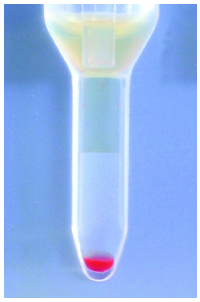
Definition

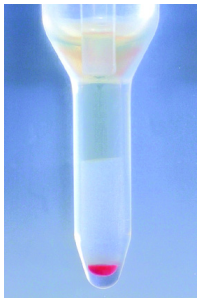
Negative Reactions: Unagglutinated red blood cells form a well-defined button in the bottom of the microtube.

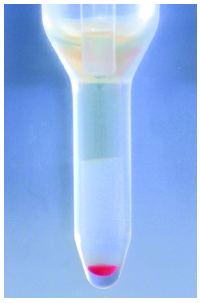


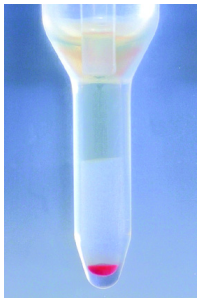

Negative Reaction


Negative Reaction


Negative Reaction


Negative Reaction


Negative Reaction


Negative Reaction

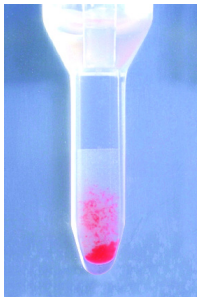
Discussion

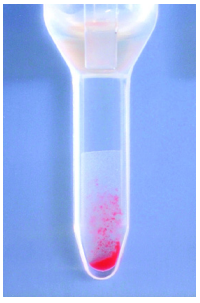
Negative reactions are represented by unagglutinated red blood cells traveling through the gel forming a button in the bottom of the microtube. Debris, fibrin, or other artifacts associated with serum, cord blood, or frozen samples may cause a few unagglutinated red blood cells to trap on top of the gel, but these tests should be interpreted as negative. For more information, refer to the troubleshooting section.

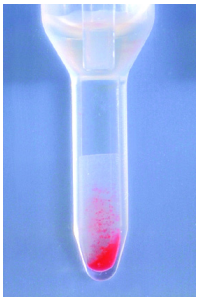
1+ Reactions

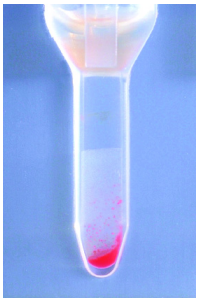
Definition

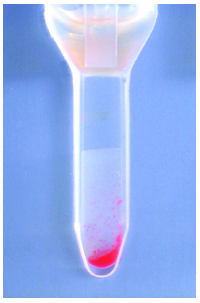
1+ Reactions:
Characterized by red blood cell agglutinates predominantly observed in the lower half of the gel microtube.
Unagglutinated red blood cells form a button in the bottom of the microtube.

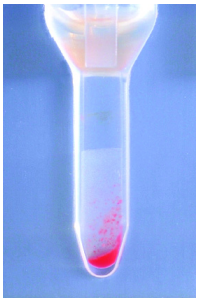

1+ Reaction


1+ Reaction


1+ Reaction


1+ Reaction


1+ Reaction


1+ Reaction

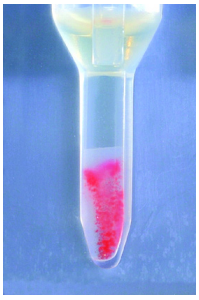
Discussion

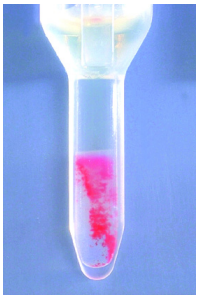
Weaker reactions may be represented by few agglutinated red blood cells in the gel microtube in the area just above the red blood cell button near the bottom of the microtube. The button associated with these weaker reactions is often disrupted.

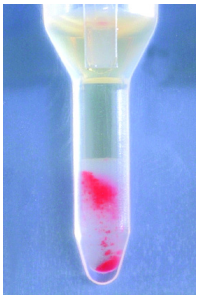
2+ Reactions

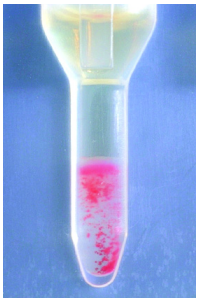
Definition

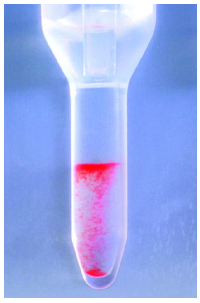
2+ Reactions:
Characterized by red blood cell agglutinates dispersed throughout the length of the gel microtube. A few unagglutinated red blood cells may be observed in the bottom of the microtube.

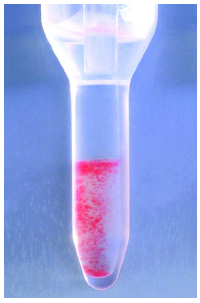

2+ Reaction


2+ Reaction


2+ Reaction


2+ Reaction


2+ Reaction


2+ Reaction

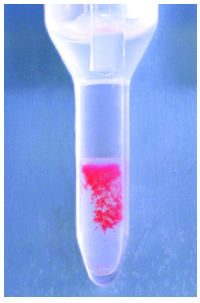
Discussion

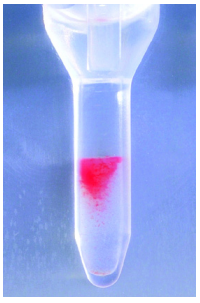
In a 2+ reaction, the agglutinated red blood cells are dispersed vertically throughout the gel in the microtube. The horizontal position of the agglutinated cells within the microtube (i.e., their relative location front-to-back or side-to-side) should not be considered. The size of red blood cell buttons in the bottom of the microtube may vary.

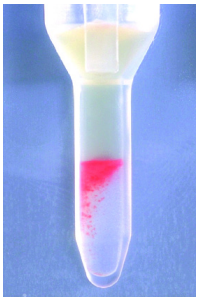
3+ Reactions

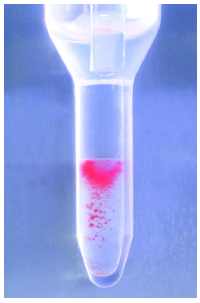
Definition

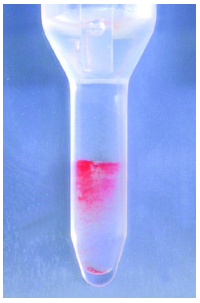
3+ Reactions:
Characterized by the majority of agglutinated red blood cells trapped in the upper half of the gel microtube.

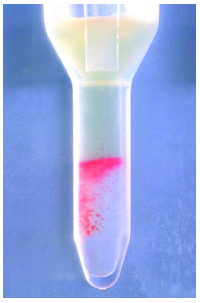

3+ Reaction


3+ Reaction


3+ Reaction


3+ Reaction


3+ Reaction


3+ Reaction

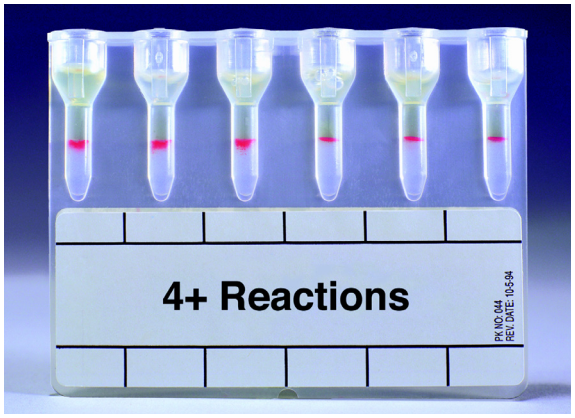
Discussion

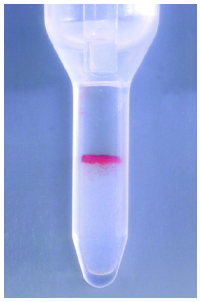
A 3+ reaction appears as a thick group of agglutinated red blood cells, or band, with some red blood cells dispersed below the predominant band in the upper half of the gel microtube. A 3+ reaction may also be characterized by an even distribution of agglutinated red blood cells in the upper portion of the gel. Occasionally, a few unsensitized red blood cells may migrate to the bottom of the microtube.

