A novel approach for evaluating antibodies against SARS-CoV-2 using menstrual blood collected from sanitary napkins before and after vaccination and evaluation of the visual napkin score

Hiromitsu Shirasawa (✉ shirasawah@doc.med.akita-u.ac.jp)

Department of Obstetrics and Gynecology <https://orcid.org/0000-0001-5300-0037>

Yukiyo Kumazawa

Department of Obstetrics and Gynecology

Shiori Kushima

Department of Obstetrics and Gynecology

Ayaka Fujishima

Department of Obstetrics and Gynecology

Wataru Sato

Department of Obstetrics and Gynecology

Kazue Togashi

Department of Obstetrics and Gynecology

Mayumi Goto

Department of Obstetrics and Gynecology

Kazumasa Takahashi

Department of Obstetrics and Gynecology

Emiko Sato

Department of Obstetrics and Gynecology

Yukihiro Terada

Akita Industrial Technology Center

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Abstract

Peripheral blood is mainly used for SARS-CoV-2 antibody testing. We evaluated menstrual blood, which can be collected noninvasively from sanitary napkins, as a specimen type for antibody testing. We also evaluated the visual napkin score and examined the relationship between the amount of menstrual blood collected. We tested napkins from 40 participants; 77.5% of samples could be tested with the antibody testing kits. In 35 unvaccinated participants, there were no IgG or IgM positive results. In five vaccinated participants, IgG was positive in 100% of samples and IgM was positive in 60%. Four antibody test kits were used in this study. The results also differed depending on the antigen targeted by each antibody. The evaluation of antibodies using menstrual blood from napkins might have public health applications in the future as napkins could be developed into wearable monitoring sensors.

1. Introduction

Various medical tests have been conducted for coronavirus disease 2019 (COVID-19), which has been raging worldwide since the end of 2019. These tests include antigen tests ¹, reverse-transcriptase polymerase chain reaction (RT-PCR) tests ², and antibody tests ³. The materials used are different for each test and include nasopharyngeal swabs, oropharyngeal swabs, saliva samples, and blood samples taken from the fingertips. From a public health perspective, these tests are chosen based on the outbreak situation in each region and the purpose of the test ⁴. In general, antigen and RT-PCR tests are used to diagnose infection in patients. IgG, IgM, and IgA antibody tests are used to confirm a history of infection. The principal antibodies against COVID-19 are the nucleoside capsid (N) and spike (S) proteins, which are structural proteins encoded by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) gene. The structural S protein is used as an antigen ^{5,6}. In addition, enzyme-linked immunosorbent assay and chemiluminescent immunoassay are generally used for quantitative antibody tests, and immunochromatography is used for simple tests such as qualitative antibody tests ^{7,8}.

The immunochromatographic method is used as a simple test rather than to confirm the diagnosis. There are many commercial anti-SARS-CoV-2 immunochromatographic test kits available in different countries ⁹. The immune response after SARS-CoV-2 infection is associated with elevated IgA, IgG, and IgM antibodies for both N and S proteins ¹⁰. There have been reports of post-onset tests using these simple kits to evaluate regional antibody prevalence and for rapid diagnostics ¹¹. On the other hand, there have been only a few reports on detecting neutralizing antibodies in response to messenger RNA (mRNA) vaccination ¹², which has been rapidly introduced in many countries since 2021, using simple test kits from various manufacturers targeting N and S proteins as antigens.

Although saliva, whole blood, serum, plasma, urine, and feces have been used as specimens for SARS-CoV-2 antigen and antibody tests, menstrual blood has not been reported to date. Menstrual blood is a bloody secretion originating from the endometrium. It contains blood, endometrial epithelial cells, and bacterial components of the uterus ¹³. The endometrial tissue is associated with natural killer (NK) cells,

T cells, and macrophages, making it an immunologically distinctive tissue ¹³. Menstrual blood is also thought to be the origin of endometriosis, which is caused by the reflux of menstrual blood into the fallopian tubes. From the immunological and inflammatory perspectives, menstrual blood is unique ¹⁴. There have been several reports on the use of menstrual blood to evaluate infectious diseases such as chlamydia and human papillomavirus as well as correlations between levels of reproductive hormones between peripheral blood and menstrual blood ¹⁵⁻¹⁷.

