

## A New Hepatitis Virus Test with Microliter-scale Fingertip Blood Collection in Japan

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We investigated whether a small amount of blood collected by fingertip blood sampling would be adequate in a mass examination for hepatitis virus infection in Japan. A cross-sectional survey was conducted at health fairs in Kasaoka City and Shodoshima Island, where participants took the hepatitis screening test. A total of 114 consecutive individuals who took the hepatitis screening test were enrolled. Twenty microliters of plasma was successfully obtained from all participants. Among the participants, two had positive results for HBs antigen and two were positive for anti-HCV; all four were >60 years old and rarely visited the hospital. Thirty-three and 38 patients chronically infected with HBV and HCV, respectively, were examined for confirmatory assays at participating hospitals. All subjects with undetectable serum levels of HBs antigen and anti-HCV had undetectable levels of both markers in fingertip blood, and the levels in serum and fingertip blood were significantly correlated ( $p < 0.01$ ). The lower detection limit of HBs antigen was defined as 0.005 IU/ml, and the cut-off value of anti-HCV was 1.0 by using 10- $\mu$ l fingertip blood samples. The fingertip blood sampling described herein may be adequate in mass examinations for hepatitis virus testing in Japan.

**Key words:** fingertip, hepatitis test, HBV, HCV, Japan

There are estimated to be 1.5 million individuals with chronic hepatitis due to hepatitis B virus (HBV) or hepatitis C virus (HCV) in Japan, including 0.3 million latent patients who are unaware of their infection [1]. Chronic infection with hepatitis virus cannot be identified if the infected individual does not take a hepatitis screening test, because hepatitis patients do not have disease-related symptoms. In Japan, a hepatitis screening test is usually offered at annual health check-ups in the workplace. However, the participation rate is generally low, because the test

is not mandatory [2]. It is necessary to educate the public about the importance of taking a hepatitis screening test [3].

Fingertip blood sampling in mass examinations has been proposed for hepatitis screening because it does not require venous blood sampling by skilled medical staff, and it enables blood collection without the uncertainty of venous blood sampling in large-scale hepatitis screening tests outside the hospital. The World Health Organization (WHO) guidelines on hepatitis B and C testing published in Feb. 2017 state that a fingertip blood sampling technique is available for both HBV and

HCV serological assays [4], and that the sensitivity and specificity of the use of whole blood samples by fingertip blood sampling compared to plasma or serum are high enough for the screenings. In Japan, however, fingertip blood sampling has not been used for hepatitis screening testing, and comprehensive evaluations of the fingertip blood sampling technique in mass examinations have not been performed according to our PubMed search.

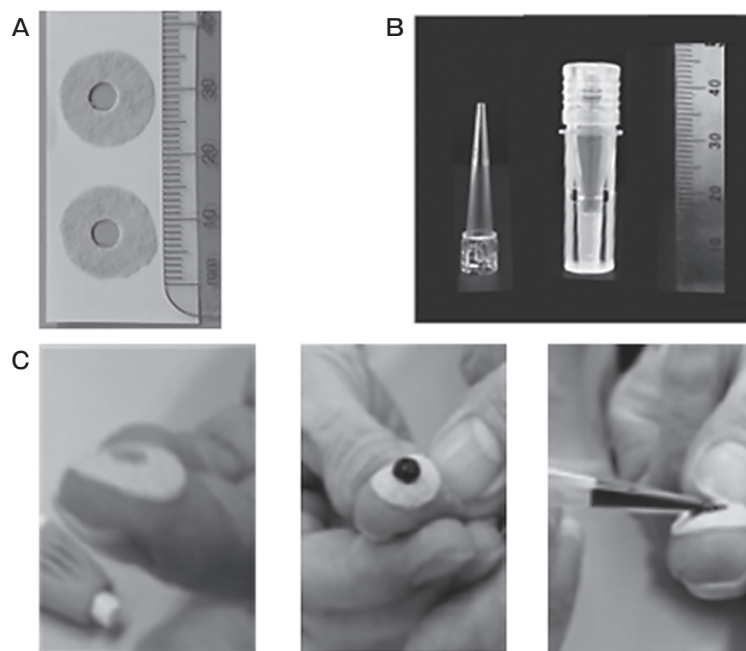
We conducted the present study to validate the measurements of HBs antigen and anti-HCV by fingertip blood sampling and to evaluate the efficiency of this sampling method in comparison with those by venous blood sampling in a mass examination for the screening of hepatitis virus infection. Fingertip blood sampling would be an efficient strategy for providing individuals with opportunities to take a more convenient hepatitis screening test in the work place.