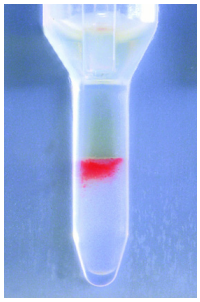
4+ Reactions

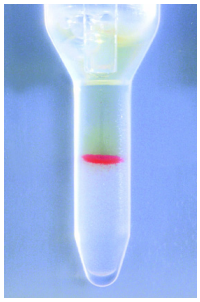
Definition

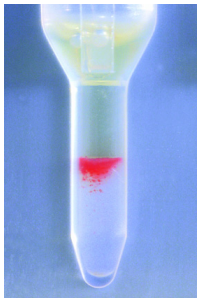
4+ Reactions:
Characterized by a solid band of agglutinated red blood cells on top of the gel. A few agglutinated red blood cells may filter into the gel but remain near the predominant band.

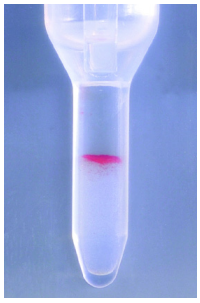


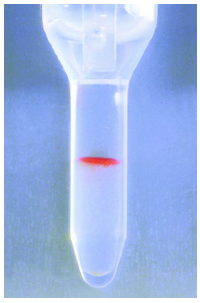

4+ Reaction


4+ Reaction


4+ Reaction


4+ Reaction


4+ Reaction


4+ Reaction

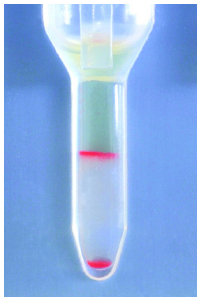
Discussion

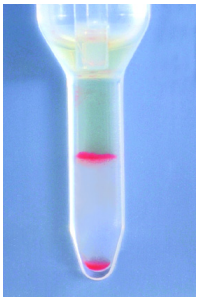
Strong 4+ reactions form a band of agglutinated red blood cells and become trapped on or near the top of the gel. The size of these agglutinated red blood cells makes it difficult for red blood cells to migrate into the gel. Occasionally, a few unsensitized red blood cells may migrate to the bottom of the microtube but the middle of the gel should remain free from agglutinated red blood cells.

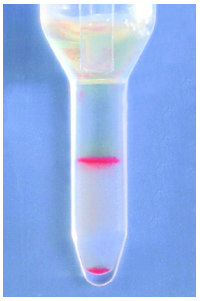
Mixed-Field Reactions

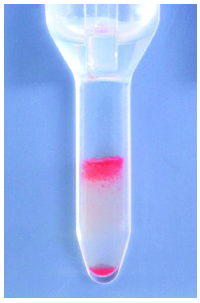
Definition

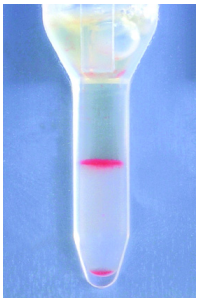
Mixed-Field Reactions: Characterized by agglutinated red blood cells on top of the gel or dispersed throughout the microtube and accompanied by a button of unagglutinated red blood cells in the bottom of the microtube.

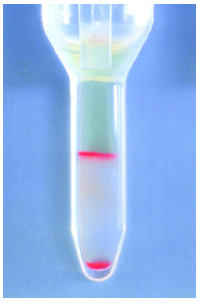

Mixed-Field Reaction


Mixed-Field Reaction


Mixed-Field Reaction


Mixed-Field Reaction


Mixed-Field Reaction


Mixed-Field Reaction

Discussion

Before interpreting reactions as mixed-field, the clinical history of the patient and the type of testing performed should be considered. Additional patient history and testing will be required for resolution (see section Troubleshooting: Reactions Associated with Recently Transfused Patients for information about recently transfused patients). With the exception of pooled red blood cells, mixed-field reactions are not possible when testing reagent red blood cells in an antibody screening or identification procedure. Not all mixed-field situations have a sufficient minor population to be detected.

ABO and Rh Card Interpretations

Definition

Monoclonal cards for ABO grouping and Rh typing are available in various configurations.

MTS™ A/B/D Monoclonal and Reverse Grouping Card

This gel card is capable of testing the forward and reverse types on a single card. The reagents are predispensed (Monoclonal Anti-A, Monoclonal Anti-B, Monoclonal Anti-D, Monoclonal Control, and MTS™ Buffered Gel in the last two microtubes). Diluted patient/donor cells and plasma are added as well as 0.8% reverse grouping red blood cells. Reaction grading is identical to all other gel card grading.

MTS™ A/B/D Monoclonal and Reverse Grouping Card:
Group O Rh-negative is shown
(patient or donor testing)

MTS™ A/B/D Monoclonal Grouping Card

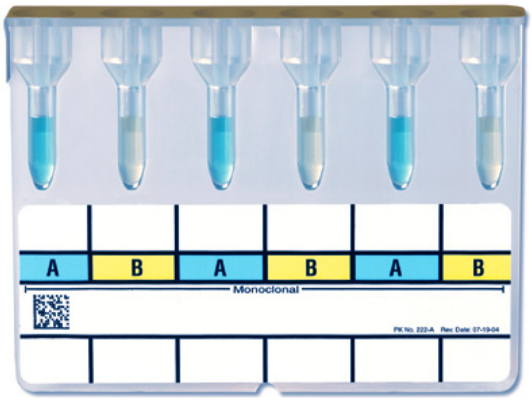
This gel card provides two forward types in a single card. This card is ideal for donor unit confirmation testing or neonatal testing that does not require reverse grouping. The reagents are pre-dispensed (Monoclonal Anti-A, Monoclonal Anti-B, Monoclonal Anti-D, Monoclonal Anti-A, Monoclonal Anti-B, Monoclonal Anti-D), and the diluted donor/patient cells are added prior to centrifugation. Reaction grading is identical to all other gel card grading.

MTS™ A/B/D Monoclonal Grouping Card:
Group AB Rh-negative and Group B Rh-negative are shown
(donor confirmation or neonate testing)

ABO and Rh Card Interpretations (continued)

MTS™ A/B Monoclonal Grouping Card

This gel card provides three ABO forward groupings in a single card. This card is ideal for donor unit confirmation testing. The reagents are pre-dispensed (Monoclonal Anti-A, Monoclonal Anti-B, Monoclonal Anti-A, Monoclonal Anti-B, Monoclonal Anti-A, Monoclonal Anti-B), and the diluted red blood cells are added prior to centrifugation. Reaction grading is identical to all other gel card grading.



MTS™ A/B Monoclonal Grouping Card:
No reaction shown.

