In this study, we investigated the correlations between biochemical and hematological test results obtained using microliter-scale fingertip blood samples collected with a newly developed blood collection device and those obtained using conventional venous blood. Eighty volunteer subjects were enrolled in this study. Blood samples were drawn from the fingertip of the ring finger by a single puncture, and 60-μL samples were promptly and accurately aspirated into a blood collection chip. Then the chip was tightly sealed in a chip container and was shaken to mix the contents without dispersion. For biochemical tests other than for HbA1c, blood was collected without anticoagulant and centrifuged to obtain 15 μL of serum which was then diluted with 190 μL of physiological saline for the assay. For hematological tests and the test for HbA1c, the sample was assayed with blood collected using EDTA-2 K. Good correlations were obtained between the test results of the assay using fingertip blood and that using venous blood. The correlation coefficients were ≥0.97 for TG, T-CHO, HDL-C, LDL-C, GLU, ALT, γ-GTP, UA, BUN, and HbA1c and ≥0.95 for WBC, RBC, Hgb, and Hct. These results suggest that our microliter-scale blood testing system is comparable to assays using venous blood and may be useful as a rapid and simple test to determine basic clinical parameters that are close to the reference intervals.

Keywords: microliter-scale blood sample, blood collection device, biochemical tests, fingertip, hematological tests

Introduction

On March 31, 2014, the Japanese Ministry of Health, Labour and Welfare announced the “Amendment of the Notification Issued based on the Act on Laboratory Test Technicians...”, officially approving self-collected blood tests at community pharmacies and similar establishments. This amendment made it possible to perform blood tests using a trace amount of blood squeezed out from a puncture site on a fingertip (hereinafter referred to as “fingertip blood”) in clinical laboratories. Micro-liter-scale blood collection is also expected to be useful...
for conducting mail-delivered sample testing or point-of-care testing in the near future.

There have been many previous reports on the measurement of blood glucose, HbA1c, and blood cell counts using microliter-scale blood samples collected from capillaries. For example, correlations between assay results using blood samples collected from a vein, such as the ante-cubital vein (hereinafter referred to as “venous blood”), and fingertip blood samples have been reported for biochemical tests. In addition, it has been suggested that hematological tests can be also measured in fingertip blood samples as long as test values are within the reference intervals established by the Japanese Committee for Clinical Laboratory Standards and if the following two conditions are met: capillary-collected blood is promptly transferred into a microliter-scale blood collection tube containing ethylenediaminetetraacetic acid (EDTA) and contamination with interstitial fluid by forced blood extrusion is avoided.

However, evaluation of the reliability of self-collected blood and mail-delivered sample testing has revealed accuracy problems caused by insufficient volumes of the blood specimens, hemolysis, and contamination with blood cell components. These problems arise from the need for a relatively large volume of fingertip blood and the difficulty in collecting blood by dripping fingertip blood from the upper part of the collecting funnel. In systems measuring diluted whole blood, the dilution ratio calculation method using internal standards poses some problems, such as accuracy issues in the method of squeezing out blood collected in EDTA-coated sponge pads and in the measurements of diluted whole blood samples of which are not necessarily consistent among patients. Moreover, microliter-scale blood collection systems using capillary blood not only require skill in the blood collection techniques, but are also time-and effort-consuming procedures; for example, an extra step is required to transfer samples into another container for mixing or centrifuging.

In order to resolve these problems with accuracy or the blood collection method in microliter-scale blood testing, we developed a new blood collection device. This device allows easy and quick aspiration of an appropriate amount of blood from the fingertip. The blood is then mixed within the blood collection chip without requiring dispensing, and the blood can then be placed in the centrifuge. In addition, we also developed a highly accurate testing method using microliter-scale blood samples collected with the device. In this article, we describe the results of our analysis of the correlation between the test values obtained from fingertip blood samples collected using our newly developed blood collection device and those obtained from venous blood, in order to assess the accuracy of our assay. Furthermore, we also examined whether our device facilitates accurate and consistent collection of fingertip blood.

Materials and Methods

Subjects

Biochemical tests and blood cell counts were performed using samples obtained from 60 and 20 adult volunteers, respectively. The blood cell counts were conducted later in samples obtained from 20 newly recruited adult volunteers because blood coagulation occurred in the majority of the fingertip blood samples during blood collection from the first 60 subjects.

