A Pilot study screening bank currency in community circulation for potential carriage of SARS-CoV-2: how safe handling the currency is?

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Abstract

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Purpose: Currency seems to represent an important vehicle for transmission of pathogenic microorganisms, thus a potential in affecting the public health. Digital transactions are more common in developed countries; however, handling cash currency is still very common in many countries including Bahrain. A recent study from Australia reported that SARS-CoV-2 may survive for 28 days on smooth surfaces (including banknotes), however the study was purely experimental and done in controlled laboratory environment. There were worldwide speculations suggesting the possible transmission of SARS-CoV-2 infection through currency notes. A recent study from Bangladesh reported presence of SARS-CoV-2 in approximately 7% of currency samples collected from the community. No such study has been performed on Bahraini currency, so the present study was proposed to screen the cash currency in circulation in Bahrain for the possible presence of SARS-CoV-2.

Method: We collected notes and coins of Bahraini currency and a few of Saudi riyals from different public sources of currency exchange. Two time points were selected for collection when percentage of tested positive cases was on its peak. All the samples were tested for the presence of SARS-CoV-2 antigen with rapid antigen detection kit and qRT-PCR method. For initial validation, 5 samples each of potential SARS-CoV-2 RNAs extracted from known positive cases and 5 samples of extracted RNA from known negative persons were tested by rapid antigen detection kit and qRT-PCR. The results of qRT-PCR were interpreted as per the interpretation chart provided by the supplier.

Results: During the study period, a total of 250 currency samples were collected including different denominations of currency notes and coins. Majority of the currency samples were collected from grocery stores followed by food outlets. Following validation, the currency samples were tested, and all turned negative for SARS-CoV-2 by rapid antigen detection test as well as by RT-PCR.

Conclusion: This study predicts on Bahraini currency posing no threat of SARS-CoV-2 transmission via currency exchange.

Keywords: Currency; SARS-CoV-2 carriage; safety; Bahrain

Introduction

Despite of passage of more than one and a half year since the start of COVID-19, the exact role of fomites (including the cash currency) in transmitting SARS-CoV-2 infection is not yet fully determined (Riddell, Goldie, Hill, Eagles, & Drew, 2020). Not only the advancing technology but also the emergence of current pandemic of COVID-19 has amplified the use of cashless mode of transactions such as use of online IT applications for money transfer or increased use of ATM/Credit Cards in day-to-day purchases by the public. Digital transactions are more common in developed countries; however handling cash currency is still very common in many countries especially in middle- and lower-income countries. Bahrain being a developed nation, cashless transactions (through either mobile applications or through ATM/Credit Cards) are quite common. However, owing to significant proportion of expatriate population working as low-income workers, cash transactions are also performed in significant proportion especially with lower currency denominations.

Though the number of cases per day were static for quite some time in Bahrain, but recently, at the time of performing this study, a surge in cases has been noticed with cases even reaching >3000/day in May, 2021, with a positivity rate of ~15% in the daily tested samples (Worldometer, 2021). Back in the early past year when the cases of SARS-CoV-2 were increasing drastically in Chinese territory (and the pandemic was not yet declared), China started decontaminating its paper currency stipulating it to be a potential source of transmission, following which, some other countries also quarantined their bank notes (https://www.thehindubusinessline.com/news/science/coronavirus-can-persist-for-four-weeks-on-paper-currency-says-research/article32831163.ece). In the queue, the Central bank of Bahrain on 23rd March 2020 passed directives to disinfect all the incoming currency notes to limit the risk of SARS-CoV-2 transmission. As per the directives, money changers were required to disinfect the currency notes with ultraviolet radiations or by isolation of currency notes for 72 hours (https://www.cbb.gov.bh/media-center/cbb-instructs-money-changers-to-disinfect-currency-notes-and-wholesale-imported-notes-to-curb-the-spread-of-the-coronavirus-covid-19). However, in October 2020, a study from Australia reported that SARS-CoV-2 may survive for 28 days on smooth surfaces (such as glass of the mobile phones and plastic bank notes) at lower temperature (Riddell et al., 2020). On the contrary, Bank of England after commissioning a research reported that handling currency notes poses low risk of spreading SARS-CoV-2 (https://www.theguardian.com/world/2020/nov/24/bank-notes-pose-low-risk-of-spreading-covid-19). These studies looked for the survival of SARS-CoV-2 under strict laboratory environment after seeding the currency notes with virus in controlled conditions and repeatedly testing over time looking for the survival of virus particles (Harbourt et al., 2020; Riddell et al., 2020).

