Clinica Chimica Acta 413 (2012) 192–197

Contents lists available at SciVerse ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/clinchim

Development of an assay of seven biochemical items, HbA1c, and hematocrit using a small amount of blood collected from the fingertip

Sugimoto Shinya [a,b,*], Akimoto Masaru [a], Hayakawa Akira [b], Hokazono Eisaku [b], Osawa Susumu [b]

[a] Physical Screening, Inc., Japan
[b] Clinical Chemistry, Division of Biochemical Science and Tecnology, Department of Health Sciences, Graduate School of Medical Sciences, Kyushu University, Japan

ARTICLE INFO

Article history:
Received 17 July 2011
Received in revised form 15 September 2011
Accepted 15 September 2011
Available online 22 September 2011

Keywords:
Fingertip blood
Choline
Tyramine
Hematocrit
HbA1c

ABSTRACT

Background: Lifestyle-related diseases in Japan account for 30% of the entire medical expenditure of the country and cause 60% of all deaths. For the prevention of lifestyle-related diseases, medical examination by laboratory tests on metabolic syndrome is important.
Methods: To undertake examination by collection of blood from a fingertip, we developed the "Well Kit". About 65 μl of blood collected from a fingertip was diluted with buffer solution, which contained two internal standard materials. The kit also separated corpuscles and diluted plasma with a special filter. It measured the obtained diluted plasma using the JCA-BM2250.
Results: This measurement system was evaluated for the quantitative analysis of 8 items. The uncertainties of tested items of this measurement system were 1.7% to 6.4%. The coefficients of correlation of all tested items between this measurement value and the venous plasma sample value were 0.876–0.991, and hematocrit was 0.958.
Conclusions: This system for testing blood collected from a fingertip is simple to use and can be applied in testing for metabolic syndrome. In addition, this testing system is useful in the medical examination of the personal healthcare and inhabitants.

1. Introduction

It is necessary to maintain health and longevity in nations with decreasing and aging populations such as Japan and other developed countries. To this end, prevention of lifestyle-related diseases is important for improved health and effective control of medical costs. For prevention of lifestyle-related diseases, monitoring by examination of factors related to metabolic syndrome is important.

In Japan in 2008, a specific new medical checkup with a focus on metabolic syndrome was initiated [1,2]. In terms of inspections that can be carried out by subjects themselves, over-the-counter (OTC) appliances are widely used, but they can only be used to analyze certain factors, such as glucose, and the general overall health of the individual cannot be determined [3]. Therefore, we have developed a fingertip blood collection system for use in medical checkups in Japan [4]. This examination system was also reported [5] in Europe.

We produced an improved Well Kit as a test kit using blood collected from a fingertip of the subjects themselves. The testee collects blood according to the manual, isolates diluted plasma, and mails the Well Kit to the laboratory center. Then, inspection of samples that arrive at the laboratory center is undertaken and the obtained results are subsequently reported by mail or e-mail, among others. This measuring system involves the determination of various items in diluted plasma from blood collected from a fingertip and added to a dilution buffer. Therefore, it is necessary for an internal standard to be added to the buffer to obtain the dilution ratio of the plasma. In the past, glycerophosphate was used as an internal standard in buffer solutions for obtaining the dilution ratio (DR). This internal standard undergoes hydrolysis by alkaline phosphatase, which must be added to EDTA, and phosphoric acid, which is an inhibitor of this enzyme [4].

Thus, in this case, we chose choline as the internal standard material for the estimation of plasma dilution, which is almost absent in blood and is stable after addition to blood. A fingertip blood collection system, which uses tyramine as a diluted internal standard material for whole blood dilution, was newly developed. Moreover, the hematocrit value can be calculated by using these internal standards [6]. Furthermore, we developed a new plasma separation appliance for the diluted whole blood and evaluated its general performance as a measurement system of collected blood.