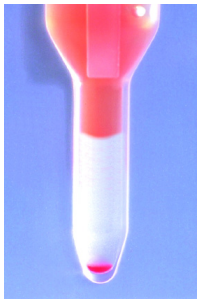
Discussion

Most blood types will demonstrate 4+ reactions with weaker reactions potentially representing weak subgroups or weak representations of naturally occurring Anti-A and Anti-B. ABO discrepancies are resolved in the usual manner according to the facility's Standard Operating Procedures (SOPs). No control is necessary when using monoclonal reagents, except when a Group AB Rh-positive is tested. If all blood grouping results for a given sample are positive, a control will be necessary to rule out false positive reactions due to spontaneous agglutination of the red blood cells.

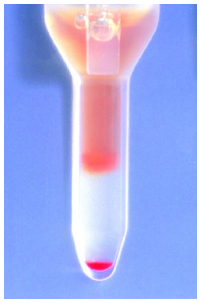
Other Reactions

Hemolysis, Weak Reactions, Abnormal Position of Agglutinated Cells

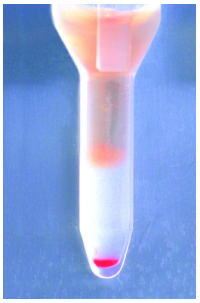
As with any test method, other results may occur that may not appear as typical negative, 1+, 2+, 3+, or 4+ reactions. If hemolyzed samples are used, the liquid portion above or just into the gel will appear pink or red, depending upon the degree of hemolysis.



1. Grossly Hemolyzed Sample



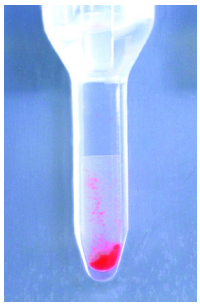
2. Hemolyzed Sample



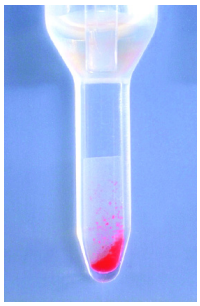
3. Hemolyzed Sample

Illustrations 1, 2, and 3 demonstrate negative test results using hemolyzed samples of varying degrees. If a hemolytic reaction occurs during an indirect antiglobulin test, the microtube may appear similar with the exception that few to no red blood cells will appear in the gel.

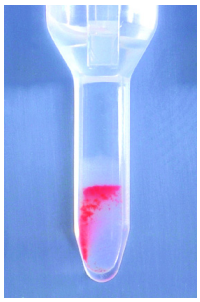
Some antigen-antibody reactions may appear weak. Refer to Illustrations 4 and 5.



4. Weak Positive Reaction



5. Weak Positive Reaction



6. Positive Reaction, Abnormal Position of Red Blood Cell

These positive reactions contain a few agglutinated red blood cells in the middle of the gel or a few agglutinated red blood cells just above the disrupted red blood cell button. These are considered positive and should be graded 1+.

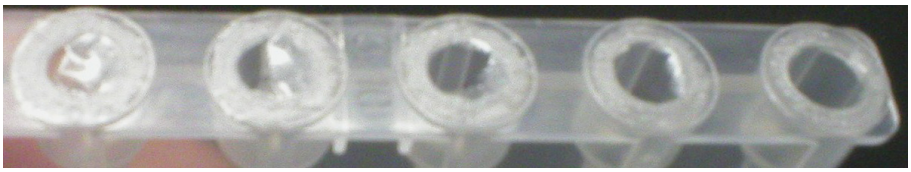
Positive reactions that show agglutinated red blood cells beginning in the middle of the gel may occur if users wait longer than 10-15 minutes prior to initiating incubation or centrifugation of the gel cards. Refer to Illustration 6. Since red blood cell agglutinates are dispersed throughout the gel, the reaction should be graded 2+.

Visual Inspection of Gel Cards Before Use

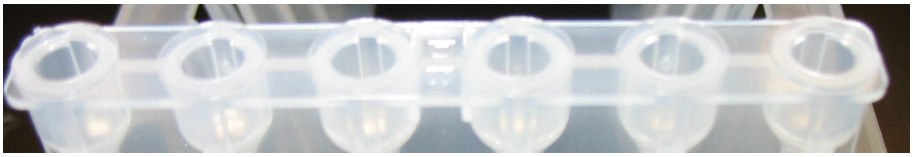
Before performing the ID-MTS™ Gel Test, visually inspect all gel cards for any damage during shipment or handling. Each microtube should have a clear liquid layer on top of the opaque gel. Variations in the liquid and/or gel levels between microtubes may normally be observed. Any gel card that shows signs of drying, discoloration, bubbles, crystals, or other artifacts should not be used in testing. Check to see if the foil seals appear damaged or opened.

Inspection of Gel Cards after Removal of Foil

Visually inspect all ID-MTS™ Gel Cards to ensure that residual film does not block the opening of any microtube after removal of the foil.



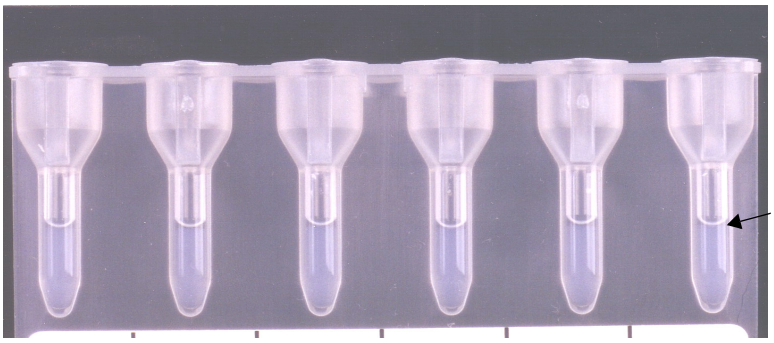
Microtubes exhibiting residual film. This card is unacceptable for use.



Microtubes with no residual film. This card is acceptable for use.

Gel Cards with No Liquid Layer above the Gel

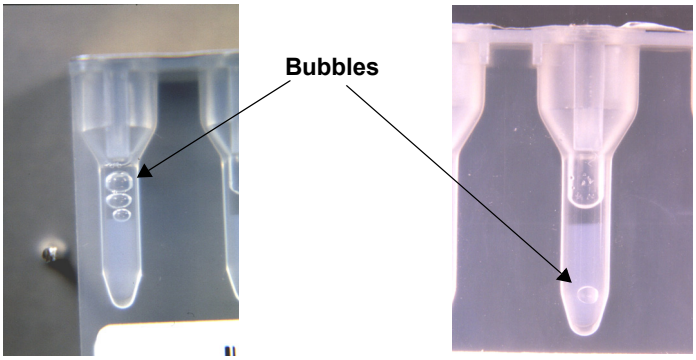
Do not use a gel card where the liquid level in the microtubes is at or below the gel matrix.



Visual Inspection of Gel Cards Before Use (continued)

Gel Cards with Bubbles in the Microtubes

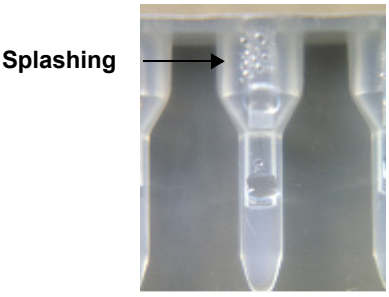
Bubbles may appear in the liquid level above the gel or within the gel itself. Do not use a gel card that contains bubbles in the microtubes.

