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TRANSFUSION

Testing for the presence of SARS-CoV-2 RNA in presymptomatic blood donors

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is responsible for the COVID-19 pandemic. The breadth of symptoms greatly varies in SARS-CoV-2–infected individuals, some may be asymptomatic, while other may experience a combination of cough, fever, and breathing difficulties associated with pneumonia in both lungs.[1] In addition, symptoms can take up to 14 days to appear after exposure to SARS-CoV-2.[1] SARS-CoV-2 RNA can initially be detected in the upper respiratory tract, 1 to 2 days before the onset of symptoms. The virus can persist for 7 to 12 days in moderate cases and up to 2 weeks in severe cases.[1] Furthermore, patients with few or no symptoms have detectable levels of viral RNA in the oropharynx highlighting the potential for virus transmission during the incubation period.[2] SARS-Cov-2 RNA can also be detected in the blood of a small proportion of people with COVID-19[1,3,4] but the presence of SARS-CoV-2 RNA and infectious virions in blood during the presymptomatic period of infection remains to be established. At Héma-Québec, from the start of the pandemic, we implemented precautionary measures to reduce the theoretical risk of transmission of SARS-CoV-2 by blood transfusion. Among those measures, all donations from donors who reported symptoms related to COVID-19 in the next 3 days after their donation were recalled from the hospital or withdrawn from the inventory, whether or not a diagnosis of COVID-19 was confirmed at that time. If the COVID-19 diagnosis was confirmed, the implicated units were included in this study. For some other donations, Héma-Québec was directly alerted by public health authorities after a confirmed COVID-19 diagnosis. Again, such products were

TABLE 1 Characteristics of donors identified as COVID-19 after donation and results of SARS-CoV-2 RNA testing

Donor	Type of donation	Date of donation	Date of symptom onset	Nature of the symptoms	Date of COVID-19 diagnosis	Results of SARS-CoV-2 RNA test in the donation sample	Results of viral culture
1	Plasma	March 4, 2020	March 11, 2020	Cough, fever	March 27, 2020	Negative	NA
	Plasma	March 10, 2020				Positive[a]	Negative
2	Plasma	March 14, 2020	March 15, 2020	Headache, muscle pain, fever	March 19, 2020	Negative	NA
3	Plasma	March 17, 2020	March 14, 2020	Headache, runny nose, diarrhea, loss of smell	March 27, 2020	Negative	NA
4	RBCs	May 5, 2020	May 6, 2020	Fever, muscle pain, headache, diarrhea	May 8, 2020	Negative	NA
	Plasma					Negative	NA
5	Plasma	April 25, 2020	April 25, 2020	Cough, breathing difficulties, muscle pain, general weakness	May 4, 2020	Negative	NA
6	Plasma	May 11, 2020	May 14, 2020	Fever, dry cough, muscle pain, breathing difficulties, loss of smell, dizziness	May 16, 2020	Negative	NA

[a]Sample found positive by two of four laboratories.

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TABLE 2 Primers and probes

Laboratories	Primers and probes sequences	
Centre de Recherche du CHUM-Université de Montréal	SARS-CoV-2 gene E	
	E_Sarbeco_F1	5′ ACA GGT ACG TTA ATA GTT AAT AGC GT 3′
	E_Sarbeco_R2	5′ ATA TTG CAG CAG TAC GCA CAC A 3′
	E_Sarbeco_P1	5′ ACA CTA GCC ATC CTT ACT GCG CTT CG 3′(FAM-ZEN-IABQ)
	SARS-CoV-2 gene N	
	WuhanCoVN_F	5′ AAC CAG AAT GGA GAA CGC AGT G 3′
	WuhanCoVN_	5′ CGG TGA ACC AAG ACG CAG TAT TAT 3′
	CoVN_P	5′ CGA TCA AAA CAA CGT CGG CCC CAA GGT TTA C 3′ (FAM-ZEN-IABQ)
Centre de Recherche du CHU de Québec-Université Laval	SARS-CoV-2 gene N	
	F	5′ TAATCAGACAAGGAACTGATTA 3′
	R	5′ CGAAGGTGTGACTTCCATG-3′
	P	5′ GCAAATTGTGCAATTTGCGG-3′ (FAM-ZEN-IBFQ)
Plateforme RNomique-Université de Sherbrooke	SARS-CoV-2 gene E	
	E_Sarbeco_F1	5′ ACA GGT ACG TTA ATA GTT AAT AGC GT 3′
	E_Sarbeco_R2	5′ ATA TTG CAG CAG TAC GCA CAC A 3′
	E_Sarbeco_P1	5′ ACA CTA GCC ATC CTT ACT GCG CTT CG 3′ (FAM-ZEN-IABQ)
	SARS-CoV-2 gene N	
	WuhanCoVN_F	5′ AAC CAG AAT GGA GAA CGC AGT G 3′
	WuhanCoVN_R	5′ CGG TGA ACC AAG ACG CAG TAT TAT 3′
	CoVN_P	5′ CGA TCA AAA CAA CGT CGG CCC CAA GGT TTA C 3′ (FAM- ZEN-IABQ1)
Laboratoire de Santé Publique du Québec	SARS-CoV-2 gene E	
	E_Sarbeco_F1	5′ ACA GGT ACG TTA ATA GTT AAT AGC GT 3′
	E_Sarbeco_R2	5′ ATA TTG CAG CAG TAC GCA CAC A 3′
	E_Sarbeco_P1	5′ ACA CTA GCC ATC CTT ACT GCG CTT CG 3′ (FAM-ZEN-IABQ1)
	SARS-CoV-2 gene N	
	WuhanCoVN_F	5′AAC CAG AAT GGA GAA CGC AGT G 3′
	WuhanCoVN_R	5′CGG TGA ACC AAG ACG CAG TAT TAT 3′
	CoVN_P	5′CGA TCA AAA CAA CGT CGG CCC CAA GGT TTA C 3′ (FAM/ZEN/-IBFQ)