2. Materials and methods

2.1. Outline of the Well Kit

The fingertip bleeding measurement kit Well Kit (Physical Screening, Inc.) is composed of a tube with a dilution buffer solution,

* Corresponding author at: Physical Screening, Inc., Clinical Chemistry, Division of Biological Science and Technology, Department of Health Sciences, Graduate School of Medical Sciences, Kyushu University, 2-24-1 Asakusabashi, Taitou-ku, Tokyo, Japan.
E-mail address: sugimoto@pcl-g.co.jp (S. Shinya).

0009-8981/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.cca.2011.09.021

a lancet, a blood-aspiration sponge, a cylinder with a blood cell separation filter, a cap for shutting it tightly, a swab, and a band-aid. The dilution buffer solution of the blood is composed of 50 mmol/l 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (HEPES), 150 mmol/l sodium chloride, pH 7.4, 4.0 mmol/l choline chloride, and 4.4 mmol/l tyramine.

2.2. Calculation of the concentration of plasma items by dilution sample measurement

The internal standard materials (IS) of choline (IS-C) and tyramine (IS-T) are dissolved in the dilution buffer solution at known concentrations. IS concentration in this buffer solution is diluted by the addition of blood. Using this concentration change, IS-C can be used to calculate the DR of the plasma (DRp) and IS-T can be used to calculate the DR of the whole blood (DRb). The DR of the plasma and the whole blood can be calculated using the following equations. ΔAbs means "Abs — blank".

$$\text{Dilution ratio of the plasma (DRp)} = (\text{IS-C } \Delta\text{Abs.})/[(\text{IS-C } \Delta\text{Abs.}) - (\text{IS-C} + \text{sample } \Delta\text{Abs.})]$$

$$\text{Dilution ratio of the blood (DRb)} = (\text{IS-T } \Delta\text{Abs.})/[(\text{IS-T } \Delta\text{Abs.}) - (\text{IS-T} + \text{sample } \Delta\text{Abs.})]$$

The specimen concentration of original plasma can be calculated by multiplying the dilution ratio by measurements of the dilution plasma.

2.3. Measuring principle of the internal standards of choline and tyramine

The measuring principles of the choline internal standard for the plasma dilution rate and the tyramine internal standard for the whole blood dilution rate are shown in Fig. 1. The color development with oxidation condenses by peroxidase and Trinder's reagent to form hydrogen peroxide and measures the concentration of each internal standard.

2.4. Measuring reagent of choline and tyramine

The measuring reagents for choline and tyramine are manually prepared. The working reagent of choline is composed of a 1st reagent (pH 7.5, 0.1 mol/l phosphate buffer, 1.5 mmol/l N-ethyl-N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline (DAOS), 3.0 kU/l peroxidase (POD; Wako Pure Chemical Industries, Ltd., Osaka, Japan)) and a second reagent (pH 7.5, 0.1 mol/l phosphate buffer, 1.2 mmol/l 4-aminophenazone (4-AP; Wako), 7.5 kU/l choline oxidase (COD; Asahi Kasei Pharma Corporation, Tokyo, Japan)). The working reagent of tyramine is composed of a 1st reagent (pH 7.5, 0.1 mol/l phosphate buffer, 1.5 mmol/l DAOS, 6.0 kU/l POD) and a 2nd reagent (pH 7.5, 0.1 mol/l phosphate buffer, 1.2 mmol/l 4-AP, 1.0 kU/l tyramine oxidase (TOD; Asahi Kasei Pharma Corporation, Tokyo, Japan)). The measuring reagent for other biochemical items uses commercial reagents for the automated analyzer (AST (Sekisui Medical Co., Ltd., Tokyo, Japan), ALT (Sekisui), GGT (Wako), triglyceride (Mizuho Medy Co., Ltd., Tosu, Japan), HDL cholesterol (Wako), LDL cholesterol (Kyowa Medex Co., Ltd., Tokyo, Japan), and glucose (Mizuho).

