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An Automated Method for Blood Type Determination by Red Blood Cell Agglutination Assay

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A new automated method for assaying specific agglutination of red blood cells upon addition of monoclonal antibodies to whole blood test samples is presented. The method can be used for blood type determination. The method is based on lying drop photometry of blood samples with added Tsoliclone monoclonal antibodies. Mechanical vibration of drop samples promotes better cell agglutination and redistribution through the volume of the sample. Experimental studies revealed significant differences in photometric values for samples with and without agglutination. This method may provide the basis for developing a portable automatic blood-typing device.

Introduction

Blood types are characterized by a set of antigens located on red blood cell surfaces and determining their specificity [1]. There are an enormous number of these antigens. Of these, the Rhesus and ABO antigens are especially important for blood transfusion purposes [2, 3]. ABO and Rhesus typing must be carried out before any surgical procedure, organ transplantation, or transfusion of donor blood to a recipient [4-6]. Transfusion of incompatible blood leads to red blood cell agglutination. The result is that cells lose their functionality, which can lead to lethal outcomes. Incorrect assaying must therefore be excluded.

The main blood-typing method widely used in clinical practice is based on direct hemagglutination reactions with monoclonal antibodies [7]. In Russian laboratory practice, blood typing makes wide use of Tsoliclone solutions of monoclonal antibodies from the Russian supplier Gematolog. Tsoliclones are salt solutions of monoclonal antibodies for detection of human red blood cell antigens. Each reagent is strictly specific for the corresponding antigen. Red blood cell agglutination using monoclonal antibodies has long been the recommended method for identifying the blood type. However, the fact that the test results (i.e., the presence or absence of agglutination in samples) are usually assessed visually by medical staff introduces the possibility of errors. For example, in the case of weak agglutination, cell aggregation may be virtually undetectable visually and the agglutination patterns may appear only after a prolonged time period. Such samples can be taken as lacking agglutination. Work reported in [8] showed that the error rate in primary determination of blood types in medical institutions is of the order of 0.41-1.16%. Thus, the subjective factor can lead to incorrect assay results and fatal outcomes of surgical procedures. The drawbacks of this method also include large consumption of reagents and long test durations.

Currently used automated blood-typing devices are large stationary devices intended for use in large medical centers. These devices use special gel cards. The gel card method includes preparation of the blood sample (preparation of packed blood cell) and is rather expensive [9]. There is a need for blood-typing devices using smaller volumes of blood and reagents, requiring minimal sample preparation, and providing correct results in short time periods [10].

Materials and Methods

Contemporary methods for blood typing by detecting red blood cell agglutination use different approaches.

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Detection of agglutination by digital photography is used in automated microplate systems [11]. Blood typing using a microfluidic chip has been a subject of research [12, 13]. Photonic crystal fibers have been used as sensory elements for detecting agglutination [14]. An acoustic-optical method for detecting agglutination by digital image analysis has been described [15, 16]. The majority of these methods for identifying agglutination require whole-blood sample preparation or visualization of study results using video systems, which complicates the test process or requires additional computational resources for video signal processing.

We present here a method for monitoring the presence or absence of agglutination in blood samples by photometry of drop samples [17-19]. Photometry is used to record changes in the intensity of a light beam passing through the blood sample during the process of red blood cell aggregation. Aggregation occurs in the case of specific red blood cell hemagglutination on addition of the corresponding monoclonal antibody. Vibration is used to enhance agglutination in the drop sample and separation of agglutinates from the transparent part of the reaction mixture. For this purpose, the cuvette containing blood samples is placed on a vibration platform moving reciprocately in the horizontal plane. Experimental studies used vibration frequencies of 15-30 Hz and platform vibration amplitudes of 0.5-1 mm. This prototype blood-typing device was used to run a series of experiments in which significant differences in optical properties were found between samples with positive and negative agglutination. Experiments were carried out using human whole blood from volunteer donors and monoclonal antibodies from Gematolog (Russia). About 100 blood samples were investigated.

Blood samples (lying drops) with added reagent were tested in a special transparent cuvette. The cuvette containing samples was placed in the measuring chamber of the device. Each drop sample contained whole blood and Tsoliclone reagent at a ratio of 1:3 (selected experimentally). The hematocrit of the blood samples used in the study ranged from 20% to 55%. Sample volume was 16-20 μL (4-5 μL blood and 12-15 μL reagent). The light beam from the source located beneath the drop passed through the sample to reach the light detector positioned at the optical focus of the drop (Fig. 1).