Despite the fear and speculations, to the best of our knowledge, there is scarcity of studies looking for the carriage of SARS-CoV-2 on the surface of currency in circulation in community. Only a recent study from Bangladesh reported presence of SARS-CoV-2 in approximately 7% of currency samples collected from the community (Akter et al.,

2021). Therefore, the present study was performed to screen the Bahraini currency in circulation in the community for any potential carriage of SARS-CoV-2 by screening by Rapid Antigen Detection Test (RADT) and Real-time PCR (RT-PCR).

Materials and Methods

Place of study

The study was performed in the Department of Microbiology, Immunology, and Infectious Diseases, College of Medicine and Medical Sciences of Arabian Gulf University, Kingdom of Bahrain from 19/02/2021 to 01/07/2021. Ethical clearance from the Institutional Ethical Committee was obtained vide project no. E036-PI-1/21.

Currency collection

Different denominations of Bahraini currency notes viz. BHD 10, BHD 5, BHD 1, and BHD 0.5, and a few coins of 100 and 50 fils, which were in community circulation, were collected for the study. Moreover, a few Saudi riyals, as Saudi currency is accepted in Bahraini market, were also collected. The currency collections were through the help of volunteers who volunteered to participate in the study. Currency denominations from BHD 0.5 through BHD 10 are paper notes, however 100 and 50 fils are smaller denomination as a metal coin (1 BHD = 1000 fils). The currency samples were collected at two different "time-points", i.e., from 19/02/2021 to 17/03/2021 and 22/05/2021 to 01/07/2021, when obvious peaks of positive cases were noticed in Bahrain.

The currency samples were those received by the volunteer from any shopping outlets from their usual shopping and were put in small sterile plastic pouches. The samples were submitted to Department of Microbiology at an earliest but not beyond 48 hours of collection. A total of 250 different currency items were collected.

Processing of currency specimens

The currency samples were swabbed using sterile moistened swabs with TE buffer. The currency samples were swabbed simultaneously with 3 swabs taken together- one for antigen extraction for RADT, other for RNA extraction for molecular detection by RT-PCR, and the third for DNA extraction for metagenomics for Microbiome analysis. The extracted DNA from the third swab could not be subjected to metagenomic analyses due to financial constraints and thus were kept at -80 degrees for any possible analyses in the future.

Antigen detection by using Rapid Antigen Detection Kit

Possible presence of SARS-CoV-2 on the currency was looked for by testing for the presence of antigen by using commercial kit for SARS-CoV-2 manufactured by Abbott (Panbio™COVID-19 Ag Rapid Test Device). Manufacturer's protocol was followed for antigen detection. Briefly, the first swab (of the three collected) was placed into the extraction buffer provided in the kit, which was further squeezed and mixed to facilitate the antigen extraction. For pooled testing, five samples with 50µl from each were pooled into a separate extraction tube. The details of pooled samples were properly recorded. Subsequently, five drops of this pooled specimen were dispensed vertically into the specimen well on the device. Results were noted at 15 minutes.

Viral RNA Extraction

RNA from the second swab was extracted using the commercial extraction kits of Bio Basic Canada Inc and was stored at -80 degrees till tested further by RT-PCR. Manufacturer's protocol was followed for RNA extraction from viral transport medium (VTM), as the second swab was kept in VTM (Liofilchem Diagnostic®). We took 200 µl of VTM and processed for EZ0-10 Spin Column method of COVID-19 viral RNA extraction. Initially, we extracted 50 VTM samples individually, but when we got continuous negative results in PCR, we opted for pooling five VTM samples for simultaneous extraction into a 1.5 ml microcentrifuge tube with 100 µl from each sample. From this pool of samples, the same method was followed as mentioned above and samples were eluted with 50 µl of nuclease-free water. The details of pooled samples were recorded so that in case of positivity they may be tested individually.

Real Time PCR

A one-step RT-PCR was carried out with SARS-CoV-2 RT-PCR Detection kit of Bio Basic Canada Inc. The cut off Ct value as per the kit was (Ct) <37. RT-PCR was done individually in first 50 samples, but later after having continuous negative results in RT-PCR, the testing was done with 5-fold pooled samples and with a positive and a negative control on QIAGEN Rotor-Gene thermocycler machine. Interpretation was done with the help of chart given in the product information leaflet for the probe sets of 2019-nCoV N1 and 2019-nCoV N2 nucleocapsid genes (Table 1).

Table 1. Interpretation chart of RT-PCR results

2019-nCoV N1	2019-nCoV N2	IPC	Interpretation
+	+	+	Positive
+	-	+	Positive
-	+	+	Positive
-	-	+	Negative
-	-	-	Invalid

Exclusion Criteria

Since our aim was to find out the possibility of presence of SARS-CoV-2 on currency in circulation among general community, currency collected from the hospital or any other healthcare settings involved in covid consultation were excluded due to safety reasons of the volunteers involved. Similarly, currency from the person who recently had COVID-19 infection or who was in contact of COVID-19 patient was excluded. Also, the currency from persons having any flu like symptoms was not included.