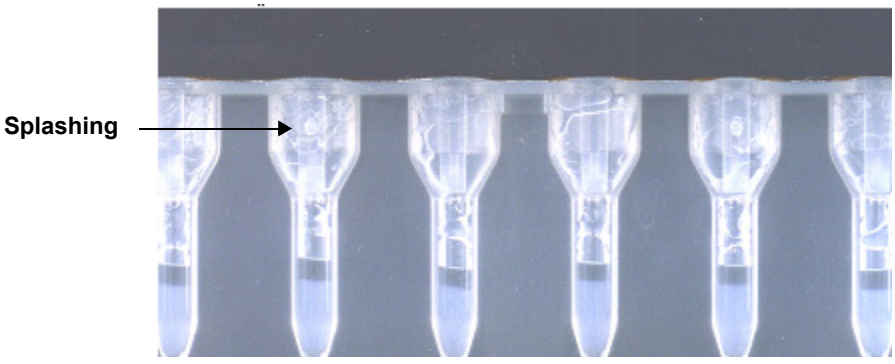


Gel Cards with Splashing in Upper Reaction Chamber

Splashing in the upper reaction chamber may occur during shipment or as a result of improper handling of the product.

The splashed liquid is easily visible upon the inspection of the gel card. Gel cards with visible splashing in the upper reaction chamber should not be used for testing.





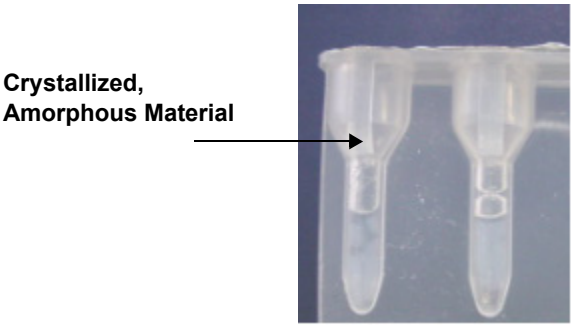
Visual Inspection of Gel Cards Before Use (continued)

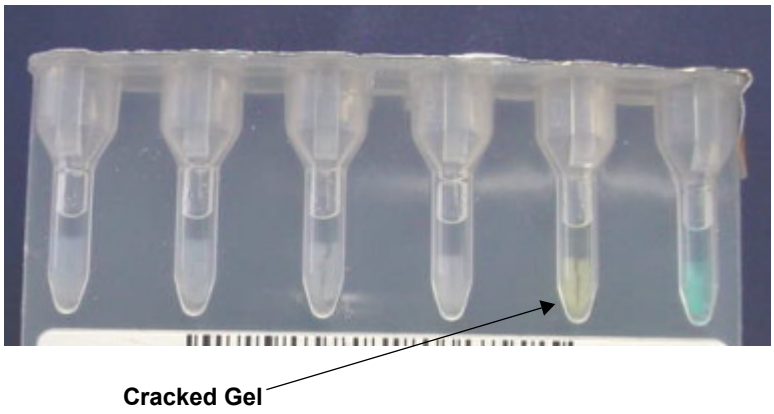
Gel Cards Showing Signs of Drying

Gel cards may show signs of drying due to improper storage, handling, or compromised integrity of the foil seal.

False-positive results may occur if a card that shows signs of drying is used in testing.

Signs of drying include visible cracks in the gel layer and crystallized amorphous material present in the microtube.

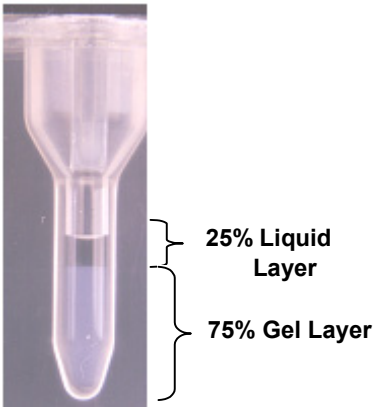




Visual Inspection of Gel Cards Before Use (continued)

Gel Cards with Microtubes Containing Low Gel Levels

When you visually inspect the microtubes in a gel card, a typical microtube should contain a proportion of approximately 75% gel layer with a 25% liquid layer on the top of the gel. The total volume of the combined gel layer and the liquid layer occupies about 75% of the space within the microtube.



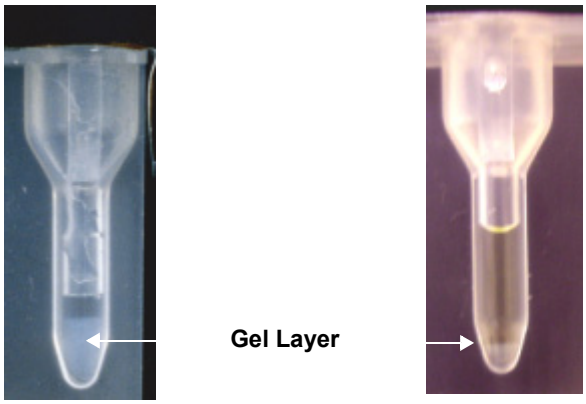
Typical Gel Microtube

Do not use microtubes in testing that have low gel levels. These could be

- Microtubes in which the total volume of the gel layer and liquid layer is reduced
- Microtubes in which the proportion of the gel layer is markedly less than 75%

Using microtubes with low gel levels may impact the results of the gel test by generating false-positive or false-negative results.

Examples of Microtubes with Low Gel Levels



Total Volume Reduced

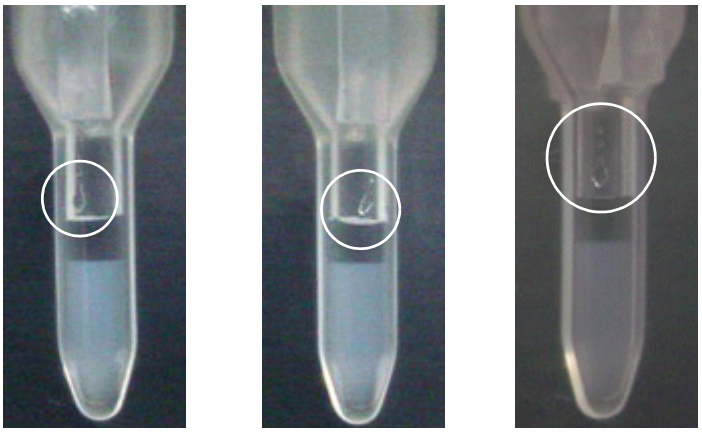
Disproportionate Gel Layer and Liquid Layer

Visual Inspection of Gel Cards Before Use (continued)

Other Cards Acceptable for Use

Gel cards are filled using an automated process. Six (6) stainless steel needles are used to deliver a measured volume of the gel-liquid mixture into each microtube. After filling, the cards are sealed and labeled and then inspected using a state-of-the-art optical inspection system designed to reject cards containing unacceptable defects, such as bubbles, cracks, debris, unacceptable fluid/gel levels, and labeling issues. During the filling process, the filling needles may touch the side of the microtube leaving a residue as noted in the pictures below.

Examples of Microtubes with Residue



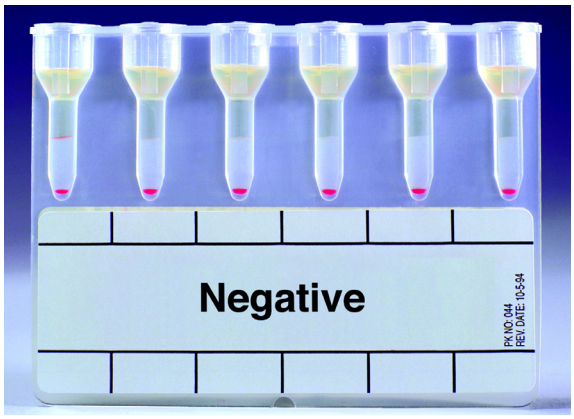
This characteristic occurs on a routine basis during the filling process and should not be confused with bubbles and splashing resulting from physical trauma. (Refer to page 17 of this guide.) It is important to note that no evidence of physical trauma is evident in gel cards demonstrating this characteristic; no bubbles or splashed liquid are visible in the upper reaction chamber and each microtube shows a clear liquid layer on top of the opaque gel. Product testing, such as in-process, final product, and stability testing, has confirmed that this residue has no affect on gel card performance and/or test results.

