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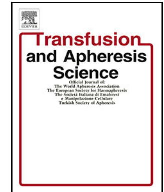

A new approach to detection of incomplete antibodies using hydrogel chromatography medium

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ABSTRACT

In assays for incomplete antibody detection, several washing steps are required to remove unbound globulins which may cause false negatives. Here, we present an improved approach employing hydrogel chromatography medium (HCM) in the detection of incomplete antibodies. After a rapid single-step centrifugation, incomplete antibodies, attached to red blood cells (RBCs), were separated from the reaction mixture using HCM and sedimentation. This method obviates the need for multiple centrifugation steps found in conventional Tube-Coombs tests. The HCM-Coombs tests may have a wide range of applications for incomplete antibody detection.

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1. Introduction

In clinical blood group antibody screening and identification, recipients, who have a history of blood transfusions or pregnancy, as well as donors, should all undergo incomplete antibody testing. Incomplete antibody detection is one of the most important elements for a clinical diagnosis of several diseases, including hemolytic disease in newborns, autoimmune hemolytic disease, drug immune hemolytic diseases, and so on. Anti-human globulin (AHG) test (Coombs T) is the traditional method used to detect incomplete antibodies [1–3]. There are many AHG tests, such as Tube Coombs test (Tube-Coombs T), Microcolumn gel immunoassay Coombs test (MGIA-Coombs T), and the solid-phase red cells adherence Coombs test (solid-phase Coombs T) [4–6]. In clinical settings, however, their application is restricted because of innate defects. In Tube-Coombs T, after the reaction between the red blood cells (RBCs) and serum, several washings are required so that the unbound globulins do not neutralize the AHG and cause a false-negative result [7]. The MGIA assay overcomes the tedious washing process, it has high sensitivity, and the results are easier to interpret, and more samples can be processed simultaneously. One disadvantage of this method is some fibrin, particulates or other artifacts may trap red blood cells at the top of the gel columns erroneously leading to an abnormal result. Another is gel particle activity is easily influenced by temperature, which also produces false positive results. In addition, the expense of the MGIA assay is cost prohibitive for mainstream clinical use. In the solid-phase red cell adherence test, the short viability span of indicator cells is the key factor prohibiting this method from being widely used [5,8–11].

To avoid tedious washings of RBCs in Coombs T, as well as to take advantage of the respective merits of these techniques, we have established a new immune experimental technique to detect incomplete antibodies using Hydrogel

Abbreviations: RBCs, red blood cells; AHG, anti-human globulin; Coombs T, anti-human globulin (AHG) test; Tube-Coombs T, Tube Coombs test; MGIA-Coombs T, microcolumn gel immunoassay Coombs test; Solid-phase Coombs T, solid-phase red cells adherence test Coombs test; HCM, hydrogel chromatography medium; BSA, bovine serum albumin; HRP–AHG, horseradish peroxidase–AHG; TMB, tetramethylbenzidine.

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chromatography medium (HCM). The HCM is in gel state, and it has a higher density (1.14 g ml^{-1}) than general cells. HCM can also be potentially employed in other immunology experiments, such as ELISAs or flow cytometry, which relate to the particulate antigen.

2. Material and methods

2.1. Reagents, instrumentation, and blood sources

Anti-D, goat anti-human IgG (goat AHG), bovine serum albumin (BSA), horseradish peroxidase–AHG (HRP–AHG), and tetramethylbenzidine (TMB) were all purchased from Sigma Aldrich (USA). Microcolumn gel immunoassay (MGIA) anti-human globulin cards were purchased from Changchun Boxun (China). Glass tubes (external diameter × length: 7.5 mm × 150 mm) were purchased from Sangon, China. 2 M H_2SO_4, normal saline (homemade), hydrogel chromatography media (patent CN201310710675.0) were prepared in our lab. Samples were separated with a centrifuge (TD-3A, Changchun Boyan Company, China). OD values were obtained from a microplate reader (Multiskan MK3, Thermo, USA).

Whole blood samples (types A, B, O, and AB) and plasma samples (types A, B, O, and AB) were provided by the Jiangsu Province Blood Center. Donors are in accordance with "National Blood Donor Health Check criteria".