The 60 subjects whose samples were collected for the biochemical tests consisted of 28 men and 32 women with a mean age of 37.2 years (range, 22–70). All the subjects were notified in advance about the necessity of fasting, in principle, prior to the blood sampling. However, after blood collection, we found that four subjects had had breakfast on the day of the test. Of the 60 subjects, 3 subjects with hypertension and 1 subject each with diabetes mellitus, gout, dyslipidemia, manic depression, allergy, and epilepsy were on medications for the respective disorders.

The 20 subjects for the blood cell counts consisted of 16 men and 4 women with a mean age of 40.6 years (range, 27–68). Of these subjects, one with hypertension was on medication.

Methods

1) Test parameters

Biochemical tests covered 14 parameters (TP, ALB, AST, ALT, γ-GTP, TG, T-CHO, HDL-C, LDL-C, BUN, CRE, UA, GLU, and HbA1c).

Blood cell counts covered 5 parameters (WBC, RBC, Hgb, Hct, and PLT).

The full names of the abbreviations used above for the test parameters are shown in Table 1.

2) Instruments and devices

1 Instruments for fingertip blood collection

We used blood collection chips, containers for centrifugation and blood collection, and blood collection tape (all MBS Co., Ltd. products) for fingertip blood collection, as shown in Fig. 1.

Two types of new blood collection chips were used: blood collection chips without any blood coagulation accelerant or anticoagulant (hereinafter referred to as “plain blood collection chips”) and blood collection chips coated with 150 μg of EDTA-2 K as an anticoagulant (hereinafter referred to as “EDTA blood collection chips”).

The BD Microtainer® Safety Lancet (Japan Becton, Dickinson and Co., Tokyo, Japan) was used to puncture the fingertip. In addition, we used nonwoven fabric soaked with Niprocrin isopropanol 70 vol.% to disinfect
Table 1. Reagent manufacturers and parameters employed in the assays performed using fingertip blood.

<table>
<thead>
<tr>
<th>Item</th>
<th>Abbrev.</th>
<th>Supplier</th>
<th>Modified method parameter</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Sample Vol (μL)</td>
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<tr>
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<td>ALB</td>
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<td>Alanine transaminase</td>
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<td>γ-GTP</td>
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<td>BUN</td>
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<tr>
<td>Platelet</td>
<td>PLT</td>
<td>HORIBA</td>
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Fig. 1 Microliter-scale blood collection devices.
(a) Tape for blood collection. The tape is made of elastic urethane nonwoven fabric with an acrylic adhesive. The tape diameter is 13 mm with a hole of 4.5 mm in diameter.
(b) From left to right: Blood collection chip, a chip container for centrifugation to separate serum for biochemical tests, a chip container for hematological tests, and a chip container for HbA1c evaluation. The blood collection chip is made of acrylonitrile butadiene styrene, and the chip containers for centrifugation are made of polypropylene. The surface of the blood collection chip is treated to reduce the contact angle. These three types of chip containers are identical in shape and size, but are colored differently according to the type of additive used for the blood collection chip. Each container is 10 mm in diameter and 42 mm in height, and each chip has a graduated scale to visually confirm the 60-μL volume of collected blood. The container for centrifugation is filled with 30 μL of a separating agent in resin form. The inner walls of blood collection chips for hematological tests and for HbA1c evaluation are coated with 150 μg of EDTA-2 K.
the fingertip, and also Nipro-pad S (all by Nipro Co., Ltd., Osaka, Japan) as a bandage to protect the fingertip puncture site after the procedure was complete.

(2) Instruments for venous blood collection;
Blood was collected using a holder which was connected with a needle (21- or 22-gauge). Blood collection tubes containing a separating medium and blood collection tubes containing EDTA-2 K (NEO-TUBE®; all by Nipro Co., Ltd.) were used for biochemical tests and blood cell counts, respectively.

(3) Centrifuge system
A small portable centrifuge, FlexiFuge (Argos Technologies, Elgin, IL, USA), was used to centrifuge the fingertip blood samples.