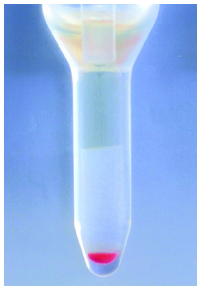
Troubleshooting

Reactions Associated with Sample Quality Issues

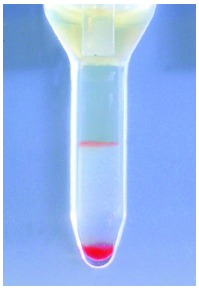
A few unagglutinated red blood cells may be observed on the top of the gel with some negative reactions. These reactions may be associated with the following sample quality issues:

- Samples with clots, particulates, or other artifacts
- Freshly collected serum samples
- Samples previously frozen

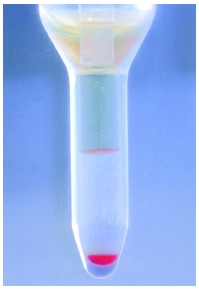




7. Negative Reaction, No Top Line Observed



8. Negative Reaction, Top Line Observed



9. Negative Reaction, Top Line Observed

Reactions Associated with Recently Transfused Patients

The potential exists for unexpected or mixed field results (see section Mixed Field Reactions: Discussion) in samples from recently transfused patients, bone marrow transplant patients, and patients with blood group chimerism[1] due to the existence of two distinct, separable populations of red blood cells in the sample. Mixed field and unexpected reactions due to transfusion last only for the life of the transfused red blood cells. After hematopoietic transplantation, the mixed-field reaction usually disappears when the patient's own red blood cells are no longer produced. Mixed-field reactions that arise through blood group chimerism may persist throughout the life of the individual.

1.Fung, Mark K., et al. (ed) Technical Manual, 18th Ed. Bethesda, MD: American Association of Blood Banks, 2014; Chapter 12, ABO Discrepancies (Red Cell Testing Problems), pg. 298.

Discussion

Samples should be free from debris or particulates prior to use in the gel system. A typical negative reaction is shown in Illustration 7.

Clots, particulates, or other artifacts may cause some red blood cells to become trapped at the top of the gel.

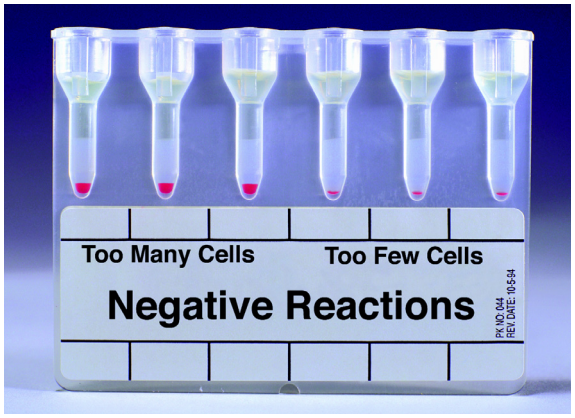
Freshly collected serum samples may have a line/circle of unagglutinated red blood cells at the top of the gel. These red blood cells will appear very pink and faint and do not have the same red, clumping appearance as a positive test result. See Illustrations 8 and 9. These results should not be considered a mixed-field.

Samples previously frozen should be mixed well and centrifuged before use.

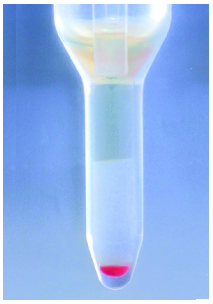
Troubleshooting (continued)

Too Many or Too Few Red Blood Cells in a Negative Reaction

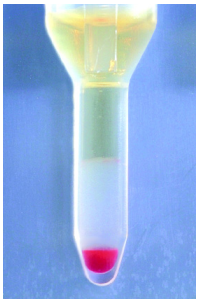
Too many or too few red blood cells in the microtube may be a direct result of using an improperly prepared red blood cell suspension, or adding incorrect quantities of red blood cells to the upper reaction chamber of the microtube.



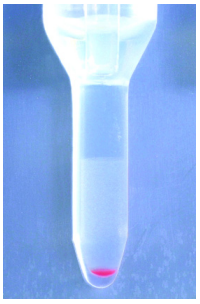
Variations in red blood cell concentration may affect the sensitivity of the test.



10. Negative Reaction, Normal Red Blood Cell Button



11. Negative Reaction, Too Many Red Blood Cells



12. Negative Reaction, Too Few Red Blood Cells

Discussion

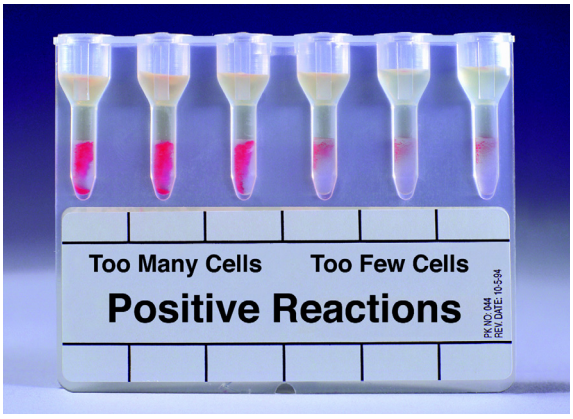
Normal Red Blood Cell Button: Illustration 10 shows the appearance of a normal red blood cell button. This button was derived by adding 50 µL of a 0.8% red blood cell suspension to the gel card as part of the indirect antiglobulin test procedure.

Negative Reaction With Too Many Red Blood Cells: Illustration 11 shows a large red blood cell button caused by adding red blood cells from an improperly prepared red blood cell suspension. Too many red blood cells may cause difficulty making correct test interpretations. If the red blood cell button is large enough, weak reactions may be missed due to masking of red blood cell agglutinates in the bottom portion of the microtube.

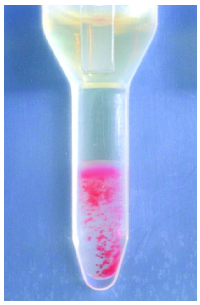
Negative Reaction With Too Few Red Blood Cells: Illustration 12 shows a small red blood cell button caused by adding red blood cells from a very weak red blood cell suspension. If too few red blood cells are added to the gel card, there may not be enough red blood cells to make a valid test interpretation.

Troubleshooting (continued)

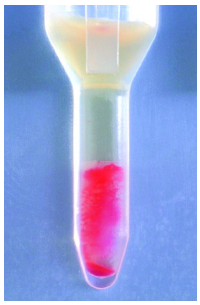
Too Many or Too Few Red Blood Cells in a Positive Reaction



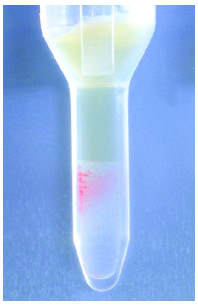
Too many or too few red blood cells in the microtube may be a direct result of using an improperly prepared red blood cell suspension, poor mixing of red blood cell suspension, or adding incorrect quantities of red blood cells to the upper reaction chamber of the microtube. Variations in red blood cell concentration may affect the sensitivity of the test.



13. Positive Reaction Typical 2+ Reaction



14. Positive Reaction, Too Many Red Blood Cells



15. Positive Reaction, Too Few Red Blood Cells

Discussion

Typical 2+ Positive Reaction: Illustration 13 shows a typical 2+ reaction. This was derived by adding 50 µL of a 0.8% red blood cell suspension, along with serum containing antibody, to the gel card as part of the indirect antiglobulin test procedure.

