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Original Article

Formulation of solution to prevent lysis time erythrocyte and detecting blood type

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A B S T R A C T

Background: In the examination of blood type using the tube method, a test cell containing 5% erythrocytes of the solution volume is required. The blood type test using the tube method is carried out in hospital blood banks and in the educational world during practical activities. The obstacle in making a 5% erythrocyte solution is that erythrocytes are lysed so that it is made every day. In addition, making a 5% erythrocyte solution takes a long time so it is not efficient.

Purpose: This study aims to find a solution that can maintain erythrocytes for a long time and detecting blood type.

Method: This experimental study involved the preparation of 120 tubes containing 5% erythrocyte suspensions from blood types A, B, AB, and O. The samples were diluted in 10 different test solutions, including CPD, ACD, EDTA 5%, Heparin 3%, NaCl 0.9% + glucose (0.025%, 0.05%, 0.1%, 0.2%, and 0.3%), and NaCl 0.9% Daily lysis observations were performed using vortex mixing, centrifugation, and color assessment. The solution with the most extended stability was further tested for ABO blood grouping using the tube method to analyze agglutination patterns.

Results: The test solution that can maintain erythrocytes for a long time is the citrate phosphate dextrose (CPD) solution. This solution can prevent erythrocytes for a long time and does not damage erythrocyte antigens. It can also detect blood types well, making blood type examination time more efficient.

Conclusion: CPD solution successfully prevents erythrocyte lysis for a longer time compared to other solutions and functions in detecting blood type antibodies.

INTRODUCTION

Blood type determination is a critical component of pre-transfusion screening to ensure donor-recipient compatibility, with the tube method widely preferred for its high sensitivity compared to the slide method. However, this method requires 5% erythrocyte suspensions of blood types A, B, AB, and O to detect serum antibodies. This process involves multiple washing steps with 0.9% NaCl solution and repeated centrifugation to assess cell integrity. If hemolysis occurs, the entire process must be restarted, significantly increasing the time required for testing and underscoring the need for a more efficient approach.[1,2]

In blood banks, erythrocyte cells are preserved using solutions such as Citrate Phosphate Dextrose (CPD), Citrate Phosphate Dextrose Adenine (CPDA), and Additive Solution-3 (AS-3).[3] These solutions maintain cell viability and prevent hemolysis by stabilizing cell membrane tonicity, with phosphate playing a key role in retaining intracellular phosphate. Typically, these preservative solutions constitute approximately 15% of the total blood volume in storage bags. [5] Blood stored in EDTA tubes can last up to four days; however, morphological changes and increased hemolysis are observed over time, limiting its application in prolonged testing scenarios.[6] Previous studies have explored using CPD, CPDA, and EDTA solutions as erythrocyte preservatives.

However, these studies often did not systematically evaluate the duration for which erythrocytes remain intact or the effect of varying blood-to-preservative ratios. In light of this, the present study aims to address these gaps by formulating and testing an improved erythrocyte preservative solution. This solution is designed to maintain erythrocyte integrity under conditions optimized for osmolality, pH, and temperature that match the permeability properties of the erythrocyte membrane. Daily observations will be conducted to monitor signs of hemolysis.

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The ultimate goal of this study is to develop a solution that not only prolongs erythrocyte stability but also streamlines the preparation of test cells for ABO blood type determination using the tube method. By reducing the time and effort required for this process, the proposed solution could enhance the efficiency of blood type testing in both clinical and educational settings.

METHOD

Study Design

This study is an experimental study in the laboratory to find a solution that maintains erythrocytes for a long time and detects blood type.[7]

Setting

The study was conducted in May 2023-September 2024, at the hematology laboratory of STIKes Mitra Keluarga.

Formulation Solution

The test solutions were formulated with specific compositions as follows. The Citrate Phosphate Dextrose (CPD) solution was prepared by dissolving 2.63 g of sodium citrate dihydrate, 0.327 g of citric acid monohydrate, 2.55 g of dextrose monohydrate, and 0.251 g of sodium dihydrogen phosphate in water for injection to a total volume of 100 ml, followed by homogenization until completely dissolved. The Acid Citrate Dextrose (ACD) solution consisted of 2.2 g of sodium citrate dihydrate, 0.8 g of citric acid monohydrate, and 2.45 g of dextrose monohydrate, dissolved in water for injection to a final volume of 100 ml.

