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A study for reducing effect of hematocrit on measurement of an analyte in whole blood

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Abstract

In this paper the presence an analyte in a sample of whole blood comprises the step of treating the sample with a nonlytic hypertonic salt composition to reduce the hematocrit by reducing the size of the red blood cells. In optical detection systems, the smaller red blood cells create greater scatter, which allows a more accurate correction to be applied in a dual-wavelength detection system.

Keywords: Habitat loss, habitat fragmentation, conservation, population decline

Introduction

In the analyte in a sample of blood with improved accuracy and precision by reducing the effect of hematocrit on the measurement. The invention further relates to a test article useful in performing the method, and test kits comprising the test article. It has become increasingly important to medical Science to be able to quantify the chemical and biochemical components in whole blood. Such ability is important in testing for exposure to hazardous materials, intoxicants, and therapeutic drugs, and in diagnostics. In some applications, it is important for a lay person to be able to perform the test outside a laboratory environment, with rapid and accurate results. For example, diabetics must test samples of their own blood for glucose several times a day to moderate their diet and medication. The test results must be both rapidly available and accurate. Assays for glucose in either plasma or whole blood can employ either oxidative or reductive chemistries that employ either colorimetric or electrochemical detection systems. Other analytes of interest in blood include cholesterol, triglycerides, ethanol, lactic acid, beta hydroxybutyrate, ketone bodies, and fructosamine.

Test kits for the determination of glucose and other analytes in blood are well known in the art. Such test kits usually involve a test article Such as a test strip or microfluidic device impregnated, coated, deposited, or printed with one or more chemicals that react in the presence of glucose to cause a dose-dependent response that may be measured by electrochemical or optical methods, or any combination thereof. Optical measurements can include transmittance, absorbance, and reflectance methods. Electrochemical measurements can include amperometric or coulometric methods.

It is well known that variations in hematocrit between whole blood samples used in diagnostic tests can interfere with accurate measurement of an analyte. Whole blood hematocrit (abbreviated hct) is a measure of the percentage of whole blood volume occupied by the red blood cells. It is also referred to as the packed cell volume, or the proportion of red blood cells to plasma. The interference caused by the hematocrit variation can arise from at least three factors: 1) interference with the detection of an optical signal used in analyte measurement by reflectance, absorbance or scatter of light; 2) interference with the rate of the chemical reaction by obstructing the diffusion of analyte within a whole blood sample; and 3) interference by reducing the amount of fluid available in a sample for adequate rehydration of a dried reagent on a test strip or other test article. Thus some test kits of the prior art require the user to dilute the sample, or require that the red blood cells be filtered out of the sample or lysed prior to applying the sample to a test device, or are designed such that these functions are carried out by the device itself without user intervention.

It is yet another object of the invention to provide a method for analyzing a sample of whole blood for an analyte that reduces interferences caused by variations in hematocrit among the various blood samples analyzed, and a test kit and an article useful in the method.

It is another object of the invention to provide a method for analyzing a sample of whole blood

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for an analyte that does not require blood to be lysed to reduce interference by variations in hematocrit, and a test kit and an article useful in the method.

It is another object of the invention to provide a method for analyzing a sample of whole blood for an analyte that does not require blood to be diluted to reduce interference by variations in hematocrit, and a test kit and an article useful in the method.

The use of nonlytic hypertonic salt solution reduces the mean cell Volume of the sample by reducing the particle size of the red blood cells. This can be seen in FIG. 1

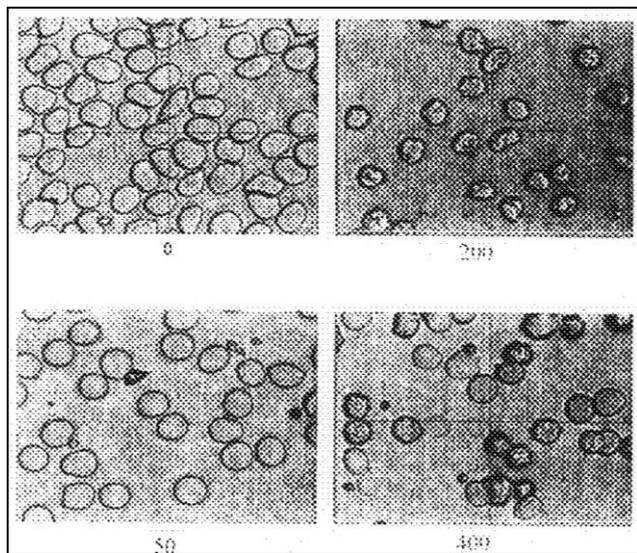


Fig 1: Effect of buffer salt compositions on red blood cell morphology

Which show red blood cell morphology in samples of whole blood having hematocrit levels of about 40% treated with solutions of NaHEPES at concentration levels of 0 mM, 50 mM, 100 mM, 200 mM, and 400 mM, respectively. Five hundred microliter aliquots of NaHEPES buffer prepared at 50, 100, 200, and 400 mM were added to 1.5 ml microfuge tubes and the liquid was evaporated using a SpeedVac concentrator (Savant Instruments, Inc.) at 30° C. Each aliquot of dried buffer salt was then reconstituted with whole blood samples prepared at various hematocrits. The samples of 20, 40, and 60% hematocrit whole blood were targeted and prepared by mixing packed cells and plasma. A small sample of each aliquot was used to prepare a blood Smear on a glass microscope slide so that the morphology of the red blood cells could be observed under the microscope. It may be seen that at higher buffer concentrations the red blood cells take up a substantially smaller volume of the sample. These smaller red blood cells increase the scatter of light from the sample.