Positive Reaction With Too Many Red Blood Cells: Illustration 14 shows a 2+ reaction with too many red blood cells. This was caused by adding red blood cells from an improperly prepared red blood cell suspension. Too many red blood cells may cause difficulty making correct test interpretations. It is possible when red blood cell suspensions are too concentrated that weaker reactions may be observed due to the increase in the antigen-antibody ratio.

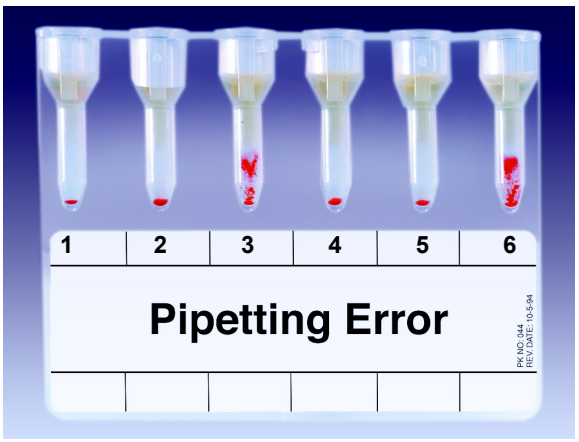
Positive Reaction With Too Few Red Blood Cells: Illustration 15 shows a 3+ reaction with too few red blood cells caused by adding red blood cells from a very weak red blood cell suspension. When red blood cells are too low in concentration, they become difficult to see.

Troubleshooting (continued)

Pipetting Errors

Indirect AHG tests should have a total of 75 µL of reactants added to the reaction chamber, 50 µL of the red blood cell suspension, and 25 µL of the test sample.

- If a pipetting error of serum/plasma occurs, the microtube will exhibit a difference in the liquid levels in the upper reaction chamber following centrifugation.
- If a red blood cell suspension pipetting error occurs, the size of the red blood cell button will be reduced.



16. Pipetting error visible in microtubes 1 and 2.

17. Microtube 1
Too few red blood cells

18. Microtube 2
No serum/plasma added

19. Microtube 5
Negative reaction—Correct volumes of reactants

Discussion

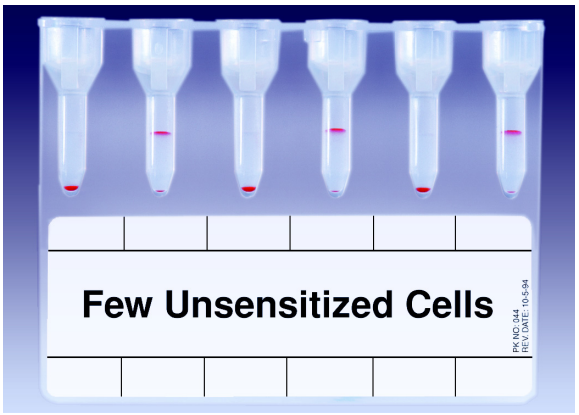
Illustration 16 shows the effect of pipetting errors in microtubes 1 and 2; however microtubes 3, 4, 5, and 6 are acceptable. Microtubes 3 and 6 exhibit a 2+ reaction; microtubes 4 and 5 show a negative reaction.

Illustration 17 shows a situation where too few red blood cells are present in the microtube. Illustration 18 shows a situation where no serum/plasma has been added to the microtube. Illustration 19 highlights the negative reaction with correct volumes in microtube 5 (in Illustration 16) for comparison with Illustrations 17 and 18. If a pipetting error is recognized, the test must be repeated.

Troubleshooting (continued)

Strongly Positive Result with a Few Nonreactive Red Blood Cells

Gel tests exhibiting 3+ or 4+ reactions may also contain a small number of unagglutinated red blood cells that form a small red blood cell button in the bottom of the microtube.



Discussion

These results could represent a mixed-field reaction in which the majority of red blood cells present in the test sample are strongly reactive and the minority of red blood cells nonreactive.

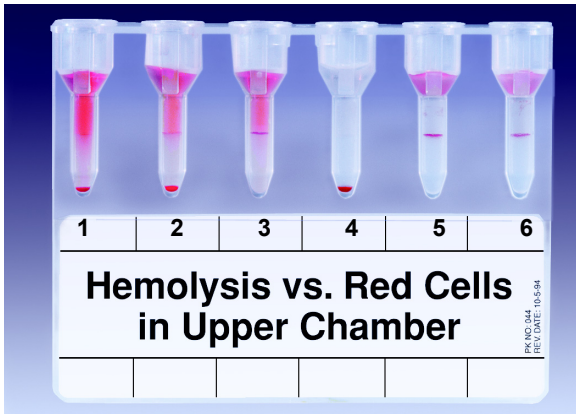
If the red blood cells used for testing are from a single source (i.e., reagent screening or panel red blood cells), this reaction cannot be the result of a true mixed-field reaction. If red blood cells are not sensitized during incubation, no antigen-antibody complex will form. These nonreactive red blood cells will travel through the gel microtube and form a small red blood cell button in the bottom of the microtube. Generally, improper mixing of the red blood cells with the serum/plasma sample is the cause of this appearance.

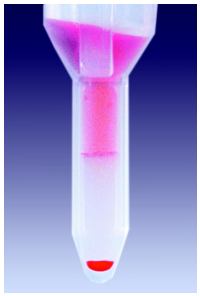
Care must be exercised in interpretation if the sample used for testing was an incompletely clotted whole blood sample. In such cases, the red blood cells noted above the gel microtube may be trapped within a large fibrin aggregate. Tests should be repeated, either with a different blood sample that avoids coagulation difficulty (plasma), or by using serum that is completely clotted and well spun.

Troubleshooting (continued)

Hemolyzed Sample vs. Red Blood Cells in the Upper Chamber

A pink or red appearance in the upper chamber that extends to the top of the gel microtube is most likely indicative of hemolysis, either from the use of a hemolyzed sample or as a result of complement binding during incubation. A pink or red appearance noted only in the upper chamber is more apt to be due to the presence of intact red blood cells.





20. Hemolysis in Microtube 1

21. Negative Test in Microtube 4

22. Red Blood Cells in Upper Chamber in Microtube 5

Discussion

The first two microtubes in this gel card demonstrate the appearance of hemolysis. See Illustration 20. The red blood cells are in a button at the bottom of the microtube; no agglutinins are noted in the gel microtube.

- If the red blood cell button is of normal size and the original sample is noted to be hemolyzed, these two tests should be interpreted as negative.
- If the red blood cell button is decreased and the original test sample is free of hemolysis, these tests may be indicative of a positive test due to in-vitro hemolysis and further investigation should be performed.

The third microtube demonstrates a 4+ reaction and the original sample is noted to be hemolyzed.

Troubleshooting (continued)

Hemolyzed Sample vs. Red Blood Cells in the Upper Chamber (continued)

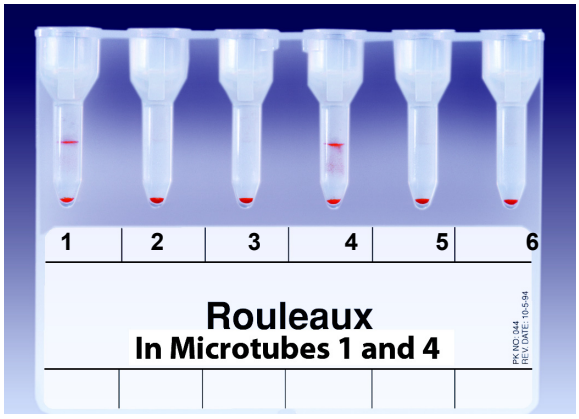
The fourth microtube (see Illustration 21) shows a normal negative gel test, i.e., red blood cell button at the bottom of the microtube, with no hemolysis or red blood cells noted in the upper chamber.