The 5% EDTA solution was made by dissolving 5 g of disodium EDTA salt in water for injection to reach a volume of 100 ml. The 3% heparin solution was formulated by mixing 300 µL of heparin (5,000 IU/ml) with water to inject 99.7 ml. The NaCl 0.9% + glucose solution was prepared by combining 0.9% NaCl with glucose at varying concentrations (0.025%, 0.05%, 0.1%, 0.2%, and 0.3%) in distilled water to a total volume of 250 ml. All solutions were prepared and homogenized for use in erythrocyte stability testing.

Experimental Procedure

Blood samples were obtained via venipuncture from the median cubital vein. 120 tubes containing 5% erythrocyte solutions were prepared by diluting blood samples from each blood type (A, B, AB, and O) in 10 different types of solutions. These solutions included CPD, ACD, EDTA 5%, Heparin 3%, NaCl 0.9% + glucose 0.025%, NaCl 0.9% + glucose 0.05%, NaCl 0.9% + glucose 0.1%, NaCl 0.9% + glucose 0.2%, NaCl 0.9% + glucose 0.3%, and NaCl 0.9%. Each solution had an average pH approaching 7.4. The total volume of each test solution was 4,750 µL, including 250 µL of erythrocytes.

After preparation, the solutions were centrifuged at 3,000 rpm for 10 minutes. If lysis occurred during this process, the preparation was repeated from the beginning. The 5% erythrocyte solutions were then stored at three designated temperatures: 0°C, 4°C, and 25°C. Daily observations were conducted to assess lysis by homogenizing each solution using a vortex, centrifuging it at 1,000 rpm for 5 minutes, and inspecting the color of the solution. A clear solution indicated no lysis, while a red solution indicated lysis. Lysed solutions were excluded from further analysis and recorded in a logbook.

The solution that demonstrated the most extended stability without lysis was subsequently tested using the ABO blood group tube method for serum or plasma examination. This procedure involved labeling four clean tubes as A, B, AB, and O, adding two drops of sample serum to each tube, and then adding one drop of the respective 5% erythrocyte solution (group A, B, AB, or O). The tubes were homogenized, sealed with parafilm, and centrifuged at 1,000 rpm for 3 minutes. Results were considered optimal if erythrocyte sedimentation was observed at the bottom of the tube without any signs of lysis or agglutination.[8]

Variables, Instruments, and Measurement

The variables measured in the study were erythrocyte lysis time, indicated by a change in the color of the solution, and the duration of stability of the erythrocyte suspension. Erythrocyte lysis was observed daily by centrifugation and recorded based on the color of the solution, while A, B, AB, O blood group testing assessed sedimentation without lysis or agglutination. All observations were documented in a notebook.

Data Analysis

This study's data analysis was conducted to determine the stability of erythrocyte suspensions under different conditions and to evaluate the effectiveness of various test solutions in preventing lysis. The analysis included descriptive statistics. Additionally, the blood type testing results were analyzed qualitatively by observing erythrocyte sedimentation and the absence of lysis or agglutination. Data were processed and visualized using statistical software, and all findings were presented in tables and graphs to facilitate interpretation.

Ethical Consideration

This research has passed the research ethics committee No.268/EC/KEPK/STIKES-PI/V/2023 and KEPK/UMP/111/V/2024

RESULTS

Characteristics of Erythrocyte Solutions

The characteristics of 5% erythrocyte suspensions in each test solution are presented in Table 1. The solutions varied in pH, temperature, and osmolality. CPD exhibited a pH of 7.11 with an osmolality of 617.52 mOsm/kg at 4°C, while ACD showed similar pH stability at 7.0 with an osmolality of 557.92 mOsm/kg at the same temperature. NaCl 0.9% combined with glucose at varying concentrations

demonstrated pH values near the physiological level (approximately 7.4) and higher osmolality, ranging from 1,237.52 mOsm/kg to 1,298.64 mOsm/kg. In contrast, EDTA 5% showed the lowest osmolality at 68.44 mOsm/kg and a pH of 8 at 25°C.

Table 1. The Characteristic of erythrocyte cells is 5% in each test solution.

