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Serum protein mediums are important factors of the manual hexadimethrine bromide (polybrene) test, experience in China

Les concentrations en protéines, facteur important du test au polybrene : expérience en Chine

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Abstract

***Background and objectives.* –** The use of the manual hexadimethrine bromide (polybrene) test in routine cross-matching after accurately detecting cell grouping and irregular antibodies is prevalent in China. This article reports the importance of serum protein mediums in the performance of the manual hexadimethrine bromide test.

***Materials and methods.* –** Blood group O red blood cells and Blood group AB and Rh positive serum were collected at random from healthy blood donators, IgG anti-D serum separated from pregnant woman, then tested with each other by the manual hexadimethrine bromide methods in routine tests and some designed corresponding tests with IgG, IgM anti-D monoclonal diagnostic reagents and some serum protein components.

***Results.* –** Red blood cells that were adjusted to 3–5% suspension by normal saline then only added in 0.7 ml low ionic medium (LIM) and two drops of polybrene solution adhere to the surface of test tubes' bottom when centrifuged, so it was difficult to perform the next approach, but the adherence disappeared when red blood cells' concentrations exceeded 20–30%. Rh positive red blood cells coated by anti-D have the same phenomenon. This adherence can be prevented by serum medium diluted from 1:128 to 1:1024 times by normal saline and hemoglobin medium diluted from 1:32 to 1:128 times, but not by albumin or immunoglobulin medium. The denary logarithm values of the greatest inhibited dilutions of serum and hemoglobin elution between antibody sensitizing red blood cells and the same pre-sensitizing red blood cells tests were no significant difference (P value > 0.05).

***Conclusions.* –** The whole serum or serum protein mediums are important factors that can influence successfully performance of the manual hexadimethrine bromide test. So appliance of the manual hexadimethrine bromide test to immunohematology laboratory, such as when performing titrations of serum or plasma, or when testing eluates for antibody activity, this adherence must be considered.

Résumé

Le test au polybrene est un test très répandu en Chine, tant pour les recherches d'agglutinines irrégulières anti-érythrocytes que pour le test de compatibilité. Cet article rapporte le rôle de la concentration en protéines afin d'augmenter les performances de ce test manuel. L'étude expérimentale a été réalisée en utilisant des anticorps anti-Rh(D) polyclonaux et monoclonaux de classe IgG et IgM. Les auteurs étudient les capacités d'adhérence des globules rouges en fonctions des solutions et en particulier en fonction des concentrations en protéines. Ainsi le test au polybrene, utilisant des solutions ajustées en protéines, se révèle un test effecace et peu coûteux pour la pratique l'immmunohématologie.

Keywords: Serum; Protein mediums; Hexadimethrine bromide (polybrene); Cross-matching

Mots clés : Test au polybrene ; Test de compatibilité ; Milieux protéiques

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1. Introduction

In the modified manual hexadimethrine bromide (polybrene) test, red blood cells are incubated with the test sera in a low ionic medium (LIM) at room temperature. Polybrene, a quaternary ammonium polymer, is then introduced to cause nonspecific red blood cell aggregation. The test tubes are centrifuged, the cell free supernatant fluid decanted, and the polybrene effect on the cells is neutralized by adding a dilute sodium citrate–glucose solution [1]. But during application of the manual polybrene method in routine cross-matching of pretransfusion testing procedures, if serum was not existent in the reaction system, 3–5% red blood cells suspended in the LIM adhere to the surface of test tubes' bottom when centrifuged in the first step of nonspecific red blood cell aggregation [2], and red blood cells coated by antibody have the same adherence phenomenon, so it is difficult to perform the next operation. The purpose of this study is to confirm the existence of this phenomenon and evaluate the effects of serum and some serum protein components act as mediums on preventing adherence phenomenon.

2. Materials and methods

2.1. The reagent kits of the manual hexadimethrine bromide test

Baso-polymatching reagent kits is the second generation production (Baso Diagnostic Inc., Zhuhai, China), this kits consists: 1. LIM: the major ingredients are the sodium salt of ethylene diamine tetraacetic acid (EDTA-2Na) and glucose; 2. Polybrene solution: the major ingredients are polybrene and the sodium chloride; 3. Resuspending solution: the major ingredients is sodium citrate–glucose solution. The routine cross-matching approach of this kits: one drop of red blood cells adjusted to 3–5% suspension by normal saline were mixed with two drops of the test sera (major side), then added in 0.7 ml LIM and two drops of polybrene solution. The test tubes are centrifuged, the cell free supernatant fluid decanted. The last step is to add two drops of resuspending solution in the test tubes. The hemagglutination results are evaluated by the naked eye and microscopy.

2.2. The designed corresponding tests

2.2.1. To confirm the existence of this adherence phenomenon

Blood group O, Rh positive red blood test cells were washed at least three times and adjusted to 3–5% suspension by normal saline. Add one drop of these red blood cell suspensions and two drops of polybrene solution and 0.7 ml LIM to each labeled tubes (vitreous or plastic). In control tests, two drops of polybrene solution and 0.7 ml LIM were separated added to control tubes. Then centrifuge these labeled tubes, read and record results. At the same time, these washed, packed red blood cells were incubated with an equal volume of the known IgG anti-D serum separated from pregnant woman for 1 h at 37 °C, then washed at least three times and adjusted to 3–5% suspension by normal saline, at last, the detecting results by direct antiglobulin test were positive. Use these red blood cells coated by anti-D to repeat the above tests.

2.2.2. The effects of concentrations of red blood cells without existence of serum or serum proteins mediums

Dilute washed, packed red blood cells and red blood cells coated by anti-D to 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% and 100% concentration by normal saline, respectively, then add one drop of these red blood cell suspension and two drops of polybrene solution and 0.7 ml LIM to every labeled tubes. Centrifuge these tubes, observe the inhibition adherence phenomenon and record the results.

2.2.3. To evaluate the effects of serum and some serum protein components

Add two drops of blood group AB, Rh positive serum between 3% and 5% anti-D sensitizing red blood cells and the same pre-sensitizing red blood cells suspension test tubes, then repeat the whole manual hexadimethrine bromide (polybrene) test approach, read and record results. Consecutive double dilute blood group AB, Rh positive serum by normal saline, then add 3–5% anti-D sensitizing red blood cells and the same pre-sensitizing red blood cells, respectively, observe the inhibition adherence phenomenon and record the greatest dilution of test sera that test tubes produced the adherence phenomenon and compare the differentia of inhibition dilutions. Use IgG (Shanghai Blood Center, Shanghai, China), IgM anti-D monoclonal diagnostic reagents (Diagnostic Scotland, Edinburgh, UK) and 20% serum albumin solution (Shanghai Blood Center) to substitute group AB, Rh positive serum to repeat the above tests. At the same time, some washed, packed red blood cells were frozen for half an hour at –30 °C and thawing subsequently at 37 °C, collect these hemoglobin elution then add one drop of 3–5% anti-D sensitizing red blood cells and the same pre-sensitizing red blood cells suspensions to two drops of these elutions, repeat the whole manual hexadimethrine bromide test approach, read and record results.

2.3. Statistical analysis

Statistical analyses were performed with paired-samples *t*-test to compare the denary logarithm values of the greatest inhibited dilutions of serum or serum protein components between antibody sensitizing red blood cells and the same pre-sensitizing red blood cells tests. All reported *P* values are two-sided.

3. Subjects studied

Blood group O red blood cells and Blood group AB, Rh positive serum were collected at random from healthy blood