The light source for the studies was a near-IR LED. During the time period specified by the software, the change in the intensity of the light beam passing through the drop of blood was measured. The light detector signal was amplified and transmitted to an ADC (USB 6009, National Instruments) and then to computer. Data were automatically plotted on the computer display (using a program written in LabVIEW) as voltage vs. time graphs reflecting changes in the intensity of the light passing through the sample. The intensity of the light arriving at the detector, as noted above, was determined by the optical properties of the transilluminated sample and changed over time depending on the processes taking place in the drop sample, i.e., the presence or absence of red blood cell agglutination in the sample. The presence or absence of agglutination in samples was also checked visually after the test. A blood sample diluted with physiological saline at the same ratio as blood in the experimental samples was used as the control sample with negative agglutination.

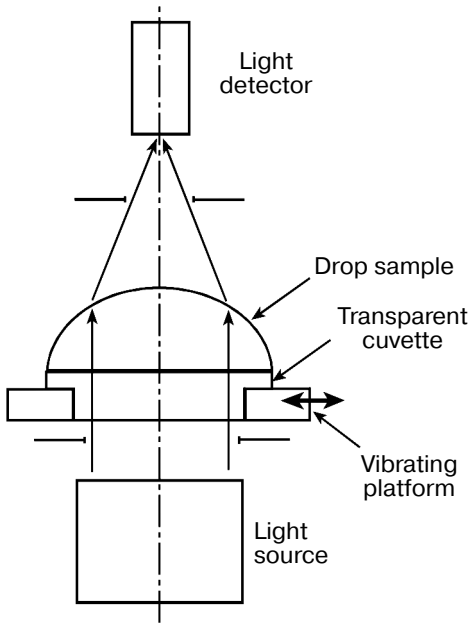

Fig. 1. Drop photometry procedure.

Experimental Results and Discussion

The studies showed that when agglutination did not occur in the sample, the intensity of light passing through the sample (Fig. 2a, curve 1) was close to that of the light passing through the control sample (Fig. 2a, curve 2). When red blood cell agglutination occurred, the optical density of the sample dropped significantly (Fig. 2a, curve 3). In this case, the voltage at the light detector output was at least 2-3 times greater than the voltage in the control channel. Such behavior of the photometric curves was typical of all samples with agglutination. The only difference was in the signal increase rate (Fig. 2b). This

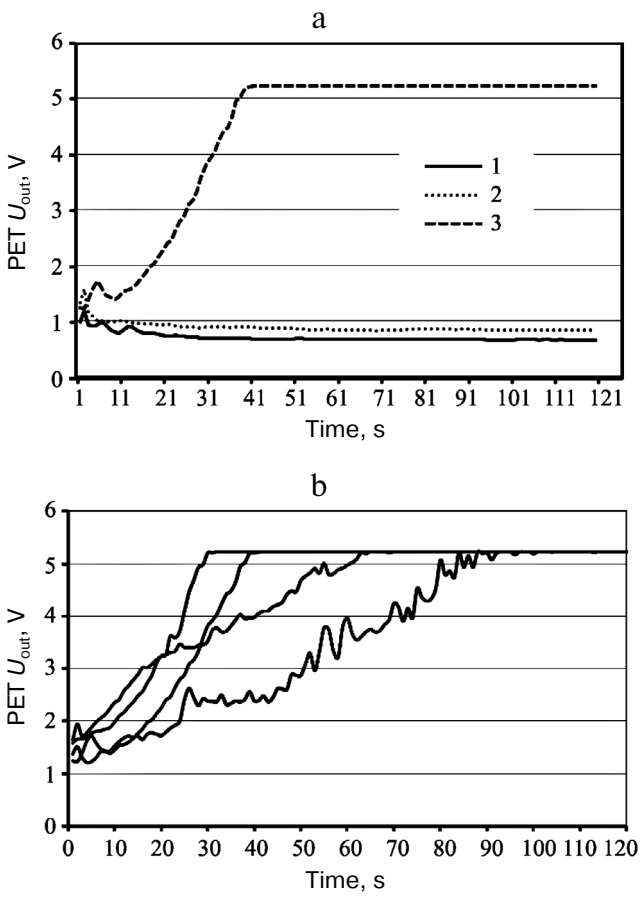

Fig. 2. Output signal of the photoelectric transducer (PET) of the device (reflecting changes in light transmission by the drop sample). The maximum signal level at the ADC (and, as a consequence, the signal level on the plot) is limited to 5 V. a) No agglutination (curve 1), control sample (curve 2), with agglutination (curve 3); b) comparison of curves for several samples with red blood cell agglutination.

can be explained by differences in the rate of red blood cell agglutination in the samples.

Samples containing agglutinates (positive tests) showed decreases in scattering properties. The medium became more transparent and the quantity of light transmitted increased. However, this factor is not definitive in this method. Use of vibration and cuvettes of special shape increased the transmittance of test samples in their central part as a result of agglomeration of small agglutinates into larger agglomerates and redistribution of these around the periphery due to vibration-induced currents in the sample (Fig. 3).

As clearing of the central part of the drop due to sample agglutination proceeded, the drop (acting as a lens) started to focus light passing through the sample on the detector positioned at the focal point of the drop. This led to a significant increase in the light flux falling on the detector.