(4) Testing systems and reagents
The biochemical tests were carried out with a JCA-BM6070 automated biochemical analyzer (JEOL Ltd., Tokyo, Japan) and blood cell counts were measured using a Microsemi LC-667CRP Hematology Analyzer (Horiba, Ltd., Kyoto, Japan). The manufacturers of the reagents used for these tests are shown in Table 1.

3) Specimen collection

(1) Methods of blood collection
Both the fingertip and venous blood samples were collected by nurses. The collected blood samples for the biochemical parameters were transported in a refrigerated state and were analyzed within a day. The blood samples for blood cell counts were analyzed immediately after collection. All the blood samples were collected from fasted patients, although water intake and routine medications were allowed. The following information was entered in the survey form: the name, age, and sex of the subject; whether or not the subject had eaten; diseases for which the subject was receiving treatment or follow-up care; and the medications taken.

For the biochemical tests, a blood collection tape was attached to the tip of the ring finger and the finger was then punctured. Blood was collected using an EDTA blood collection chip for HbA1c measurement and using a blood collection chip for the other biochemical parameters. For the blood cell counts, a blood collection tape was attached to the tip of the ring finger and the finger was then punctured. Blood was collected using an EDTA blood collection chip.

Venous blood collection was performed immediately after the fingertip blood collection.

(2) Procedures before measurement of specimens
[1] Fingertip blood collection
The procedures for fingertip blood collection are shown in Fig. 2. Sixty microliters of blood was collected for measurement of the biochemical parameters other than HbA1c using a plain blood collection chip. The chip was placed into a centrifuge container that was then tightly closed. For HbA1c measurement, 60 µL of blood was collected using the EDTA-2 K blood collection chip and placed into a container that was then tightly closed. The container was held in one hand and shaken by repeatedly snapping the wrist in a downward motion (hereinafter referred to as “snap-shaken”).

For the blood cell counts, 60 µL of blood was collected using an EDTA blood collection chip and snap-shaken. Then, the blood sample was dispensed into the blood cell count containers designated by Horiba, Ltd.

[2] Venous blood collection
For the biochemical tests, 5 mL of the collected blood was placed in blood collection tubes containing separating medium. For the HbA1c measurement, 2 mL of the collected blood was placed in EDTA-2 K blood collection tubes. After the tube was shaken, the blood sample was dispensed into the blood cell count containers designated by Horiba, Ltd.

[3] Centrifugation method
For biochemical tests, the fingertip blood specimens were stored either in the centrifuge containers or the blood collection containers in a refrigerated state and were centrifuged at 4,700 g for 5 minutes.

The venous blood specimens were stored in a refrigerated state in the blood collection tubes containing a separating medium for biochemical tests or the EDTA-2 K blood collection tubes for HbA1c measurement and were then centrifuged at 2,000 g for 10 minutes.

(3) Measurements
[1] Fifteen microliters of serum from the fingertip blood was diluted with 190 µL of physiological saline, giving a defined dilution rate of 13.7-fold. At each dilution, the volume of each sample and the amounts of reagents were adjusted for each measurement parameter according to the modified method (Table 1). The measurements were performed using a biochemical testing system with which a new calibration curve was generated in the dilution range for each parameter. Measurement values were obtained by multiplying the observed values by the dilution ratios.

[2] HbA1c was measured using blood cells separated from the centrifuged EDTA fingertip blood in the biochemical testing system.

[3] Venous blood samples were measured without dilution by routine methods in the biochemical testing system. Also, HbA1c was measured using blood cells separated from the EDTA venous blood in the biochemical testing system.

[4] Five blood cell count parameters were measured in EDTA fingertip blood and EDTA venous blood dispensed into the containers designated by Horiba Ltd. using a Mi-
crosemiLC-667CRP.

4) Analytical methods

For each test parameter, the test values obtained from the fingertip blood and the venous blood were plotted on a two-dimensional graph. Then, the correlation coefficients for all plots were obtained and were compared.

Results

Using our newly developed blood collection device and blood collection method, we were able to promptly collect an appropriate volume of blood for the measurements of biochemical parameters and HbA1c by a single puncture of the fingertip in all 60 subjects. Similarly, the blood collection for blood cell counts was successful in all 20 subjects. Since blood coagulation occurred in the majority of the specimens collected for blood cell counts in the first 60 subjects, another 20 volunteers were newly recruited and fingertip and venous blood samples were collected from them for the blood cell counts.