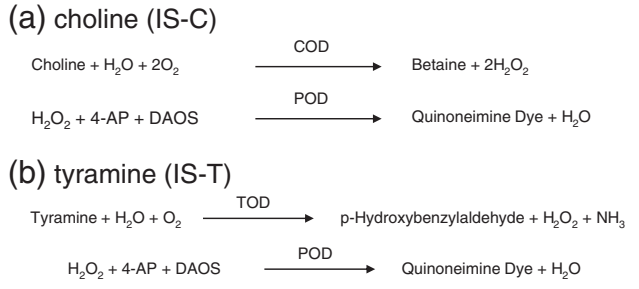

Fig. 1. Reaction equations of two internal standard materials. (a) Choline (IS-C), (b) tyramine (IS-T). COD: choline oxidase, 4-AP: 4-aminophenazone, DAOS: N-ethyl-N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline, POD: peroxidase, TOD: tyramine oxidase.

2.5. Specimens

Fingertip blood and EDTA venous blood of 95 volunteers and patients obtained according to the collection manual were used. The bleeding of fingertip and vein was carried out within 10 min.

2.6. Sampling method and method of processing blood

The bleeding from a fingertip was carried out using Well Kit. About 65 μl of blood was dispersed into the blood-aspiration sponge. The sponge was dropped into 200 μl of dilution buffer solution and they were mixed well. Diluted blood was separated by the cylinder, which has a separate membrane. The diluted plasma was diluted about sixfold using the buffer solution. Biochemical items and internal standard of the diluted plasma, which was provided by using the filter, were measured. On the other hand, the hemolysate of the exclusive reagent (Tosoh Corporation, Tokyo, Japan) was added to the blood cells, which were separated in the Well Kit, and glycohemoglobin was measured in HLC-723G7. In addition, after venous blood was collected in the EDTA blood collection tubes, the plasma was obtained by centrifugation (1200×*g*, 10 min).

2.7. Instrument and measuring conditions

Biochemical automated analyzer JCA-BM2250 (JOEL Ltd., Tokyo, Japan) was used for the measurement of the biochemical items and internal standards. However, for this instrument, measurement is performed after dilution of the sample 5 times with saline. For the measurement of each item of the biochemical test and internal standard using the JCA-BM2250 automated analyzer, measurement conditions were optimized for the dilution plasma sample as follows: AST sample volume 25.0 μl, reagent 1 volume 35.0 μl, reagent 2 volume 8.8 μl, wavelength 340/410 nm; ALT 25.0 μl, 35.0 μl, 8.8 μl, 340/410 nm; GGT 25.0 μl, 35.0 μl, 11.7 μl, 410/545 nm; triglyceride 11.5 μl, 48.0 μl, 24.0 μl, 545/805 nm; HDL cholesterol 8.0 μl, 52.0 μl, 17.3 μl, 596/694 nm; LDL cholesterol 10.0 μl, 50.0 μl, 16.6 μl, 596/694 nm; glucose 6.5 μl, 54.0 μl, 18.0 μl, 340/410 nm; choline 4.0 μl, 60.0 μl, 20.0 μl, 596/694 nm; tyramine 7.5 μl, 60.0 μl, 30.0 μl, 596/805 nm. HbA1c was measured using glycohemoglobin analyzer HLC-723 G7 (Tosoh) with a special reagent and the prescribed measurement specifications. The hematocrit of the original whole blood was measured using a hematology analyzer XE-2100 (Sysmex Corporation, Kobe, Japan) with the special reagent and the prescribed measurement specifications.

2.8. Calibrator

Calibration was carried out using the dilution buffer solution and certified reference materials that had been diluted 5-fold with the buffer solution. The certified reference materials used were JCCLS CRM-001b (AST, ALT, and GGT), JCCRM 223-25 VI (TG), JCCRM 223-25 V (HDL-c), JCCRM 223-25 VII (LDL-c), and JCCRM 521-9HH (glucose) from Reference Material Institute for Clinical Chemistry Standards (ReCCS, Kawasaki, Japan).