In photometric measurements, the transmittance of the medium also increased in the flat horizontal cuvette in which red blood cell agglutination took place. However, there were significant fluctuations in light flux due to a nonuniform distribution and displacement of aggregates in the optical measurement area (Fig. 4). This hindered analysis of the curves and their usefulness for detecting agglutination in the sample.

Focusing of light by the drop had the result that an integrated light flux reached the detector. Local fluctuations in the light flux in different parts of the drop had little effect on the overall signal level recorded by the detector.

Agglutination increased the signal recorded using the detector by a factor of 2-3 compared with the initial signal level at the moment of sample application or relative to the signal in the control channel. When aggluti-

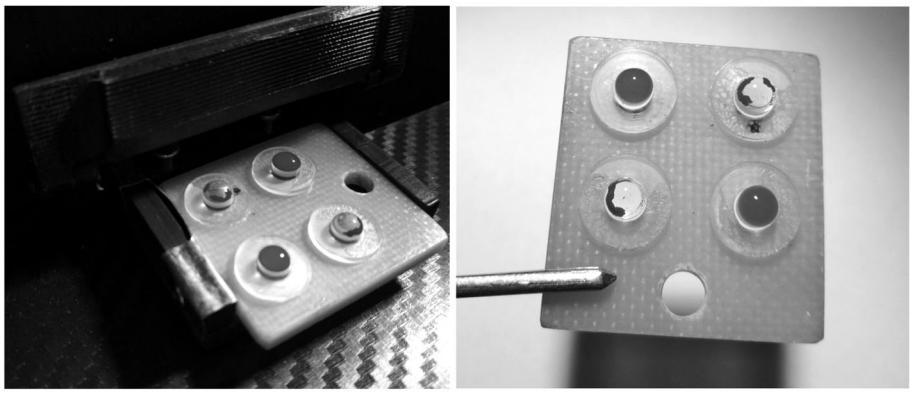

Fig. 3. Photographs of the cuvette with drop samples after applying vibration for 1 min. When agglutination occurs, the agglutinates are distributed around the periphery of the base of the drop.

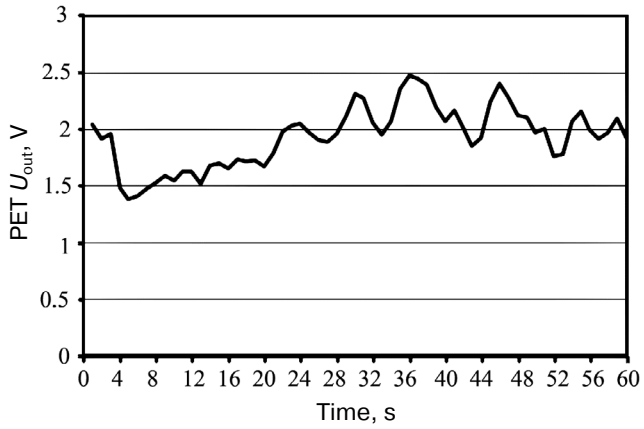

Fig. 4. Output signal of the photoelectric transducer of the device during specific agglutination of red blood cells in the plane-parallel horizontal cuvette transilluminated vertically.

nates did not form in the sample (negative reactions), vibration of the drop simply stirred the sample. The medium became turbid after stirring of whole blood with added reagent, and the signal from the output of the light detector decreased. For samples with negative agglutination (Fig. 2a, curve 1), the transmittance after stirring was close to that of the control sample (Fig. 2a, curve 2).

Thus, analysis of the experimental photometric curves indicates that a two-fold increase in the signal from the sample channel relative to the control channel can be taken as a criterion for the presence of agglutination in the sample.

Conclusions

This study of blood typing by drop photometry is a pilot study and will be continued. The experimental results demonstrated that the method of drop photometry with sample vibration used here allows the presence or absence of agglutination in whole blood samples with added monoclonal antibodies to be identified. This instrumental method for detecting agglutination may provide the basis for developing an automatic blood-typing device.

Significant distinctive features of the method proposed here are the use of small volumes of biological material and monoclonal antibodies, the opportunity for automatic detection of agglutination, the lack of a sample preparation stage, the absence of subjectivity, and a short analysis time. If agglutination is strong, the agglutination assay takes no more than 30 s, while weak agglutination can be detected in 2-3 min. The method requires a small volume of whole blood (about 20 μL) for analysis, which is extremely important for blood typing in children and people with anemia or large traumatic blood loss.

Further refinement of the method will make it possible to achieve higher sensitivity and specificity and to detect weak agglutination in study samples with high levels of confidence. For this purpose, experimental studies should be carried out to determine the optimal parameters for the optical measuring system and develop the appropriate technique and algorithm for processing the assay results. These tasks will be addressed in our future work.

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