This study involved antibody testing of menstrual blood collected from sanitary napkins with multiple immunochromatographic kits. We have been evaluating sanitary napkins from asymptomatic non-COVID-19 women since before mRNA vaccination began. We also performed antibody testing on menstrual blood from women who had received mRNA vaccination and compared results from several antibody test kits. Unlike invasive blood collection methods, menstrual blood collected non-invasively from participants can be used to evaluate the presence of antibodies against SARS-CoV-2. Our report is the first to show an association between menstrual blood and the presence of neutralizing antibodies acquired via mRNA vaccination and the usefulness of menstrual blood as a sample type for detecting SARS-CoV-2 antibodies, considering the volume of blood in sanitary napkins.

2. Results

In this study, we collected one napkin each from 40 participants and attempted to collect their menstrual blood from the napkins. The distribution of visual napkin score (VNS) in the 40 participants is shown in Table 1. As shown in Table 1, higher VNS indicates more menstrual blood collected. Statistically, VNS 3 or higher resulted in significantly higher menstrual blood collection than VNS 1 ($p<0.01$). In addition, VNS 5 napkins had more menstrual blood collection than VNS 2 samples, but there was no significant difference after Bonferroni correction ($p=0.070$). With VNS 1, collection of menstrual blood was extremely difficult, and antibody test kits could not be tested for all eight participants. On the other hand, 31 of 32 participants (96.9%) with VNS 2 or higher could be tested with one or more antibody test kits. In 5 of 40 participants, menstrual blood was collected after at least 1 dose of mRNA vaccination (Comirnaty). The overall antibody testing rate, defined as the percentage of participants who could be tested with at least one antibody test kit, was 77.5%.

Table 2 summarizes the antibody test kit results using menstrual blood. In this study, Kit A was most frequently used, with 31 tests performed. Kit D, which was added after the study began, had the lowest number of tests, with 21 participants. For all kits, 100% of tests with menstrual blood had a positive control line. On the other hand, there was a difference in positive IgG and IgM lines among the kits, as shown in Table 2. All participants who tested positive for IgG and IgM had received a COVID-19 mRNA vaccine. A participant tested 13 days after the second vaccination was IgG positive with Kit A and IgG and IgM positive with Kit C. A participant tested 16 days after the first vaccination was positive for IgG and IgM with Kits A and C. A participant tested 15 days after the first vaccination was positive for IgG with Kit A, IgG and IgM positive with Kit C, and weakly IgG positive with Kit D. Two participants who received the second vaccination were tested 21 and 48 days later, respectively. Only IgG was positive with

Kits A and C in these participants. With Kit B, there were no positive IgG or IgM lines in participants regardless of vaccination status. We summarized these results in Table 3.

3. Discussion

This study has several significant findings. First, it was possible to collect enough menstrual blood from sanitary napkins to perform antibody testing in 77.5% of participants. The previous study by Magnay et al. evaluated menstrual blood on sanitary napkins as a pictogram and reported a relationship with menstrual blood loss and a pictogram¹⁸. Our study found for the first time that napkins with VNS 2 or higher can yield sufficient menstrual blood for multiple antibody tests with syringe aspiration. A sanitary napkin has been reported to be a useful specimen for PCR-based detection of *Chlamydia trachomatis*¹⁵. Alary et al. showed that a sanitary napkin simply worn for 4 hours without menstrual blood can be a PCR specimen for *Chlamydia trachomatis*, with sensitivity of 93.1% and specificity of 98.9%, suggesting that sanitary napkins are useful as self-collection devices. The study showed that VNS 3 or above was associated with significantly higher volume of menstrual blood collected than VNS 1.

A recent report used menstrual cups to collect menstrual effluents. It examined CD45⁻ and CD45⁺ cell populations using flow cytometry¹⁹. One of the novelties of this study is that menstrual blood absorbed by sanitary napkins was recollected and used as a sample to detect SARS-CoV-2 antibodies. Second, this study was novel because no evaluation using sanitary napkins or menstrual blood has been reported to date. This is also the first time that multiple antibody test kits have detected IgG and IgM against SARS-CoV-2 in menstrual blood collected from sanitary napkins. In this study, the upper limit was set to 980 µl of menstrual blood. The upper limit was reached in three participants, each with VNS 4 or 5. However, even with this upper limit, an average of 364 µl of menstrual blood can be collected. Thus, menstrual blood may be an attractive non-invasive specimen for SARS-CoV-2 antibody evaluation. For antibody confirmation after mRNA vaccination, a study using saliva has recently been reported²⁰. Ketas et al. showed that IgG and IgA against the S protein could be detected in saliva after vaccination. Several attempts to identify SARS-CoV-2 RNA in stool have also been reported^{21,22}. Although various specimens have been used, the current gold standard for evaluating antibodies against SARS-CoV-2 remains serum-based evaluation. However, very recently, a trial on remote early detection of SARS-CoV-2 infection using a wearable device has been reported²³. We believe that this study demonstrates that menstrual blood collected from sanitary napkins can be helpful as a wearable device to determine the presence of SARS-CoV-2 antibodies in public health.

