The Implementation of the Capture® Workstation in the Transfusion Medicine Student Laboratory

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Abstract

Solid-phase technology has revolutionized antigen-antibody testing in the transfusion medicine laboratory. The Capture® Workstation is a semi-automated solid-phase technology that provides a highly sensitive method for in vitro antibody screen and identification testing. The identification of antibodies in clinical samples is important for reducing the likelihood of a hemolytic transfusion reaction. The purpose of this project is to familiarize transfusion medicine students with the use of Capture® Workstation solid-phase technology as an alternative to both tube and gel typing methods. Ten plasma samples were tested for unexpected IgG antibodies to red blood cells using the Capture-R® Ready-Screen® three-cell screening system. Samples with a positive screen result were further tested to identify the most probable antibody using the Capture-R® Ready-ID® system. Of the ten samples tested, samples #2, #3, #8 and #9 were positive for antibodies against red cell antigens. The following antibodies were identified from the panel: sample #2 (anti-K), sample #3 (anti-E), sample #8 (anti-D) and sample #9 (anti-c and anti-E). Although the antibody results from the Capture-R® Ready-ID® system correlated with the samples' historical results, an extended typing panel is required to definitively identify the antibodies in samples #8 and #9 and to rule out the least probable antibodies. The Capture® Workstation solid-phase system was successful in screening plasma for unexpected IgG antibodies and identifying the most probable antibody present in the sample. The Capture® Workstation serves as a sensitive and specific semi-automated method that can be used by transfusion medicine students for in vitro antibody screening and antibody identification testing.

Introduction

Transfusion medicine practice centers around the identification of antigen-antibody reactions using the following three methodologies: tube typing, gel technology and solid-phase technology. The tube method is a familiar concept that utilizes glass test tubes to test for ABO antigen (forward) typing, ABO serum (reverse) typing, direct antiglobulin testing (DAT) and indirect antiglobulin testing (IAT). Although the tube method is considered the gold standard of transfusion medicine testing, the implementation of automation in the laboratory has reduced human error, standardized technique and improved turnaround times.[1] Both gel and solid-phase technology have been used for antibody identification studies and provide better sensitivity and specificity compared to manual tube methods.[2]

In the gel method, the microtube is composed of an upper reaction chamber and a narrow column containing 75% dextran acrylamide beads. The gel column acts as a filter to the resulting red cell agglutinates. The unagglutinated cells easily pass through the column and settle to the bottom, but the agglutinated cells remain suspended on the surface of the beads because of the agglutinate size. The purpose of this laboratory exercise is to familiarize transfusion medicine students with the use of Capture® Workstation solid-phase technology as an alternative to both tube and gel typing methods.

The Capture® Workstation is a semi-automated solid-phase technology that provides a highly sensitive method for antibody screen and identification testing.[3] The highly sensitive and specific nature of solid-phase technology makes it an effective method for identifying clinically significant antibodies that may cause hemolytic transfusion reactions.[4, 5] The solid-phase technology involves the use of a microtiter plate wells that are pre-coated with a specific red cell antigen. Low ionic strength solution (LISS) and patient plasma are added to the well. If antibodies to the specific antigen are present in the patient's plasma, those antibodies will readily bind to the antigens on the well. The LISS used in the solid-phase method is different from what is used in other methods because it contains a protein indicator that serves as a process control, ensuring that patient plasma was added to the well.

After the addition of LISS and patient plasma, the microtiter plate is first incubated and then washed to remove any unbound proteins that may be present in the reaction well. Anti-IgG indicator cells are added, and the wells are centrifuged. The indicator cells bind to the IgG antibodies that are bound to the antigens on the well surface. The indicator cells allow for the antigen-antibody reaction to be visualized. A positive reaction is indicated by a smooth, uniform spread of the indicator cells on the bottom of the well. In contrast, a negative reaction results in a pellet being formed at the bottom of the well. A bench light may be used to help visualize and grade the resulting reactions.

Methods

Antibody screening and identification tests were performed to detect the presence of unexpected IgG antibodies in plasma. This solid-phase method required the use of a 37°C incubator, semi-automated plate washer and centrifuge designed specifically for the microtiter plate wells. A total of ten patient plasma samples were tested for the antibody screening assay. To begin the process of the antibody screening assay, microtiter plate wells pre-coated with lysed group O screening cells were obtained from the Capture-R® Ready-Screen® kit. Two drops of Capture low ionic strength solution (LISS) were added to each well followed by one drop of positive control serum, negative control serum and patient plasma into each designated well. The bromocresol purple dye in the LISS reagent changed color from purple to blue as an indication that serum or plasma was successfully added to each well. The test wells were incubated at 37°C for 20 minutes and then washed using the P1 setting on the semi-automated plate washer. One drop of anti-IgG Capture-R Ready indicator red cells was added to each well, and the test strips were centrifuged at 530 x g for 2 minutes. The resulting antigen-antibody reactions were visualized using an illuminated surface lamp, and the reactions were graded accordingly. Samples with a positive antibody screening result were followed up with an antibody identification test using a selection panel cells from the Capture-R® Ready-ID® kit. The Capture-R® Ready-ID® kit included microplate wells prepared from fourteen group O donors that expressed frequently inherited antigens. Two additional wells represented the experimental positive and negative controls. The same test method and materials used for the antibody screening assay was used for the antibody identification assay, and the resulting antigen-antibody reactions were graded accordingly.