The fifth and sixth microtubes (see Illustration 22) contain intact red blood cells adhering to the walls of the upper reaction chambers. The red blood cells that came in contact with the Anti-IgG reagent in the gel microtube have agglutinated and resulted in a 4+ reaction. Those red blood cells remaining in the upper chamber have adhered to the side of the reaction chamber. This adherence may occur because there is a high protein level in the sample being tested or because there is increased static electricity in an environment with low humidity.

Troubleshooting (continued)

Rouleaux

Rouleaux are aggregations of red blood cells in a characteristic “stacking” pattern. Rouleaux can occur if sufficient quantities of abnormal proteins are present in the test sample and may infrequently cause difficulties in gel test interpretation.



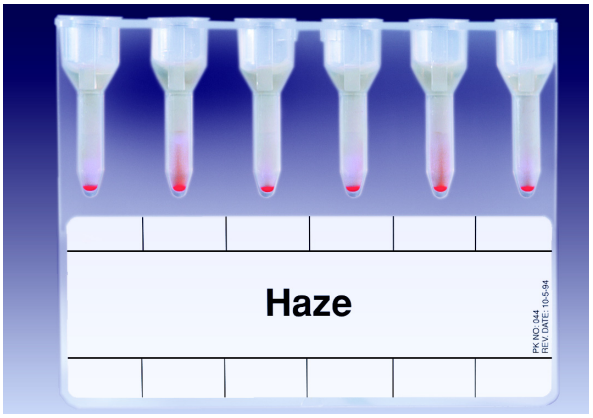
Discussion

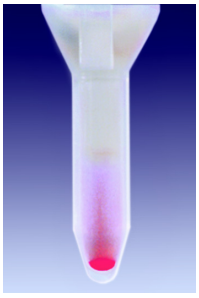
Rouleaux caused by serum or plasma with abnormally high concentrations of protein (such as in patients with multiple myeloma or Waldenstrom’s macroglobulinemia or from patients who have received plasma expanders of high molecular weight) may infrequently cause difficulties in ID-MTS™ Gel Test interpretation. False positive results or hazy reactions may occur with these samples but are rare. If false positive reactions (e.g. Rouleaux, cells coated with immunoglobulins, etc.) occur in the control gel, the blood group cannot be established with this card. Additional testing will be necessary to resolve this false positive reaction. If the control test is positive, the test cells should be washed several times in warm saline and retested. If the control test again gives a positive reaction, a valid interpretation of the results obtained cannot be made. Laboratories are advised to consult their approved procedures.

Troubleshooting (continued)

Haze or Pink Color in Gel

Occasionally, gel tests may appear hazy or pink with no evidence of red blood cell agglutination. These reactions differ from typical positive or negative results.





Haze

Haze

Haze

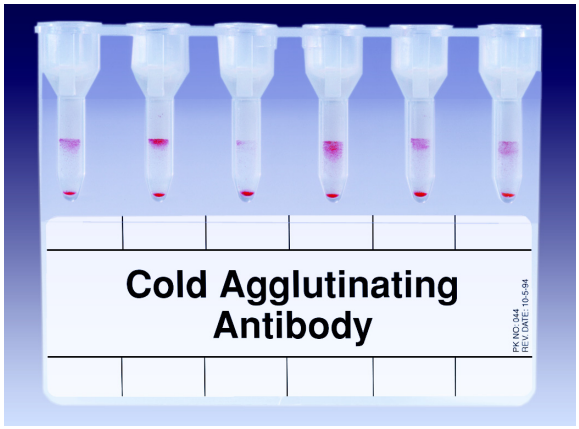
Discussion

Hazy or pink color in gel tests may be associated with rouleaux which has been induced by high levels of abnormal proteins in the patient's serum or plasma. Although rouleaux does not usually occur in gel tests because unbound proteins remain above the gel and are removed from contact with the red blood cells during centrifugation, high levels could cause a coating of the red blood cells during incubation. The resulting rouleaux may cause difficulties in the interpretation of a gel test. Information regarding the patient's diagnosis and total serum protein may help confirm this explanation. Refer to page 30 for additional information and instructions.

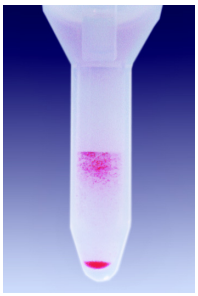
Troubleshooting (continued)

Direct Agglutinating Antibody

In antibody detection or identification tests, samples containing strong, direct agglutinating antibodies may give the appearance of a mixed-field reaction. As illustrated in the photo to the right, the most common direct agglutinins are cold-enhanced IgM antibodies.



Cold Agglutinin



Cold Agglutinin

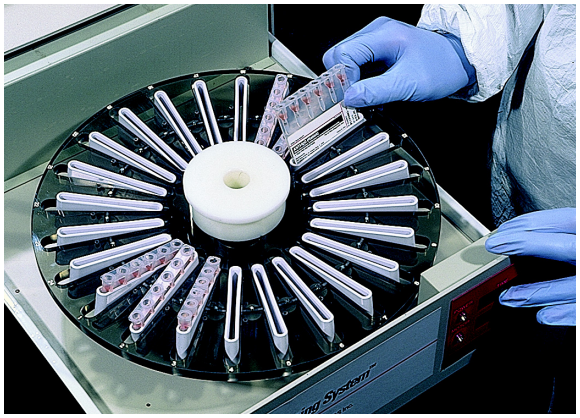
Discussion

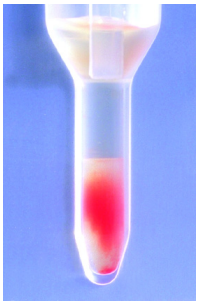
Direct agglutinins may give a mixed-field appearance in Anti-IgG gel antibody detection or identification tests. It may be helpful to perform a room temperature or 37°C test procedure using the MTS™ Buffered Gel Card. Reactivity in these tests indicates the presence of direct agglutinating antibody. Reactivity only at room temperature or reactivity that is stronger at room temperature would be consistent with a strong IgM cold agglutinin in the test sample. These reactions are not truly mixed-field if the reagent red blood cell sample used for testing is from a single donor source, i.e., not a pooled red cell sample.

Troubleshooting (continued)

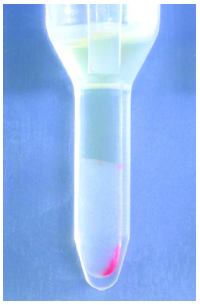
Improper Centrifugation

When centrifugation cycles are interrupted or cards are not spun at the proper angle or speed, all six microtubes of a gel card will have a similar appearance. These reactions differ from typical positive or negative results.

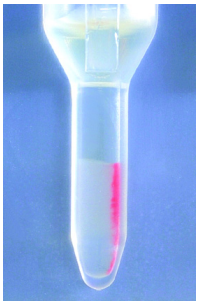




23. Interruption in Centrifugation; No Result Determined



24. Incorrect Card Position During Centrifugation; No Result Determined



25. Incorrect Card Position During Centrifugation; No Result Determined

Discussion

If the centrifugation cycle is interrupted, unagglutinated red blood cells may be observed in the gel. These red blood cells will appear dark pink and hazy. See Illustration 23. Because all six microtubes within a gel card are centrifuged under the same conditions, improper centrifugation is easily recognized by all six microtubes showing the same or similar appearance.