In this study, the results of antibody tests differed significantly between participants by vaccination status. Non-vaccinated participants were asymptomatic at the time of testing, and a total of 92 kits were tested on 26 non-vaccinated participants. All were positive for the control line only, with no positives for IgG or IgM. On the other hand, all five asymptomatic participants who received mRNA vaccination were positive for IgG with least 1 kit. Three participants were also positive for IgM in antibody tests using menstrual blood. Thus, 11 out of 20 kits were positive for IgG or IgM in the vaccinated group, which was

significantly higher than in the non-vaccinated group. In the non-vaccinated group, all 92 kits were negative for IgG and IgM ($p < 0.001$). There have been no reports on the concordance rate between antibody test kits after mRNA vaccination. In this study, neutralizing antibodies against the S protein were detected in menstrual blood. These results were helpful in determining which antibody test kit to use for evaluation.

Since saliva is a non-invasive specimen for vaccination or antibody evaluation after SARS-CoV-2 infection, studies have been conducted on its usefulness as described above^{20,24}. Like saliva, menstrual blood in a napkin can be collected non-invasively, but the napkin may have the advantage of being a wearable device. Non-invasively obtained menstrual blood has also been used as a biomarker for screening and monitoring for human papillomavirus DNA in cervical lesions¹⁶. In addition, menstrual blood has attracted attention for its immunological specificity, such as the differential expression of NK cells from peripheral blood²⁵. It is very important to investigate the immunological effects of menstrual blood on SARS-CoV-2. In this study, both IgG and IgM were detected in antibody test kits targeting the S protein in participants who have received the mRNA vaccine. In the future, it is necessary to study the response of menstrual blood after vaccination with peptide vaccines that target both the S and N proteins²⁶.

Naturally, there is debate about the usefulness of SARS-CoV-2 antibody testing after vaccination. The United States Food and Drug Administration (FDA) did not recommend antibody testing to assess immunity after COVID-19 vaccination as of July 2021 (<https://www.fda.gov/medical-devices/safety-communications/antibody-testing-not-currently-recommended-assess-immunity-after-covid-19-vaccination-fda-safety>). To date, no test kit has been approved to confirm the presence of antibodies after vaccination. It is also unclear whether qualitative assessment of IgG antibodies after vaccination can indicate immunity to SARS-CoV-2. While antigen and RT-PCR testing are certainly important in determining whether a person is infected with SARS-CoV-2, we believe that qualitative antibody testing after vaccination will become increasingly important in public health. In our study, since the antibody responses differed across kits, it may be possible to differentiate between antibodies obtained from vaccines and antibodies from SARS-CoV-2 infection based on differences in antigens from different kits used to test menstrual blood. In the future, it may be possible to embed immunochromatographic antibody test kits for the S and N proteins in the sanitary napkin itself so that the presence of antibodies can be determined by looking at the lines on a sanitary napkin. A trial for a SARS-CoV-2 detection sensor to be embedded in a face mask as a wearable device have recently been reported²⁷. In the future, it is expected that electrical signals from wearable devices such as sanitary napkins will be linked to applications to obtain biometric information.

One of the limitations of this study is that the sample size was small. It is necessary to include more participants considering that there will be several types of COVID-19 vaccinations in the future. It should also be noted that the participants in this study were from a region with a relatively low incidence of COVID-19. This study was conducted in a province with about 900,000 people, and there were only

approximately 970 cumulative cases of COVID-19 cases as of July 2021, suggesting that the risk of infection was low even in asymptomatic participants before vaccination. In addition, there are many types of sanitary napkins, and the effect of differences in absorbent polymers in different products on the composition of the menstrual blood collected needs to be further studied in the future.