## Methods

**Study population.** The cross-sectional survey was conducted among residents of Kasaoka City attending a

health fair at a local healthcare center and among residents of Shodoshima Island attending a health fair at Shodoshima Central Hospital in November 2016. The accuracy of the detection of HBs antigen and anti-HCV in fingertip blood was confirmed by the measurement of HBs antigen and anti-HCV in the fingertip blood and serum of patients with chronic HBV or HCV infection at the Departments of Gastroenterology of Okayama University Hospital and Kagawa Prefectural Hospital. The study was conducted in accordance with the Helsinki Declaration, and was approved by the ethical committees of the participating institutes (#1611-014). All of the participants provided their written informed consent.

**Specimen collection and hepatitis virus testing.** Whole blood samples by fingertip blood sampling and paired serum samples were simultaneously collected from the participants (Fig.1). For the preparation of fingertip blood samples, the fingertips were punctured with a lancet (BD Microtainer Safety Lancet; Becton, Dickinson and Company, Tokyo, Japan). Then, 60  $\mu$ l of the whole blood collected was taken up by an EDTA-2K device designed for the collection of small



**Fig. 1** Fingertip blood sampling. Round pieces of urethane tape with a central 4.5-mm hole **A** and the EDTA-2K blood-collecting device and container **B** were used for the fingertip blood sampling (MBS, Tokyo). The fingertip was sealed with a round piece of tape with a 4.5-mm hole in the center and punctured with a lancet (BD Microtainer Safety Lancet; Becton, Dickinson, Tokyo). Then, 60  $\mu$ l of the whole blood was taken up by the EDTA-2K device **C**.

blood samples (MBS Corp., Tokyo, Japan). All of the samples were kept at 4°C during their transfer to our laboratory.

The plasma portions were separated by centrifugation, and 10 µl of the separated plasma was diluted with 90 µl of diluent for the detection of HBs antigen and anti-HCV. The detection of HBs antigen and anti-HCV was done using a chemi-luminescence enzyme immunoassay with a blood analyzing instrument (HISCL HBsAg kit, HISCL HCV Ab kit, and HISCL-800, respectively, Sysmex, Kobe, Japan) according to the manufacturer's recommendations. Based on the manufacturer's specifications, the lower quantitation limit of HBs antigen is 0.03 IU/ml and the cut-off value of anti-HCV is 1.0 in the assays using 100 µl of a serum sample.

**Statistical analysis.** We compared the results of the HBs antigen and anti-HCV measurements in fingertip blood with those in the serum by determining the Pearson's correlation coefficient. The sensitivity, specificity, positive predictive value and negative predictive value of fingertip blood versus serum for HBs antigen and anti-HCV were calculated. Statistical analyses were performed using JMP Pro ver. 12.0 software (SAS Institute, Cary, NC, USA), and *p*-values <0.05 were considered significant.