Results and Discussion

Currency Samples, Source of Collection and Collection Time

During the study period, a total of 250 currency samples were collected including different denominations of currency notes and coins. The details of the collected currency samples are presented in figure 1. These samples were collected from different shopping outlets including street food outlets, grocery stores, petrol pumps, general community pharmacy outlets, school, laundry, and primary health center (See Table 2). Samples were collected at two points of time when there was an apparent peak in positive cases. The details are presented in Figures 2 & 3.

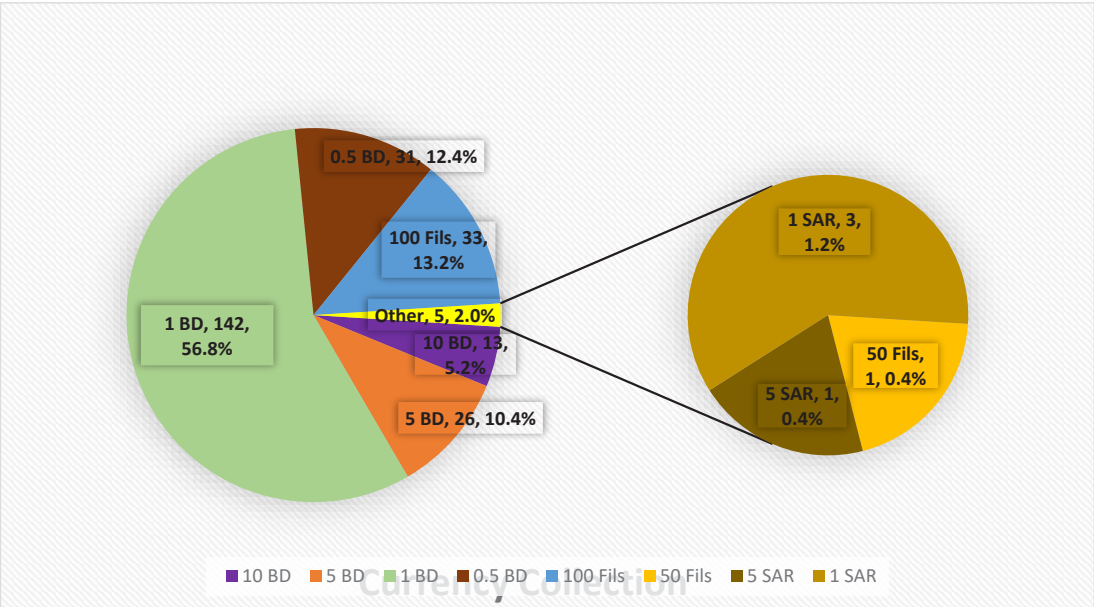

Figure 1. Chart showing the details of each denomination with collected numbers and percentage

Table 2. Sources of currency collection with respective currency denominations.

Source of Collection↓	Currency Denominations								TOTAL
	10 BD	5 BD	1 BD	0.5 BD	100 FILS	50 FILS	5 SAR	1 SAR	
Grocery	6	12	81	9	7	1	0	3	**119**
Food outlets	4	7	33	21	16		1	0	**82**
Petrol pumps	2	6	14	1	0	0	0	0	**23**
School	0	1	8	0	3	0	0	0	**12**
Pharmacy	1	0	0	0	7	0	0	0	**8**
Laundry	0	0	4	0	0	0	0	0	**4**
Hospital*	0	0	2	0	0	0	0	0	**2**
TOTAL	**13**	**26**	**142**	**31**	**33**	**1**	**1**	**3**	**250**