In conclusion, we have, for the first time, evaluated antibodies against SARS-CoV-2 in menstrual blood collected from sanitary napkins with several antibody test kits. We found that if more than 20% of the napkin area has menstrual blood on it, sufficient menstrual blood can be collected for antibody testing. We also confirmed that menstrual blood collected from a sanitary napkin could be used to detect IgG and IgM after mRNA COVID-19 vaccination. We believe that our results are a pioneering effort that has not been reported previously and will lead to better public health and development of wearable devices.

4. Online Methods

A) Menstrual blood collection

In this study, a total of 40 participants visiting the outpatient gynecology clinic of our university hospital with no symptoms related to COVID-19 provided us with one sanitary napkin containing menstrual blood. Mean age was 35.5 ± 5.4 years. The napkin they were wearing when they visited the outpatient clinic, which was on average day 2.8 ± 0.86 of their menstrual period, was used for the study. In 5 of 40 participants, menstrual blood was collected after at least one dose of mRNA vaccination (Comirnaty; Pfizer, New York, NY, USA). In this study, we used commercially available sanitary napkins that the patient usually used. Once collected from the participants, the napkins were promptly brought to the laboratory. A 5 ml syringe was used to collect menstrual blood (Figure 2a). The blood-stained area on the napkin was aspirated with a syringe in several places. The blood collected in the syringe was transferred into 300- μ l tubes (Figure 1b) and 20 μ l were used for each manufacturer's antibody testing. Excess blood was frozen and stored. In this study, antibody test kits from four companies were used. For this study, the maximum volume of menstrual blood collected was set as 980 μ l, consisting of three 300- μ l tubes and 80 μ l for four antibody test kits.

B) Napkin visual score evaluation

In this study, we used the area of blood that had adhered to the napkin as a guide for evaluating the possibility of blood collection from the napkin. Specifically, referring to the previous report by Magnay et al., the percentage of blood on the napkin was assessed in 20% increments in this study¹⁸. Figure 2 shows the classification of napkins based on the visual napkin score (VNS), with level 1 being the lowest percentage of blood on the napkin (0–20%) and level 5 being the highest category (80–100%). VNS was based on the percentage of the sanitary napkin with blood according to the consensus of two researchers.

C) Antibody testing with multiple immunochromatographic kits

We used four different antibody testing kits A–D. Kit A is the One Step Novel Coronavirus (COVID-19) IgM/IgG Test Kit (Artron Laboratories, Burnaby, Canada) (<http://www.artronlab.com/products/IFU/A03-51-322%20COVID-19AbIFU.pdf>). Kit B is the 2019-nCov IgG/IgM Detection Kit (Shionogi & Co., Osaka, Japan; Vazyme Medical Technology, China) (https://mtendoscopy.com/wp-content/uploads/2020/04/Vazyme_2019-nCoV-IgG-IgM-Detection-Kit-Instruction

-for-Use04.28.20-Final.pdf). Kit C is the Lepu Medical SARS-CoV-2 Antibody Test (Lepu Medical Technology, Beijing, China) (<http://www.artronlab.com/products/IFU/A03-51-322%20COVID-19AbIFU.pdf>). Kit D is the Cellspect COVID-19 IgG LF/RCGLF011 kit (Cellspect, Iwate, Japan) (https://9ed3a4c3-7463-4090-8d0a-9691c6a1c873.filesusr.com/ugd/ab41e7_c02b2d0a28f648bc9e76ab1cb5f525cb.pdf). Kits A–C detect both IgM and IgG against SARS-CoV-2, while Kit D detects only IgG. For all kits, 20 µl of menstrual blood were used (Figure 2c). At 15 minutes after the addition of the buffer from each kit, the control line was checked and the IgG and IgM lines were visually checked to determine whether the result was positive or negative (Figure 2D). The datasheet from each manufacturer was used as a reference to determine whether each antibody test kit recognizes N protein or S protein. The antigens, sensitivity, and specificity of each kit are summarized in Table 4, based on datasheets on the manufacturer's website. The antigens for Kits A and B were not disclosed. Initially, three different antibody test kits (Kits A, B, and C) were used, but starting from Patient 12, Kit D was also added. When the amount of collected menstrual blood was insufficient for testing with all kits, Kit A was used.

D) Statistical analysis

The two-sample *t*-test or the multiple comparisons test was performed with SPSS version 25.0 (IBM Corp, Armonk, NY, USA). The Kruskal-Wallis test was used as a nonparametric test. The Dann-Bonferroni test was used for subsequent multiple comparisons. $P < 0.05$ was determined to be statistically significant.