## Results

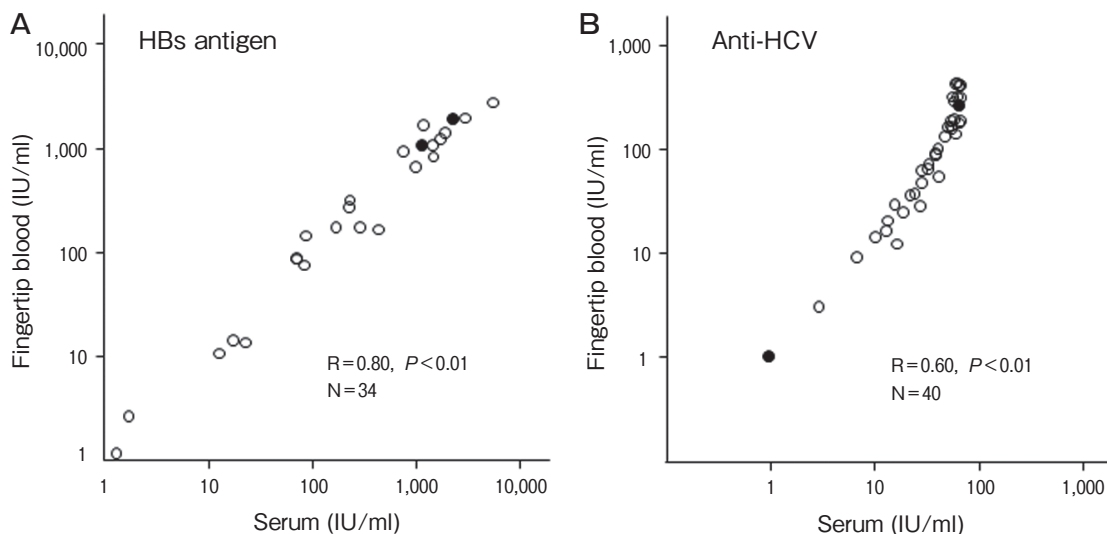
**Characteristics of study participants.** A total of 114 consecutive individuals who took the hepatitis screening test were enrolled in the study: 98 residents of Kasaoka City and 16 residents of Shodoshima Island. Their mean age was 63 years, ranging from 34 to 88 years. Among them, 86 individuals (75%) were female. In the second part of the study, 33 and 38 patients with chronic HBV and HCV infection, respectively, were enrolled at either of the 2 participating hospitals and underwent confirmatory assays for the measurement of HBs antigen and anti-HCV levels. Whole blood samples were successfully collected from all of the participants by fingertip blood sampling using the novel collection system with EDTA-2K. Each sample consisted of 60 µl of blood and provided >20 µl of plasma, a sufficient amount for each test.

**Positive rates of HBs antigen and anti-HCV among the participants.** Among the 98 residents of Kasaoka City who took the hepatitis virus test, 2 were positive

for HBs antigen (a 61-year-old female and a 79-year-old female), and their levels of HBs antigen were nearly identical between their serum and fingertip blood. Two other residents were positive for anti-HCV (a 68-year-old female and a 78-year-old female) in both the serum and fingertip blood. This was the first time that any of these four participants had taken a hepatitis virus test, and they did not have any other complications or liver disease-related symptoms. The positivity rates were thus 2.0% for HBs antigen and 2.0% for anti-HCV. Among the 16 residents of Shodoshima Island who took the hepatitis virus test, none showed positive results for HBs antigen or anti-HCV.

**Accuracy of detection of HBs antigen in fingertip blood.** All of the patients with HBs antigen >0.03 IU/ml in the serum had detectable levels of HBs antigen in their fingertip blood samples (*n*=34; Fig. 2A). Significant correlations were observed between the levels of HBs antigen in the serum and those in the fingertip blood (*R*=0.80, *p*<0.01). One patient with less than the lower quantitation limit in the serum (0.02 IU/ml) had an undetectable level of HBs antigen in fingertip blood. All 150 test participants who had undetectable HBs antigen in the serum also had undetectable HBs antigen in the fingertip blood samples. These results showed that the sensitivity and specificity of detectable HBs antigen were 97.1% and 100%, respectively. The positive predictive value and negative predictive value were 100% and 99.3%, respectively. The lower detection limit of HBs antigen was defined as 0.005 IU/ml by using 10 µl of fingertip blood samples.