* Primary Health Centre involved in consulting covid patients were not included

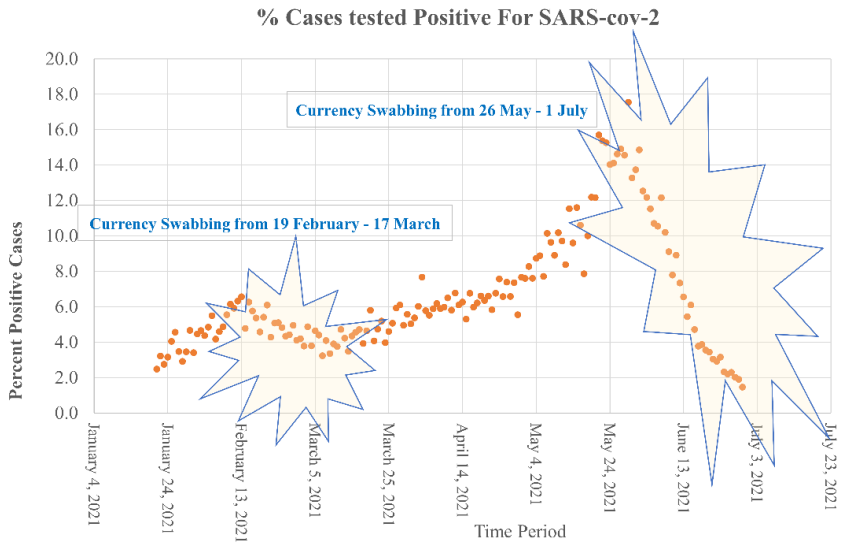

Figure 2. Chart shows the time slots of currency collections.

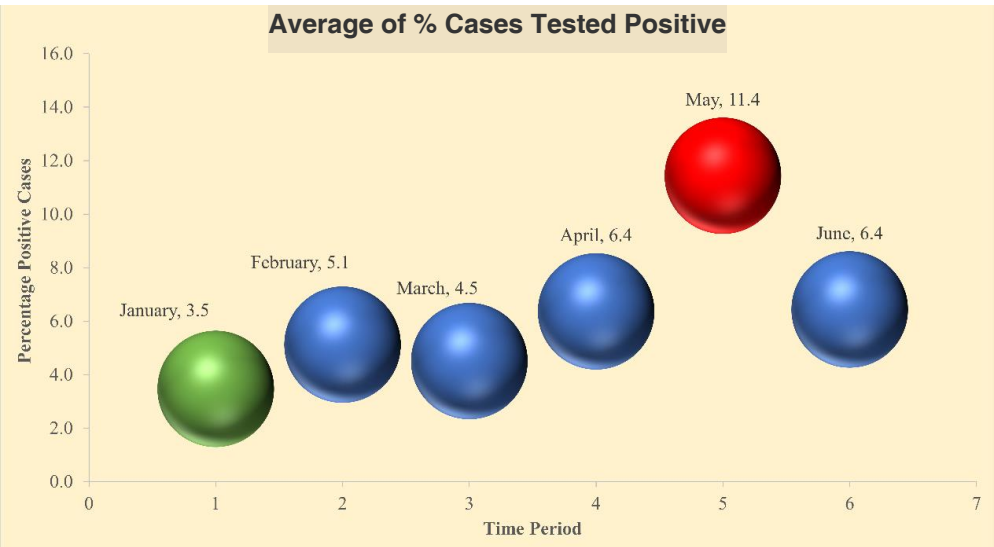

Figure 3. Chart shows the average percentage of positive cases monthly during the study period.

Results of RADT

Currency samples were tested with commercial rapid antigen test device as described above. Rapid Ag test device is a lateral flow assay widely used as a fast and effective test to screen SARS CoV-2 with nasal swab as a point of care testing (POCT) tool(Salcedo, Harmon, & Herrera, 2021). We screened our samples with swabs rubbed on currency bills after soaking with TE buffer. Initially, testing was carried out with individual samples but when we repeatedly got negative results, we opted for pooling five samples into a 1.5 ml tube. Pooling of 5-fold to 20-fold swabs for RADT is already proved authentic by Berking et al (Berking et al., 2021) and Salcedo et al(Salcedo et al., 2021). All the currency samples were found negative by rapid antigen detection kit. Validation of RADT was based on appearance of control line in the individual test device.

Validation of qRT-PCR

Although commercial kits are already approved by different organizations but that doesn't mean the test is valid for all applications. Especially when you are dealing with the fomites unlike swabbing humans. Moreover, there are several factors which can alter the assay's performance. To determine the performance characteristics, we carried out validation tests with some positive and negative samples. To check the validity of our qRT-PCR method, we obtained 5 extracted RNA samples of known positive cases and 5 samples of known negative cases from a diagnostic laboratory. These samples were subjected to real time PCR for amplification. The RT-PCR assay kit uses three probe sets for SARS CoV-2 detection. Two of the probe sets are for the detection of SARS CoV-2 nucleocapsid genes N1 and N2 and the third probe set is used for internal positive control (IPC) detection. Results are shown in Table 3.

Table 3. Validation report of RT-PCR method.