There were no bleeding nor wound problems after the puncture with the lancet. However, 5 of the 15 subjects who responded to the questionnaire distributed after the blood collection reported to having felt pain at the time of or after the puncture. Blood collection was suspended in one subject in whom venous blood collection was difficult after fingertip blood collection. As a result, 59 of the 60 subjects were included in the analysis of correlations between the results of the fingertip and venous blood assays.

After collecting fingertip blood into the collection chip, each chip was placed into a centrifuge container that allowed the separating agent to get inside the blood collection chip. After centrifugation, serum was appropriately separated from all the blood samples for the biochemical tests with no visually detectable contamination by blood cells. However, in one subject, chyle was found in the serum separated from the fingertip blood, but there was no evidence of hemolysis or fibrin contamination. Nonetheless, in this sample also, the quantitative dispensing of serum was carried out without any problem.

Correlations between the test values of the biochemical parameters obtained from the fingertip-derived diluted
serum and the venous blood-derived serum are shown in Fig. 3. Correlations between the results of blood cell counts obtained from the fingertip and venous blood specimens are shown in Fig. 4. The average test values for the fingertip and venous blood assays, along with their differences, are shown in Table 2.

In the biochemical tests, the correlation coefficients were $\geq 0.97$ for ALT, $\gamma$-GTP, UA, BUN, TG, T-CHO, HDL-C, LDL-C, GLU, and HbA1c, with slopes of approximately 1.0, thereby demonstrating a good correlation between the results of the assays on the two types of specimens. For AST, the correlation coefficient was 0.945, showing a relatively good result, but the average difference between the results for the fingertip and venous blood varied from $-6.0$ to $4.4$ U/L. For CRE, the results were favorable, with a correlation coefficient of 0.959, and the average difference between fingertip and venous blood test values was 0.05 mg/dL, with a maximum difference of 0.1 mg/dL. However, the values tended to be higher in the fingertip blood than in the venous blood.

For TP and ALB, although the correlation coefficients were 0.915 and 0.918, respectively, the average differences between fingertip and venous blood test values were 0.13 g/dL and 0.07 g/dL, respectively, demonstrating very similar test values for the two assays.

The blood cell counts showed good correlations for WBC, RBC, Hgb, and Het, with correlation coefficients $\geq 0.95$. However, the PLT value obtained using fingertip blood varied according to the blood collection status, and tended to be somewhat lower than the values obtained using venous blood.

**Discussion**

In this study, the test values obtained with fingertip blood collected using our newly developed method were compared with those with venous blood collected using the conventional method. There have been some problems in assays using fingertip blood samples. Unlike venous blood, fingertip blood is a mixture of blood from capillaries, venules, and arterioles, so the blood may be contaminated with interstitial fluid. In addition, small amounts of blood are used for the analysis of fingertip blood specimens after dilution, which may affect the test results.

In our study, however, a good correlation was generally observed between fingertip and venous blood test values for the 14 biochemical parameters, although the fingertip test values of AST and CRE varied to a small extent and tended to be slightly lower in the low concentration ranges of these test items.

However, the correlations were rather low for the assays of TP and ALB. This may be explained by the fact that the variation in individual values became more apparent since all the data fell in a narrow range because we used healthy volunteers as the subjects whose TP and ALB values fell within the reference intervals (6.5–7.9 g/dL and 3.9–5.1 g/dL, respectively). In addition, the relatively low correlations in the low concentration ranges for AST and CRE may be attributable to reduced measurement accuracy as the concentrations of these substances became low due to the dilution. Moreover, for blood glucose, no significant difference was observed between the fingertip and venous blood assays in this study, which is consistent with a previous report.

In the blood cell count measurement, we reviewed the procedures and were able to successfully prevent coagulation of the specimens by thoroughly mixing the contents by snap-shaking after the fingertip blood collection. Yang, et al. reported a difference in the WBC count between fingertip blood and venous blood, and also that by repeated collection there was a decrease in the WBC count in fingertip blood. In the present study, there was no significant difference in the WBC count across specimens, and our fingertip blood collection method allowed us to collect specimens by a single puncture, obtaining consistent results for the blood cell counts. However, for PLT, the fingertip blood assay tended to yield lower values. In the collection of fingertip blood, blood squeezed out from the puncture site pools at the puncture site before being aspirated into the tip. Therefore, some specimens took a relatively long time before being aspirated. This may have allowed platelet aggregation in the specimens, resulting in a lower PLT count. Thus, for PLT counts, the correlation can be further improved by ensuring rapid blood collection and prompt measurement.