Solution	pH	Temperature (^{0}C)	Osmolality (mOsm/kg)
CPD	7.11	4	617.52
ACD	7	4	557.92
EDTA 5%	8	25	68.44
Heparin 3%	7	25	330
NaCl 0.9% + Glucose 0.025%	7.4	4	1.237
NaCl 0.9% + Glucose 0.05%	7.4	4	1.243
NaCl 0.9% + Glucose 0.1%	7.24	4	1.254
NaCl 0.9% + Glucose 0.2%	7.43	4	1.276
NaCl 0.9% + Glucose 0.3%	7.06	4	1.298
NaCl 0.9%	7.23	4	287

Duration of Erythrocyte Stability

The longest erythrocyte stability, as indicated by the absence of lysis, was observed in NaCl 0.9% + glucose solutions stored at 4°C, particularly at glucose concentrations of 0.2% and 0.3% (Figure 1). These solutions maintained stability significantly longer than others. CPD and ACD also showed reasonable stability but were less effective compared to NaCl 0.9% + glucose solutions. EDTA 5% and Heparin 3% demonstrated shorter stability durations, highlighting their limitations in maintaining erythrocyte integrity.

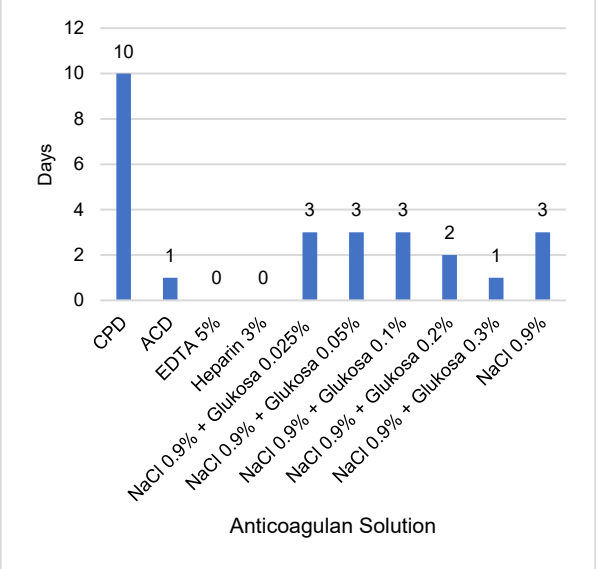

Figure 1. The longest lysis sign of erythrocyte cells is 5% in each test solution.

ABO Blood Type Testing

The ABO blood type testing using the tube method with CPD-preserved erythrocytes yielded reliable results (Figure 2). In serum from blood group O, agglutination was observed in A, B, and AB erythrocyte cells, consistent with the presence of antibodies A and B. Conversely, serum from blood group AB showed no agglutination across all erythrocyte groups due to the absence of antibodies in the serum. These findings confirm the effectiveness of CPD-preserved erythrocytes in ABO compatibility testing.