E) Ethical considerations

After obtaining approval from our institutional review board (approval number 2412, March 2020), written informed consent was obtained from all participants who participated in the study.

Declarations

Author contributions

H.S. conceived the concept of this study, conducted most of the research, wrote the manuscript, created the figures and tables, and oversaw this study. Y.K., A.F., W.S., and K.T. were responsible for the collection and processing of research materials and analysis of experimental data. S.K. provided advice on research concepts, collected research materials, analyzed data, and created figures. E.S., M.G., and K.T. analyzed the research materials and assisted in preparing figures and tables. Y.T. made overall corrections to the manuscript and figures.

Competing interests

All authors have no conflicts of interest to disclose for this study.

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Tables

Table 1. Summary of 40 participants based on visual napkin score

	VNS 1	VNS 2	VNS 3	VNS 4	VNS 5	Total	
Number of participants (n)		8	9	8	8	7	40
Average menstrual blood volume collected (μl)	0	110±77 ^a	412±245 ^a		610±359 ^a	774±310 ^a	364±372
Antibody testing rate (%)	0	88.9	100	100	100	77.5	

^a indicates significant difference vs. VNS 1.

VNS, visual napkin score; Antibody testing rate, percentage of participants whose menstrual blood could be with at least one antibody test kit.

Table 2. Summary of antibody test results with menstrual blood

Antibody kit	Number of participants tested	Control positive (%)	IgG positive (%)	IgM positive (%)
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Kit A	31	31(100)	5 (16.1)	1 (3.2)
Kit B	30	30 (100)	0 (0)	0 (0)
Kit C	30	30 (100)	5 (16.7)	3 (10.0)
Kit D	21	21 (100)	1 (4.8)	-

Vaccination status

Unvaccinated	26	26 (100)	0 (0)	0
Vaccinated	5	5 (100)	5(100)	3 (60.0)

Kit A, Antron One Step Novel Coronavirus (COVID-19) IgM/IgG Test Kit; Kit B, Shionogi IgG/IgM Antibody-test Kit for COVID-19; Kit C, Lepu Medical SARS-CoV-2 Antibody Test; Kit D, Cellspect COVID-19 IgG LF/RCGLF011.

Table 3. Antibody test results from each kit in vaccinated patients

Kit	Patient	1	2	3	4	5
Kit A, IgG/IgM		+/-	+/+	+/-	+/-	+/-
Kit B, IgG/IgM		-/-	-/-	-/-	-/-	-/-
Kit C, IgG/IgM		+/+	+/+	+/+	+/-	+/-
Kit D, IgG		-	-	+	-	-
Days since vaccination		13	16	9	48	21
mRNA vaccine dose number		2	1	1	2	2

Kit A, Antron One Step Novel Coronavirus (COVID-19) IgM/IgG Test Kit; Kit B, Shionogi IgG/IgM Antibody-test Kit for COVID-19; Kit C, Lepu Medical SARS-CoV-2 Antibody Test; Kit D, Cellspect COVID-19 IgG LF/RCGLF011.

Table 4. Antibody type, antigen, sensitivity, and specificity of each kit according to the datasheet

Kit	Detectable antibody type	Antigen	Sensitivity (%)	Specificity (%)
A	IgM, IgG	ND	97.2	97.9

B	IgM, IgG	ND	91.5	97.0
C	IgM, IgG	S protein	98.9	97.6
D	IgG	N protein	87.0	100

Kit A, Antron One Step Novel Coronavirus (COVID-19) IgM/IgG Test Kit; Kit B, Shionogi IgG/IgM Antibody-test Kit for COVID-19; Kit C, Lepu Medical SARS-CoV-2 Antibody Test; Kit D, Cellspect COVID-19 IgG LF/RCGLF011; S protein, spike protein; N protein, nucleoside capsid protein; ND, not disclosed.

Figures

Figure 1

The visual napkin score was based on percentage of the area on the sanitary napkin with blood. Menstrual blood area percentage of approximately 0–20% was classified as level 1, approximately 20–40% as level 2, approximately 40–60% as level 3, approximately 60–80% as level 4, and approximately 80–100% as level 5.

Figure 2

A) Aspiration of menstrual blood from a napkin with a 5-ml syringe. B) Menstrual blood collected in 300- μ l tubes. C) Use of an antibody testing kit with menstrual blood. D) Results of the four antibody testing kits in a participant who has received mRNA vaccination.