recalled and included in this study if the symptoms had started no later than 3 days after donation. As of March 10, 2020, six donors have been identified as confirmed cases of COVID-19 after donating blood (Table 1). One of the donations was a whole blood donation which was converted into a red blood cell (RBC) and a plasma component and the other five donations were for plasma intended for fractionation. None of the components from these donors were transfused. In one of the six cases (Donor 3), upon questioning the donor, it was established that mild symptoms of COVID-19 were present at the time of donation but disregarded by the donor at the time as nonsignificant. All donors had a mild to moderate infection and none were hospitalized. Samples from these donations were sent to four independent laboratories in order to test for SARS-CoV-2 viral RNA. Viral RNA extraction was performed using a commercial viral DNA purification kit (QIAamp Viral RNA Mini Kit, Qiagen, Toronto, Ontario, Canada) and real-time reverse transcription polymerase chain reaction (RT-PCR) assays targeting the N regions of the viral genome was performed by all of the laboratories. Three of four laboratories added a real-time RT-PCR assays targeting, in parallel, the N and E regions of the viral genome to increase the

specificity of the assay. The primer and probe sequences, used by the four laboratories, are listed in Table 2. Among the six samples tested, only one was found to be weakly positive for the E gene (36.1 cycle threshold; cycle threshold of a positive result was ≤40) by two of the four testing centers (Table 1). This was a plasma donation dedicated to fractionation. This discrepancy among the testing laboratories was related to the volume of plasma used to isolate the viral RNA. Indeed, by using a larger (up to 280 μL vs 140 μL) volume of plasma during the viral RNA isolation, these two laboratories were able to get a weak positive result by PCR. The infectivity of the SARS-CoV-2 found in this donation was evaluated using Vero E6 cell line[5] and was found to be noninfectious. A plasma donation given 6 days earlier by this same donor was also recalled, tested, and found to be negative for SARS-CoV-2 RNA. Interestingly, for the donor who had mild symptoms at the time of her donation, this latter was tested negative for the presence of SARS-CoV-2 RNA. All six donors were also tested for the presence of antibodies against SARS-CoV-2 and all were negative, confirming that they were in the early phase of infection. These results are consistent with those reported by Chang and colleagues from China,[4] where only four donors out of more than 7000 were found to be weakly positive for SARS-CoV-2 RNA in their blood.

In conclusion, our data indicate that SARS-CoV-2, like other respiratory viruses such as SARS-CoV and MERS-CoV, is present in very limited amount if not totally absent in blood products donated shortly before the onset of symptoms in infected individuals. Consequently, the risk of transmission of COVID-19 by blood transfusion, if it exists, would appear to be negligible.

CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

Lionel Loubaki[1,2]
Pierre Gantner[3]
Amélie Pagliuzza[3]
Hugues Fausther-Bovendo[4]
Gary Kobinger[4]
Nicolas Chomont[3]
Marc Germain[1]

[1]*Héma-Québec, Affaires médicales et Innovation, Québec, Québec, Canada*
[2]*Department of Biochemistry, Microbiology and Bioinformatics, Laval University, Québec, Québec, Canada*
[3]*Centre de recherche du CHUM, Axe Immunopathologie, Montréal, Québec, Canada*
[4]*Centre de recherche du CHU de Québec-Université Laval, Axe maladies infectieuses et immunitaires, Québec, Québec, Canada*

Correspondence
Lionel Loubaki, Héma-Québec,Affaires Médicales et Innovation, 1070 avenue des Sciences-de-la-Vie, Québec, QC G1V 5C3, Canada.
Email: lionel.loubaki@hema-quebec.qc.ca
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ORCID

Lionel Loubaki https://orcid.org/0000-0002-4744-5387

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Decrease in serum antibodies to SARS-CoV-2 in convalescent plasma donors over time

To the Editor

Convalescent plasma, collected from donors who have previously recovered from viral illnesses, has been used to treat patients with a variety of emerging infectious diseases. This product has become more prominent during the current COVID-19 pandemic, including with the recent emergency use authorization from the Food and Drug Administration, but the longevity of