S. no.	Color of Peak	Sample*	Target Probe			Interpretation
			N1	N2	IPC	
1		S1	+	+	+	Positive
2		S2	+	+	+	Positive
3		S3	+	+	+	Positive
4		S4	+	+	+	Positive
5		S5	+	+	+	Positive
6		S6	-	-	+	Negative
7		S7	-	-	+	Negative
8		S8	-	-	+	Negative
9		S9	-	-	+	Negative
10		S10	-	-	+	Negative
11		Positive CTRL	+	+	+	Positive
12		Negative CTRL	-	-	+	Negative

* S1-S5 were extracted RNA of known positive samples
S6-S10 were extracted RNA from known negative samples

The two target probes i.e., 2019-nCoV N1 and 2019-nCoV N2, tested positive against all known positive RNA samples while no negative sample showed any amplification for the same. IPC probe set showed good amplification for all PCR reactions (Figures 4-6).

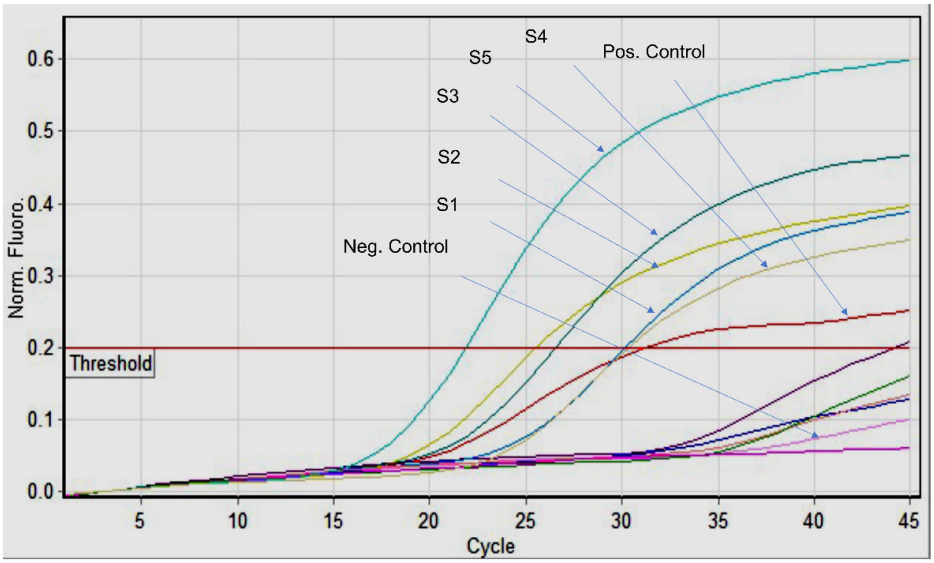

Figure 4. RT-PCR validation graph for target probe 2019-nCoV N1

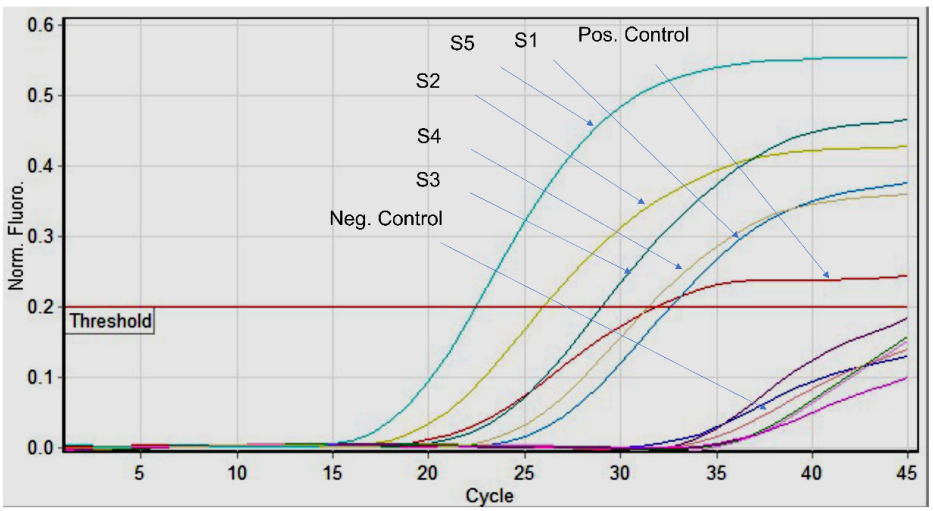

Figure 5. RT-PCR validation graph for target probe 2019-nCoV N2

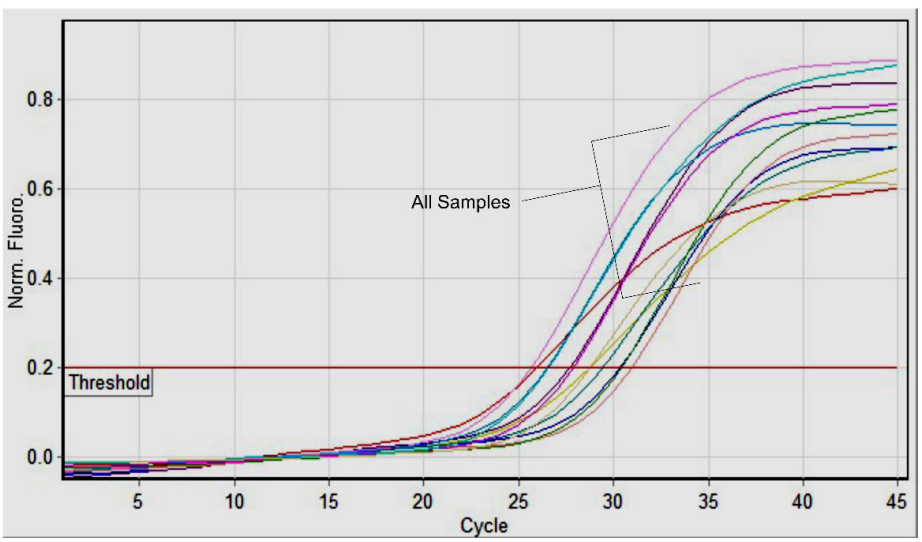

Figure 6. RT-PCR validation graph for target probe IPC

Results of RT-PCR

RNA from all 250 samples was extracted following all precautions under aseptic conditions. Having tested 50 samples negative, we applied the strategy of pooling samples in 5-fold quantity and amplified with qRT-PCR targeting the three probe sets as described previously. The customized and resource-saving concept of pooling samples is an efficient, cost effective and time saving approach. Pooling of 4/5 samples is recommended by many researchers,(Garg et al., 2021; Mulu et al., 2021; Sami, Perween, Khan, & Khan, 2021). The cycle threshold value was considered as an indicator of amplification. Although, the cycle threshold value (Ct) <37 as positive and Ct >37 as negative was proposed by the manufacturer in product information leaflet but in pooling approach, the Ct value is found to be higher with pooled samples corresponding to deconvoluted samples(Lopez-Lopes et al., 2020; Sami et al., 2021). Sami et al found a mean difference of 0.96 in cycle threshold (Ct) of pooled and deconvoluted positive samples(Sami et al., 2021). Nonetheless, there are other studies which found a Δ Ct higher than this(Garg et al., 2021). Therefore, we considered cut off (Ct) <40 to be reasonable for deconvolution. And any pooled group indicating a rise in peak beyond (Ct) >37 and (Ct) <40 was deconvoluted to test individually.

In our currency samples, the Ct values for qRT-PCR reactions remained (Ct) >40 resulting negative results (Figures 7-9). These findings suggest that no SARS CoV-2 was carried by these currency notes and coins tested.