Results

Figure 1 Essential Equipment for the Capture® Workstation. A. Semi-automated Plate Washer B. Microtiter Plate Centrifuge C. 37°C Plate Incubator

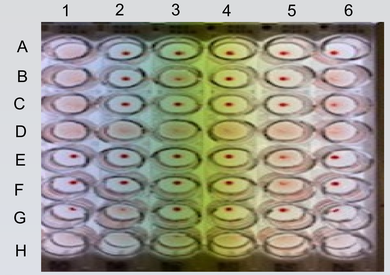

Figure 2 Capture-R® Ready-Screen® System Plate Map

Table 1 Capture-R® Ready-Screen® System Results

Row	Sample	Result
A1 to D1	Capture R Positive control serum	Pos (cell I, II & III)
E1 to H1	Capture R Negative control serum	Neg
A2 to D2	Sample #1	Neg
E2 to H2	Sample #2	Pos (cell III)
A3 to D3	Sample #3	Pos (cell II)
E3 to H3	Sample #4	Neg
A4 to D4	Sample #5	Neg
E4 to H4	Sample #6	Neg
A5 to D5	Sample #7	Neg
E5 to H5	Sample #8	Pos (cell I & II)
A6 to D6	Sample #9	Pos (cell II & III)
E6 to H6	Sample #10	Neg

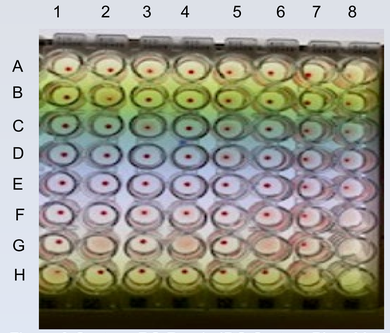

Figure 3 Capture-R® Ready-ID® System Plate Map

Table 2 Capture-R® Ready-ID® System Results

Column	Sample	Most probable antibody
1 & 2	Sample #2	anti-K
3 & 4	Sample #3	anti-E
5 & 6	Sample #8	anti-D
7 & 8	Sample #9	anti-c and anti-E

Discussion

Pre-transfusion testing is an essential method that is used to help reduce the likelihood of hemolytic transfusion reactions from occurring in patients. Although the conventional tube method is considered the gold standard in transfusion medicine testing, automated methods are reliable and provide results that are consistent with the conventional tube method. The goal of the study was to familiarize transfusion medicine students of the use of the Capture® Workstation as an alternate technology to the conventional tube method and gel method. The present study showed that the Capture® Workstation serves as a sensitive and specific semi-automated method for antibody screening and antibody identification in the transfusion medicine laboratory.

The sensitivity of the assay to IgG antibody was demonstrated by the Capture-R® Ready-Screen® three-cell screening system. The antibody screening results from the Capture® Workstation correlated 100% to the patient antibody history from previous tube testing. In addition to the patient results, quality control results for both positive and negative controls were acceptable. Conventional tube methods resulted in weak antibody screening cell reactions in sample #8; however, solid-phase technology produced strong, positive reactions for the sample in screening cells I and II. The specificity of the assay was demonstrated by the Capture-R® Ready-ID® kit. Fourteen group O donors that expressed frequently inherited antigens were used as the screening cells. The assay identified the most probable antibody in all four of the positive samples; however, further testing using the extended panel is recommended to definitively rule out other antibodies in sample #8 and sample #9.

The present study has limitations that should be identified. The major limitation of the study is that the Capture® Workstation is designed to only identify unexpected IgG antibodies, not IgM antibodies. Another limitation is erroneous test results can occur from inadequate incubation periods, improper centrifugation, inadequate washing of the testing wells or the omission of test reagents.

Conclusion

Pretransfusion testing is useful for detecting and identifying clinically significant antibodies to red cell antigens that could potentially cause a hemolytic transfusion reaction in transfused patients. The Capture® Workstation solid-phase system was successful in screening plasma for unexpected antibodies and identifying the antibody present in the sample. Future research will include the use of the Capture-R® Ready-ID® Extend I (D-positive panel) and Capture-R® Ready-ID® Extend II (D-negative panel) systems for identifying antibodies that are not easily identified using the Capture-R® Ready-ID® system.

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