2.2. Test procedure

In this study, RBCs were mainly used as a reaction model to detect the separating effect of the HCM. The reaction mixture including RBCs and plasma samples was transferred to the HCM surface, and the RBCs, in the presence or absence of incomplete antibodies, were separated from the reaction liquid under the action of low centrifugal force (1000 rev min^{-1}, 1 min, 1000 rev $min^{-1} \approx 95\,g$, RCF $(g) = 1.12r\,(\text{rev min}^{-1}/1000)^2$). RBCs require less than 100 *g* centrifugal force for sedimentation, while proteins require a minimum of 3000 *g* centrifugal force for sedimentation [12]. We chose the 1000 rev min^{-1} centrifugal force to ensure that the reacted RBCs sediment through the HCM, to the bottom of the tubes, and the reactant, including nonspecific proteins, remain above the HCM. After discarding the reactant and HCM, without disrupting the pellet, AHG was added to the test tube and centrifuged again. The agglutination was visible after centrifugation (Fig. 1).

Using this procedure, other particulate antigens may be separated; after separation the next step would be adding a marked secondary antibody for incubation, and then continuing on to the last step.

2.3. Application of HCM in the separation of small molecule dyes and RBCs

Methylene blue (Mw 799 Da) and RBCs were diluted with normal saline to 1% and 3%, respectively. Afterwards, 100 µl diluted methylene blue and RBCs were added to the glass tubes preloaded with 2 ml HCM. After centrifuging at 1000 rev min^{-1} for 1 min, the separation of the Methylene blue and RBCs was observed. In parallel, methylene blue (100 µl) was added to glass tubes preloaded with HCM. The tubes were incubated in a 37 °C water bath for 1 h, and then the diffusion of methylene blue in HCM was observed.

2.4. ABO blood group antibody detection

Whole blood (types A, B, O, and AB) were diluted to 3% with normal saline, and A, B, O, and AB plasma types were prepared. Sixteen tubes preloaded with 2 ml HCM each were also prepared. 1 drop (1 drop ≈ 50 µl in this study) of plasma was added to each tube, and then 1 drop of diluted RBCs was added to the corresponding tubes. The tubes were then centrifuged at 3000 rev min^{-1} for 1 min. After removing the

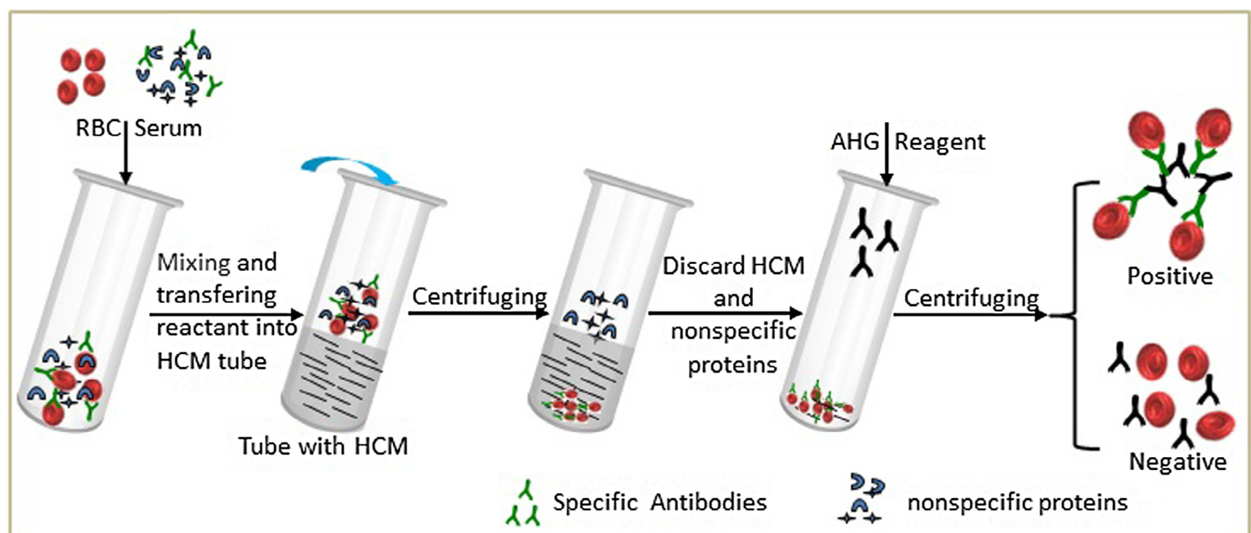

Fig. 1. Flow chart of experimental procedure for incomplete antibody detection using HCM.

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