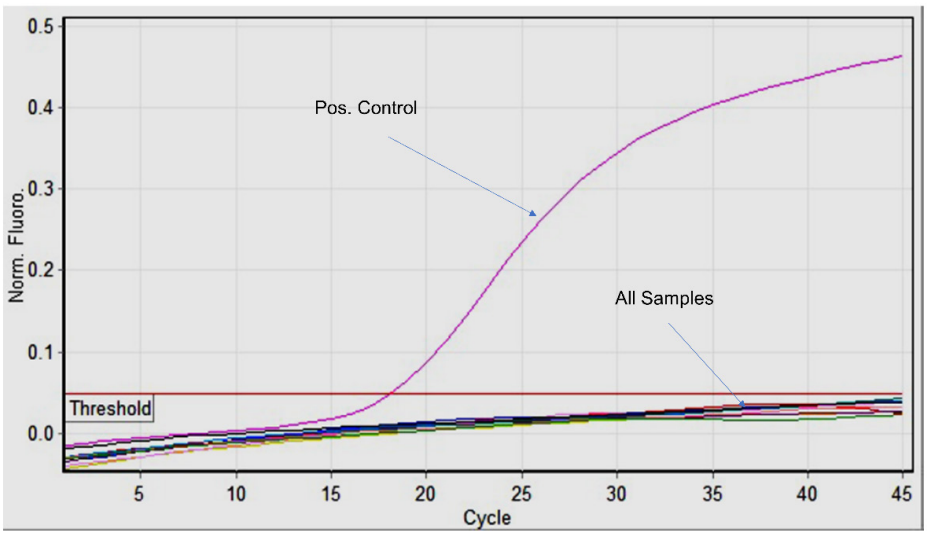

Figure 7. RT-PCR graph showing results for target probe 2019-nCoV N1

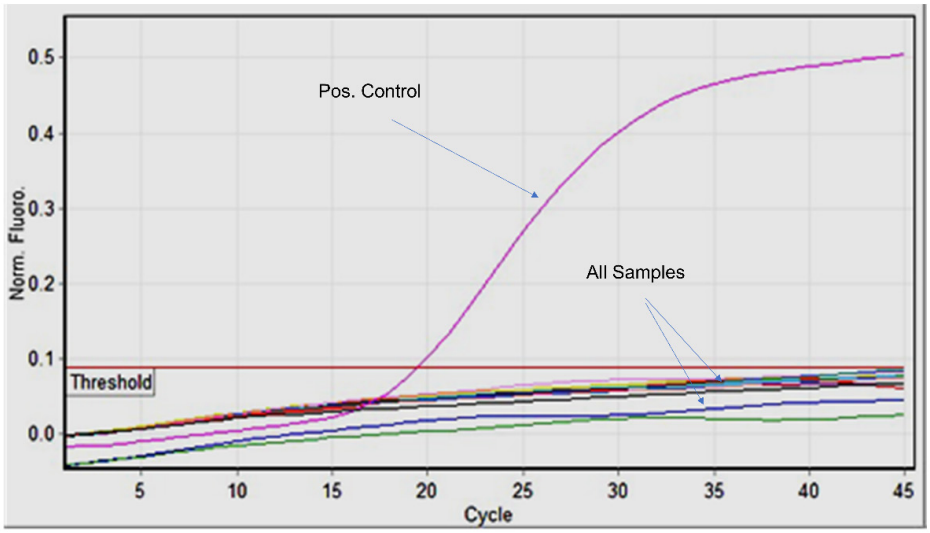

Figure 8. RT-PCR graph showing results for target probe 2019-nCoV N2

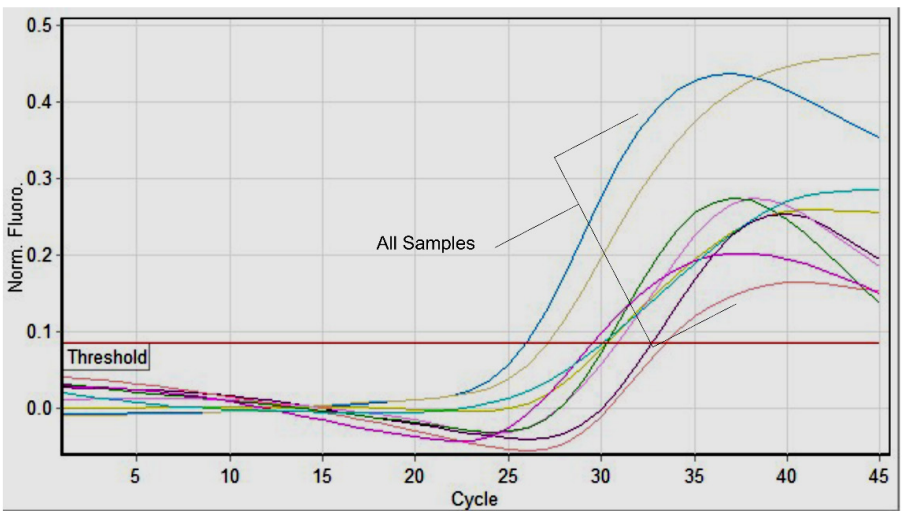

Figure 9. RT-PCR graph showing results for target probe IPC

Conclusion

Presence of virus particles on fomites like currency notes may affect the epidemiology of SARS CoV-2, exaggerating the current situation and posing a threat to healthy individuals. However, there is a paucity of research studies focusing on survival kinetics, especially on transmission dynamics of SARS CoV-2 particles on currency notes. Our study is an addition to throw further lights on transmission dynamics and suggest that there are less chances of transmission of COVID-19 infection via currency in general, and by Bahraini currency in particular. However, a recent study on Bangladeshi bank notes demonstrated presence of SARS-CoV-2 RNA on 7.29% (31/425) currency samples (Akter et al., 2021). It had been speculated that microbes may be transmitted through fomites, including bank notes and that the stability or survival of these microbes depend on numerous factors such as environment, temperature(Riddell et al., 2020), moisture, currency material and initial microbial load (Akter et al., 2021).

Said that, non-detection of SARS-CoV-2 in our collection could be affected because of these reasons, especially the Bahrain climate having high temperature and the material composition of Bahraini and Saudi currency.

Limitations of the Study

Obviously, there were limitations in our study. It was a pilot study done only on 250 currency samples and larger sample size may be required in future studies. Furthermore, samples were obviously collected from clean areas due to safety reasons of the volunteers. Hospital premises dealing with COVID-19 patients were not explored so these hospital and large number of pharmacies are to be included in future studies. Furthermore, relatives and friends who were in touch with patients should have been included for currency collection.