**Accuracy of the detection of anti-HCV in fingertip blood.** As shown in Fig. 2B, all of the patients with anti-HCV >1.0 in the serum also had detectable levels of anti-HCV in their fingertip blood (*n*=40). The anti-HCV levels in the fingertip blood samples were significantly correlated with those in the serum (*R*=0.60, *p*<0.01). All of the 145 test participants with undetectable anti-HCV in the serum also had undetectable anti-HCV in their fingertip blood samples. These results showed that the sensitivity, specificity, positive predictive value, and negative predictive value were all 1.0. Three patients had anti-HCV levels between 1 and 10 in both their serum and fingertip blood. These patients had undetectable HCV RNA in a further examination. The cut-off value of anti-HCV was defined as 1.0 by using 10 µl of fingertip blood samples.



**Fig. 2** HBs antigen and anti-HCV results. A scatter diagram of the measurement of HBs antigen levels in the fingertip blood and paired serum samples is shown **A**, along with a scatter diagram of the measurement of anti-HCV levels in the fingertip blood and paired serum samples **B**. The positive results of HBs antigen and anti-HCV are shown in the closed circle.

## Discussion

This cross-sectional survey for a hepatitis screening test evaluated the use of a fingertip blood sampling technique in a mass examination for hepatitis virus infection in Japan, by validating the measurement of HBs antigen and anti-HCV in the resulting samples from the mass examination. The present study is the first to confirm that fingertip blood sampling would be useful in mass examinations for hepatitis virus infection in Japan. Fingertip blood sampling removes the necessity of venipuncture and would be adequate for the tests of HBs antigen and anti-HCV that are commercially available in Japan.

Previous investigations revealed that dried blood samples from fingertip blood sampling could be successfully used for hepatitis screening tests [4,5]. We used the novel blood-collecting device described herein, which successfully and promptly collected a sufficient amount of whole blood by capillary action rather than aspiration, without clotting or hemolysis, from all of the participants. The resulting samples were sufficient to detect HBs antigen and anti-HCV adequately. In addition, several participants volunteered to perform self-administered blood sampling under the close supervision of the medical staff, and were successful in the self-administration.

In terms of the positive rates of HBs antigen and

anti-HCV among the participants in Kasaoka City, all 4 individuals who had positive results of HBV or HCV were >60 years of age, and had no complications or liver disease-related symptoms. These results suggested that hepatitis virus tests should be encouraged among elderly people in good health who rarely visit hospitals. The fingertip blood sampling technique in this study may become a versatile and efficient strategy for identifying latent hepatitis patients. Moreover, the availability of hepatitis screening by self-blood sampling from a fingertip under the close supervision of medical staff outside a hospital setting, followed by consultation with a hepatologist as needed, might increase the number of participants in hepatitis screenings in the future.

To assess the accuracy of the detection of HBs antigen and anti-HCV in the fingertip blood samples, we compared the quantities of HBs antigen and anti-HCV between the fingertip blood samples and paired serum samples. In all of the participants with undetectable HBs antigen or anti-HCV in the serum, HBs antigen or anti-HCV was also undetectable in fingertip blood samples, and significant correlations of the values were observed between fingertip blood samples and the paired serum ( $p<0.01$ ). The lower detection limit of HBs antigen was defined as 0.005 IU/ml and the cut-off value of anti-HCV was 1.0 by using 10  $\mu$ l of fingertip blood samples, which should be sufficient for hepatitis screening tests in clinical use.

In conclusion, fingertip blood sampling may be useful in mass examinations in Japan. A fingertip blood-sampling technique using a novel system permitted the collection of microliter-scale whole-blood samples, which should be adequate for the measurement of anti-HCV and HBs antigen.

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