Additionally, if cards are not properly seated in the card holders and not allowed to spin at a 90 degree angle during centrifugation, a line of red blood cells may stream down one side forming a “J” appearance. See Illustrations 24 and 25. These tests should be repeated.

In these situations, do not recentrifuge these gel cards under any circumstances. The recentrifugation of gel cards may dissipate weak reactions producing false negative results.

Suggested Reading

Malyska H, Weiland D. The Gel Test. Laboratory Medicine, 1994; 25:81-5.

Lapierre Y. et al. The gel test: a new way to detect red cell antigen-antibody reactions. Transfusion, 1990;30:109-113.

Fung, Mark K., et al. (ed) Technical Manual, 18th Ed. Bethesda, MD: American Association of Blood Banks, 2014.

Instructions for Use: Anti-Human Globulin Anti-IgG (Rabbit) MTS™ Anti-IgG Card (current version). Pompano Beach, FL: Micro Typing Systems, Inc.

Instructions for Use: Blood Grouping Reagent MTS™ A/B/D Monoclonal Grouping Card, (current version). Pompano Beach, FL: Micro Typing Systems, Inc.

Revision History

2016-03-15

Chapter/Section/Appendix	Page	Description
Front Cover	N/A	▪ Updated company logo. ▪ Placed “Rx ONLY” symbol on US Licensed prescription drug product labeling per the FDA Modernization Act of 1997 (FDAMA).
Mixed Field Reactions	12	▪ Discussion: Inserted “(see section Troubleshooting: Reactions Associated with Recently Transfused Patients for information about recently transfused patients)” at the end of the second sentence.
Troubleshooting	21	▪ Added section entitled “Reactions Associated with Recently Transfused Patients.”
Suggested Reading	34	▪ Updated the reference to the AABB Technical Manual
Back Cover	N/A	▪ Updated company logo.

2010-06-04

Chapter/Section/Appendix	Page	Description
Entire guide	N/A	▪ Updated trademarks.
Introduction	4	▪ Replaced “package insert” with “Instructions for Use,” and described how to obtain IFUs. ▪ Removed statement claiming improved sensitivity, specificity and reliability for gel test.
Reading Gel Test Reactions	5	▪ Added statement that if hemolysis is observed, facility procedures should be consulted.
Interpreting Gel Test Reactions	6	▪ Added page references; arranged following pages in corresponding order. ▪ Added caution regarding clots, particulates and other artifacts.
2+ Reactions	9	▪ Clarified Discussion of 2+ reactions.

2010-06-04 (continued)

Chapter/Section/Appendix	Page	Description
Other Reactions	15	▪ Changed heading from “Hemolysis\Weak Reactions\Other” to “Hemolysis, Weak Reactions, Abnormal Position of Agglutinated Cells.”
Visual Inspection of Gel Cards Before Use	16	▪ Added instruction to inspect cards for residual film after removing foil. Added photos showing acceptable and unacceptable gel cards.
Troubleshooting: Pipetting Errors	24	▪ Specified which microtubes exhibit pipetting error in Illustration 16.
Troubleshooting: Rouleaux	28	▪ Specified which microtubes exhibit rouleaux in graphic. ▪ Clarified definition and discussion of rouleaux; using verbiage from MTS™ Instructions for Use.

2008-12-09

Chapter/Section/Appendix	Page	Description
Entire guide	N/A	▪ Updated product references. ▪ Changed “agglutinates” to “agglutinated red blood cells” or “red blood cell agglutinates.”
Front Matter	ii	▪ Added copyright date 2008.
Introduction: Gel Test Principle	4	▪ Expanded history of gel test; rephrased description of centrifugation.
	5	▪ Updated photograph of gel card. ▪ Added photograph of dextran acrylamide gel particles with agglutinated red blood cells.
Introduction: Interpreting Gel Test Reactions	6	▪ Corrected description of 2+ reaction by changing “A few agglutinates may be observed in the bottom of the microtube” to “A few unagglutinated red blood cells may be observed in the bottom of the microtube.” ▪ Re-ordered list of reaction levels and rearranged following pages to correspond. ▪ Replaced photograph illustrating range of reactions.
ABO and Rh Card Interpretations	13	▪ Added “monoclonal” to occurrences of Anti-A, Anti-B and Anti-D.
	14	▪ Added photo of MTS™ A/B Monoclonal Grouping Card
Troubleshooting: Pipetting Errors	24	▪ Adjusted images to more accurately depict the pipetting errors described.

2008-12-09

Chapter/Section/Appendix	Page	Description
Troubleshooting: Rouleaux	28	▪ Expanded discussion to include guidance for dealing with rouleaux, reflecting the MTS™ Instructions for Use.
Troubleshooting: Haze or Pink Color in Gel	29	▪ Added cross reference to Rouleaux section for additional information and instructions.
Suggested Reading	32	▪ Updated edition of AABB Technical Manual.

2006-04-27

Chapter/Section/Appendix	Page	Description
Front Matter	ii	▪ Added copyright date 2006.
Table of Contents	1	▪ Regenerated for this revision.
Introduction	2-3	▪ Provided additional information to consult the package insert for specific instructions for use. ▪ Edited, “Reading Gel Test™ Reactions” title.
Interpreting Gel Test Reactions	4	▪ Updated Mixed Field Note. Removed the second sentence. Added, “Additional patient history and testing will be necessary for resolution.”
Negative Reactions	9	▪ Clarified Definition section.
Mixed-Field Reactions	10	▪ Provided instruction to gather additional patient history and testing before interpreting reactions as mixed field.
ABO and Rh Card Interpretations	11	▪ Added, “...and Rh”
Visual Inspection of Gel Cards Before Use	18	▪ Added section on, “Other Cards Acceptable for Use.”
Troubleshooting: Reactions Associated with Sample Quality Issues	19	▪ Removed, “...which can be minimized by the use of samples collected in EDTA.”
Trouble Shooting: Cold, Agglutinating Antibody	28	▪ Changed the title to, “Direct Agglutinating Antibody.” ▪ Replaced “cold-reactive” with “direct” agglutinating. ▪ Clarified the Discussion section.
Trouble Shooting: Improper Centrifugation	29	▪ Clarified the Discussion section.
Suggested Reading	30	▪ Updated the reference information.
Revision History	31	▪ Revised to include listing of current revisions.

2004-10-21

Revision to Chapter/Section/Topic/Appendix	Page	Description
Entire guide	N/A	▪ Dated all pages YYYY-DD-MM. ▪ Changed the page layout using the OCD template. ▪ Edited wording for clarity. ▪ Changed the term *mixed-cell* to *mixed-field.* ▪ Used the term *red blood cells* consistently ▪ When appropriate, numbered the illustrations.
Front Matter	ii	▪ Added this page.
Table of Contents	1	▪ Added the Table of Contents.
Introduction	2	▪ Added Gel Test Principle section.
Introduction	3	▪ Added Reading Gel Test Reactions section.
Introduction	4	▪ Added Interpreting Gel Test Reactions section.
ABO Card Interpretations	12	▪ Added MTS™ AB Monoclonal Grouping Card information.
Visual Inspection of Gel Cards Before Use	14-17	▪ Added this section.
Troubleshooting Reactions Associated with Sample Quality Issues	18	▪ Renamed this section and added information.
Troubleshooting Pipetting Errors	21	▪ Added to the explanation.
Troubleshooting Haze or Pink Color in Gel	26	▪ Reworded the Discussion section.
Troubleshooting Improper Centrifugation	28	▪ Added to the explanation.
Suggested Reading	29	▪ Added this section.
Revision History	30	▪ Added this section.

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