Safety Note

All the procedures, including swabbing, antigen and genome extraction and testing for antigen by rapid kit and RT-PCR reagent preparation was done following strict precautions under biosafety cabinet in a BSL-2 level laboratory.

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Authorship contribution

Mohammad Shahid:	Conceptualization, fund Acquisition, project supervision, manuscript writing, formal analysis, data curation, review and editing
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Ali Al-Mahmeed:	Investigation, validation, manuscript writing, formal analysis, data curation, review, and writing
Mohd. Shadab:	Investigation, validation, manuscript writing, formal analysis, data curation, review, and writing
Ahmad Ramadan:	Molecular technical support, review and writing

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دراسة تجريبية على العملات البنكية المتداولة في المجتمع للكشف عن النقل المحتمل لفيروس سارس- كوفيد-2: إلى أي درجة يعد تداول العملات آمن؟

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المُستَخلَص

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هدف الدراسة: يمكن أن تمثل العملات البنكية وسيلة مهمة لنقل الجراثيم الممرضة، وبالتالي فهي لها أهمية عالية في التأثير على الصحة العامة للمجتمع. إن التعامل الرقمي للعملات منتشر بشكل واسع في الدول المتقدمة، ومع ذلك فما زال التداول بالعملات البنكية منتشر في العديد من الدول بما في تلك الدول مملكة البحرين. ولقد تبين من خلال دراسة حديثة أجريت في أستراليا، أن سارس- كوفيد2- يمكنه أن يبقى نشطا على الأسطح الملساء (بما في ذلك العملات البنكية) لمدة 28 يوما، ومع ذلك فهذه الدراسة عبارة عن تجارب علمية بحتة أجريت داخل مختبرات مهيئة بشكل عالي. وهناك تكهنات كثيرة حول العالم حول إمكانية انتقال عدوى سارس- كوفيد2- من خلال التعامل بالأوراق النقدية. وأظهرت دراسة حديثة أجريت في بنغلاديش، تواجد سارس- كوفيد2- فيما يقارب 7% من عينات الأوراق النقدية الممتحصلة من المجتمع. لا توجد دراسة مشابهة تم إجراءها على العملات البنكية في مملكة البحرين، لذلك تم اقتراح هذه الدراسة لفحص العملات النقدية المتداولة في البحرين للكشف عن امكانية تواجد سارس- كوفيد2-.

منهج الدراسة: لقد قمنا بجمع عينات من العملات الورقية والمعدنية البحرينية وبعض العملات الورقية السعودية (مجموع ما تم جمعه 250عينة من العملات) من مصادر عامة مختلفة لمواقع تبادل العملات. ولقد تم اختيار آلية مضاعفة لجمع العينات عندما تكون النسبة المئوية للحالات الإيجابية المفحوصة ذات قمة مرتفعة. لقد تم فحص كل العينات للكشف عن وجود SARS-CoV-2 بالكشف عن مولدات المضادات (Antigens) وذلك باستخدام الفحص السريع لمولادات الأجسام وكذلك باستخدام تقنية تفاعل البلمرة المتسلسل المتزامن الكمي (qRT-PCR). ولتقييم مدي دقة التقنيات المستخدمة في الكشف عن تواجد SARS-CoV-2 فقد تم فحص 5 عينات لحمض رابوزومي نووي لكوفيد2- (CoV-2 RNAs) مستخلصة من حالات إيجابية معرفة مسبقا، و5 عينات لحالات سلبية معرفة مسبقا. لقد تم الإعتماد على قوائم بيانات التعريف المرفقة بأدوات الكشف من قبل الشركة المصنعة.

النتائج: خلال فترة الدراسة، تم جمع 250 عينة للعملات البنكية والتي تتضمن فئات مختلفة من العملات الورقية والمعدنية. كانت غالبية عينات العملات تم جمعها من محلات البقالة وتليها منافذ بيع الطعام. وبعد إتمام عملية تقييم التقنيات، تم فحص جميع العينات لفيروس SARS-CoV-2 باستخدام تقنية الفحص السريع للكشف عن مولدات المضادات للأجسام (Antigens) وكذلك باستخدام تقنية تفاعل البلمرة المتسلسل المتزامن الكمي (qRT-PCR). والتي جاءت كلها بنتيجة سلبية.

الخلاصة: هذه الدراسة تتوقع أن العملات البحرينية لا تظهر أي خطر لانتقال عدوى SARS-CoV-2 من خلال تبادل هذه العملات.

مفاتيح الكلمات: العملات، حامل لفيروس SARS-CoV-2، سلامة، البحرين.