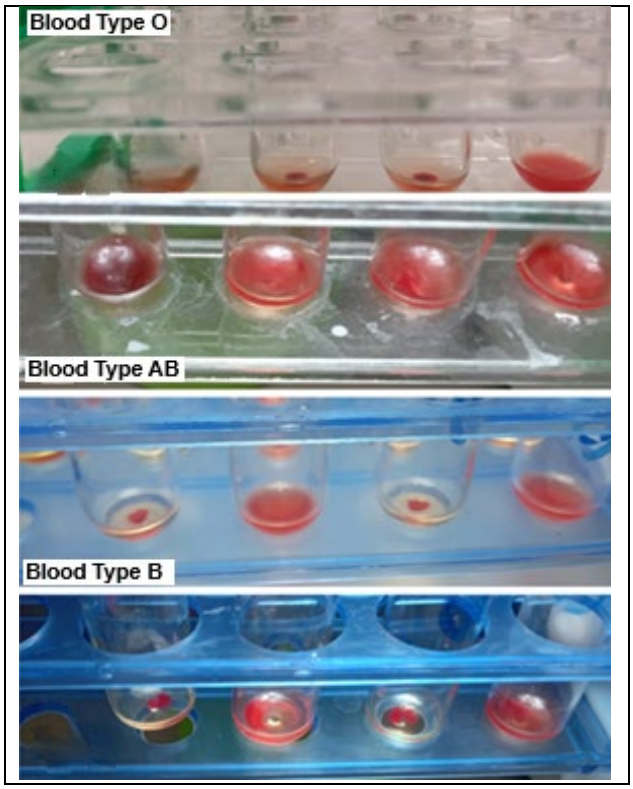

Figure 2. Results of ABO blood type serum test using tube method using 5%

DISCUSSION

The results showed that CPD solution was more effective in preventing lysis compared to solution. This is because each component in the CPD solution has a function, such as citrate functions as a calcium-binding agent that prevents blood clotting, fosfat serves to stabilize pH and maintain proper 2,3-DPG levels, and dextrose extends the shelf life of blood.[9] Adenine in CPDA solution functions to maintain the levels of adenosine triphosphate (ATP) in red blood cells, which is known as an energy source for membrane ion transport.[10] However, the research results showed that the CPDA solution has a shorter incubation period than the CPD solution in preserving erythrocytes. This is because the addition of adenine can change the pressure of the cell membrane.[11] The novelty of this study is the formulation of the composition of the CPD solution used and the comparison between the volume of the solution and erythrocytes. The disadvantage of this study is the absence of a pH solution during the storage period.

Normal blood pH levels in the body (in vivo) are in the range of 7.35–7.45. pH function in cells is not only for body metabolism, where enzymes will work optimally at the appropriate pH.[12] The pH of the solution also interacts with the cell membrane through phospholipid and cholesterol groups. Changes in lipid structure in the cell membrane

affect membrane changes.[13] A pH biosensor membrane is a specialized membrane that changes its properties in response to pH level changes.[14] Phospholipids and membrane proteins can act as biosensors for changes in pH in the environment. Changes in pH can cause lipid vesicles to migrate and change shape and can polarize phase-separated membranes.[15]

Another candidate as a local pH biosensor is the signaling phospholipid phosphoinositide (PIP). Signaling proteins bind PIP generally through a structurally conserved binding domain.[13] Phosphoinositides are lipids that function as signaling derived from phosphatidylinositol, a phospholipid found in the cytoplasmic layer of eukaryotic membranes.[16] Its function will be inhibited if the extracellular fluid contains a lot of Ca+2. Ca+2 ions are intracellular fluids that, if these ions come out, cause changes in pH in the environment.[17] Cells will be better able to survive in an acidic environment, although an acidic environment can inhibit gene electrotransfer. However, cells are able to tolerate a slightly acidic extracellular pH that allows for more efficient damage repair.[18]

The osmolality value of the hypertonic ACD solution and the CPD solution. In the hyperosmotic solutions, water diffuses out of the cell, causing it to shrink and shrivel.[19] Erythrocyte blood cells can maintain cell membranes in hypertonic solution conditions longer than in hypotonic solution conditions. However, there are changes in the morphology of erythrocytes. Changes in the morphology of erythrocytes can affect changes in the structure located on the erythrocyte membrane.[20] The predicted morphology and deformability indicate decreasing quality and viability of stored RBCs undergoing storage lesions. The loss of membrane structural integrity due to the storage lesion further degrades the cell deformability and recoverability during mechanical deformations.[21]

The results of serum tests on ABO blood type examination using the tube method using 5% erythrocyte solution showed results that were in accordance with cell tests using anti-A, anti-B, anti-AB reagents. However, changes in the shape of erythrocytes do not change the structure of the ABO antigen on the membrane.[22] The human blood group ABO systems are based on the oligosaccharide antigens A or B, located on the surface of blood cells.[23] The location of the ABO antigens on the cell surface is thought to be unaffected by the osmolality of the solution. Only the semipermeable cell membrane transports Na^+ K^+ ions to maintain cell tonicity, resulting in changes in cell size. This is an implication of the research results.

During practice, the preparation for making the solution was only once on the first day and for subsequent practice activities using the same 5% erythrocyte CPD solution. There was no need to make it again because the erythrocytes were not lysed, which really made the activity time more efficient. Erythrocyte cells in the CPD solution can detect serum according to the results of the cell test. This indicates that the CPD solution can be implied, successfully preserving and not damaging the ABO antigen on the erythrocyte membrane.

CONCLUSIONS AND RECOMMENDATION

CPD solution is one form of innovation for ABO blood type testing that makes it easier for health workers in blood banks and researchers in the field of immunohematology who use erythrocyte cells in their activities. This CPD solution can prevent erythrocytes, so it is not necessary to make it every day if you want to do an ABO blood type test. For researchers who want to use this solution, it is important to note that you should avoid adding too much NaOH and HCl to set the pH because it can affect the osmolality of the solution.

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