Several methods for fingertip blood assays have already been reported. Among them, the common blood collection method is to drop around 150 to 200 µL of fingertip blood into a small tube or onto the upper surface of a filter paper pad for blood aspiration, followed by separation of serum or plasma using a battery-operated microcentrifuge. However, in these methods, a relatively large volume of blood is required, and it is difficult to identify the volume of blood dropped. Also, a longer time is needed for the blood collection. As a result, these methods may cause some problems such as insufficient blood volume, hemolysis, contamination with blood cell components, and insufficient centrifugation. Thus, the preparation of test samples can be difficult with these methods.

In contrast, using our newly developed blood collection device, fingertip microliter-scale blood sampling was carried out smoothly without causing such problems. The inner surfaces of the blood collection chips were coated to reduce the contact angle. Therefore, by bringing the tip of the blood collection chip into contact with the lateral surface of the pooled blood, it was possible to aspirate the blood naturally without applying negative pressure with a syringe. In addition, the transparent blood collection chips with scale marks allowed visual confirmation of blood aspiration. Moreover, the use of blood collection tape prevented blood from dripping off the puncture site.
Fig. 3 Correlations between the biochemical test results obtained by fingertip blood assays and conventional venous blood assays in 59 adult volunteers.
at the time of blood collection, allowing an appropriate amount of blood to pool at the fingertip. These features made it possible to collect promptly the necessary specimen volume. Thus, we consider that our blood collection device is useful for fingertip blood collection and that it contributes to improved accuracy of the assay.
Another method has also been proposed to simplify blood collection from the fingertip. In this method, whole blood absorbed by an EDTA-coated sponge pad is soaked and diluted in a hyperosmotic buffer. Then, the diluted blood sample is subjected to a membrane separation process wherein the sample is mechanically filtered through a membrane to obtain diluted plasma. The dilution ratio of the plasma released into the diluted solution is measured post hoc using the difference in absorbance between the internal standard materials contained in the hyperosmotic buffer. However, this method may cause a change in the plasma components, because it is inevitable that water and low-molecular-weight substances in blood cells will diffuse into the diluted solution due to the hyperosmotic buffer used to avoid hemolysis during pressure application in the membrane separation process. Another problem with this method is that measurement variations, even in the common range of 2% to 3%, may greatly affect the calculated dilution ratio because of very small differences in the absorbance of the internal standard materials used to determine the plasma dilution ratio.

It has been demonstrated that our new blood collection device allows the above-mentioned problems to be overcome by the high quality of the specimen collection and by a modified micro specimen measurement method based on the existing method. We demonstrated that our assay is useful for the measurement of test values that are close to the reference intervals. Moreover, with respect to the specimens, our methods allow the collection and preparation of the necessary amounts of serum, plasma, and whole blood, meaning that the methods are applicable to a wide range of blood tests. In fingertip blood collection assays, there are few reports examining the correlation of serum test values that fall outside the reference intervals.

Further studies are necessary to investigate whether high correlations can also be obtained using our method with fingertip blood samples for which test values fall outside the reference intervals.

Conclusions

Using a new blood collection device with modified micro specimen measurement methods, we obtained relatively good correlations between biochemical parameters obtained from fingertip blood samples and from simultaneously collected venous blood samples for values close to the reference intervals. In addition, in the blood cell count measurement system using EDTA-added whole-blood samples, the accuracy of the assay is expected to further improve by our rapid fingertip blood collection methods.

Microliter-scale blood tests using simple fingertip blood collection methods with a newly developed blood collection device are considered to be useful as simple assays for health checkups and medical examinations, although there is room for further improvement in the methods for some test parameters.

Disclosure of conflict of interest

Hajime Iwasawa is on the board of directors of MBS Co., Ltd. Tomoaki Nishimura and Shota Nemoto are employees of MBS Co., Ltd. Naoki Aikawa is a medical adviser (without compensation) to MBS Co., Ltd. The remaining author declares no competing financial interests.

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