Table 1 below illustrates the effect of a hypertonic buffer salt, NaHEPES, on the hematocrit (packed cell volume). The data represent hematocrit readings taken after mixing whole blood at different hematocrit levels with different concentrations of NaHEPES buffer. The hematocrit level of each sample both before and after adding to the salt was checked using a Compur M-1 100 micro-capillary reader instrument to measure the packed cells. The data in Table 1 and FIG. 2 demonstrate that, as the cell size decreases with increasing buffer salt concentration, the packed cell volume decreases, thus decreasing the hematocrit. The theoretical trend lines shown in FIG. 2

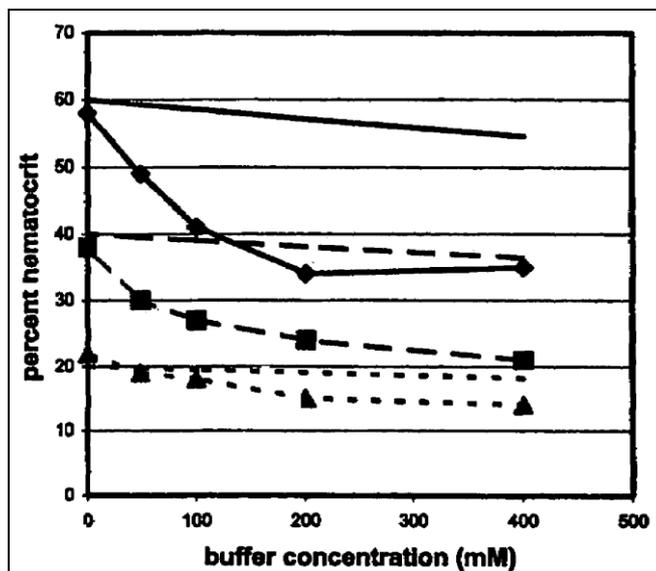


Fig 2: Effect of Buffer Concentration on Percent Hematocrit

Demonstrate what the expected hematocrit would be due to the volume displaced by the salt. It may be seen that the effect on the hematocrit is substantially greater than theoretically expected, and, at higher hct levels, the effect of increased buffer salt concentrations is substantially greater for higher hct levels than for lower hct levels. As shown in figure 3.

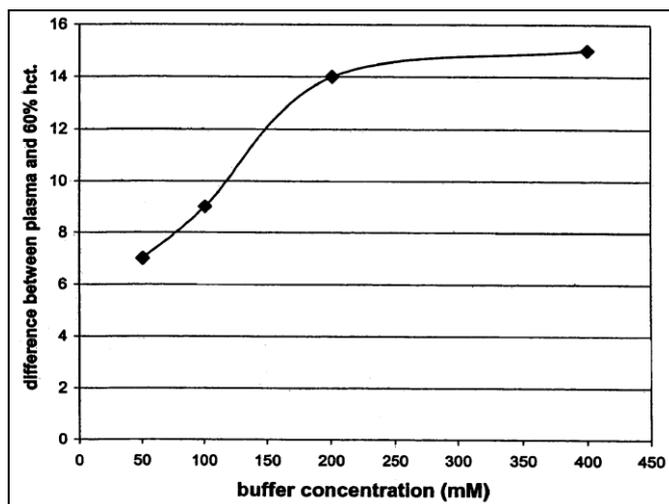


Fig 3: Effect of Salt Concentration on Reflectance at 940 nm

The effect of hypertonic buffer salt concentration on measured reflectance at 940 nm which was chosen as an example wavelength for the hematocrit correction using red blood cell scatter. In the graph, the vertical axis represents the difference between percent reflectance measured for plasma samples and for whole blood samples initially having 60% hct, each sample having the indicated buffer concentration. The data were collected by measuring reflectance of the mixture obtained after adding the blood samples to aliquots of glucose reagent buffered at the specified concentrations with NaHEPES, pH 7.5, using the apparatus described in co-pending patent application Ser. No. 60/373,583 filed Apr. 19, 2002. These data demonstrate the significant effect that increased buffer concentration has on the reflectance of a sample with high hct. The method of the instant invention is also useful in non-optical determinations, such as electrochemical determinations. The smaller red blood cells

pose less of an obstruction to the diffusion of analyte and reagents in a whole blood sample, Such that the chemical reactions necessary for analyte determination can proceed more readily, which will yield a more accurate result.

Conclusion

In this study the invention are met by the method of the instant invention, in which the hematocrit of a blood sample is adjusted before an analyte reading is made by treating the sample with a nonlytic hypertonic salt composition to reduce the mean cell volume of the blood cells in the sample.

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