This is an Accepted Manuscript for *Infection Control & Hospital Epidemiology* as part of the Cambridge Coronavirus Collection.
DOI: 10.1017/ice.2020.144

Article Type: Letter to the Editor

Environmental cleaning is effective for the eradication of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in contaminated hospital rooms: A patient from the Diamond Princess cruise ship

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Key words: SARS-CoV-2; 2019-nCoV; COVID-19; environmental cleaning; RT-PCR; infection control

To the Editor- Doctors, nurses, and other medical staff are greatly concerned about nosocomial outbreaks of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Environmental contamination is a possible source of nosocomial transmission [1, 2]. However, it is unclear how effective environmental cleaning is against SARS-CoV-2.

A 75-year-old man infected with SARS-CoV-2 was diagnosed as having COVID-19 during the quarantine period on the Diamond Princess cruise ship. He was transferred directly to our hospital on February 11, 2020. He resided in patient Room A for 2 days, then was moved to Room B, where he stayed for 19 days. After cleaning the rooms thoroughly with disinfectant (Rely^{+}On Virkon, LANXESS, or RUBYSTA in Japan), we tested a total of 15 areas that were in close contact with the patient and medical staff. Swabs were used to transfer five environmental samples from Room A and 10 samples from Room B to universal transport media (Copan, Murrieta, CA). Cleaning was conducted immediately after the patient left the rooms. Environmental sampling was conducted within 5 days and 30 min after the patient left Rooms A and B, respectively. Nucleic acids were extracted using MagMAX Viral/Pathogen Nucleic Acid Isolation Kit (ThermoFisher Scientific, Waltham, MA), and examined by real-time reverse transcription polymerase chain reaction (RT-PCR) targeting the nucleocapsid (*N*) gene of SARS-CoV-2. Seven sets of primers and probes (CDC-N1, CDC-N2, CDC-N3, YCH-N1, YCH-N2, NIID-N1, and NIID-N2) were used to detect SARS-CoV-2 as previously described (Supplemental Table 1) [3]. For the internal positive control, the human ribonuclease P 30 subunit (*RPP30*) gene was used. The patient's records, timing of cleaning and sampling, and RT-PCR results were collated.

On admission, the patient had fever (39 °C) and a mild cough (Supplemental Table 2). The chest X-ray and computed tomography scan on day 1 showed signs of pneumonia in both lungs. He received lopinavir/ritonavir and antibacterial therapy on day 2 but showed respiratory failure. He received supplemental oxygen from day 4 to 15. After careful clinical management, the patient's overall status improved. RT-PCR showed his sputum was positive for SARS-CoV-2 on day 11. Subsequently, nasopharyngeal swabs were negative on days 17, 22, and 29.

The patient stayed in Room A for 3 days, during which he had the SARS-CoV-2 infection. After cleaning Room A, five environmental samples were examined by RT-PCR. All samples were negative for SARS-CoV-2 and positive or negative for *RPP30* (Table 1).

After the patient left Room A, he resided in Room B for 20 days. Ten environmental samples were collected after cleaning. All ten samples from Room B were negative for SARS-CoV-2 and positive or negative for *RPP30* (Table 1).

SARS-CoV-2 is detectable in several types of clinical sample including Bronchial lavage fluid, nasopharyngeal swab, pharyngeal swab, sputum, saliva and stool [4, 5]. Transmission of SARS-CoV-2 via surfaces in hospitals is of great concern to medical staff and patients. Blocking the potential routes of transmission is essential for preventing the spread of SARS-CoV-2 [6]. A recent

study showed that environmental contamination can occur via contact with patients with SARS-CoV-2 and upper respiratory tract symptoms [7]. We found that, after cleaning, all areas were negative for SARS-CoV-2; therefore, thorough cleaning is sufficient for SARS-CoV-2 decontamination.

This study had several limitations. First, RT-PCR was not performed before cleaning because of the risk of nosocomial transmission. Therefore, a comparison of the viral loads of high-touch areas before and after cleaning is required. Second, this study involved a single patient, and further studies are required to confirm the findings.

In summary, our data indicate the effectiveness of environmental cleaning for SARS-CoV-2 decontamination. This information is useful for infection control strategies and may alleviate the concerns of medical staff.

Acknowledgement. We thank all of the medical and ancillary hospital staff and the patients for consenting to participate. We thank Suzanne Leech, Ph.D., from Edanz Group (https://en-author-services.edanzgroup.com/) for editing a draft of this manuscript.

Financial support. This study was supported by a Grant-in-Aid for the Genome Research Project from Yamanashi Prefecture (to M.O. and Y.H.), the Japan Society for the Promotion of Science (JSPS) KAKENHI Early-Career Scientists (grant number JP18K16292 to Y.H.), a Research Grant for Young Scholars (to Y.H.), the YASUDA Medical Foundation (to Y.H.), the Uehara Memorial Foundation (to Y.H.), and Medical Research Grants from the Takeda Science Foundation (to Y.H.).

Conflict of Interest. None.

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Table 1. Real-time PCR analysis of environmental samples

Location		**RT-PCR (number of samples)**	
Patient Room A		**SARS-CoV-2**	**Human *RPP30***
	Light switch	Negative (0/1)	Negative (0/1)
	Nurse call attached to the bed	Negative (0/1)	Positive (1/1)
	Toilet door handle	Negative (0/1)	Negative (0/1)
	Bed guard	Negative (0/1)	Positive (1/1)
Anterior Room A			
	Dust box	Negative (0/1)	Positive (1/1)
Patient Room B			
	Bed desk	Negative (0/1)	Negative (0/1)
	Bed guard	Negative (0/1)	Negative (0/1)
	Door handle	Negative (0/1)	Positive (1/1)
	Dust box, room side	Negative (0/1)	Negative (0/1)
	Dust box, corridor	Negative (0/1)	Positive (1/1)
	Control panel on mechanical ventilation	Negative (0/1)	Positive (1/1)
	Light switch	Negative (0/1)	Negative (0/1)
	Nurse call	Negative (0/1)	Positive (1/1)
	Hand soap dispenser	Negative (0/1)	Negative (0/1)
Anterior Room B			
	Sink, external rim and internal bowl	Negative (0/1)	Positive (1/1)

RPP30, ribonuclease P 30 subunit; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2

Supplemental Material

Supplemental Table 1. Sequences of the primers and probes used in the CDC, NIID, and YCH assays

Institute	Primer name	Description	Oligonucleotide Sequence (5'>3')	Modification
CDC	CDC-N1-F	2019-nCoV_N1 Forward Primer	5'-GACCCCAAAATCAGCGAAAT-3'	None
CDC	CDC-N1-R	2019-nCoV_N1 Reverse Primer	5'-TCTGGTTACTGCCAGTTGAATCTG-3'	None
CDC	CDC-N1-P	2019-nCoV_N1 Probe	5'-FAM-ACCCCGCATTACGTTTGGTGGACC-BHQ1-3'	FAM/BHQ1
CDC	CDC-N2-F	2019-nCoV_N2 Forward Primer	5'-TTACAAACATTGGCCGCAAA-3'	None
CDC	CDC-N2-R	2019-nCoV_N2 Reverse Primer	5'-GCGCGACATTCCGAAGAA-3'	None
CDC	CDC-N2-P	2019-nCoV_N2 Probe	5'-FAM-ACAATTTGCCCCCAGCGCTTCAG-BHQ1-3'	FAM/BHQ1
CDC	CDC-N3-F	2019-nCoV_N3 Forward Primer	5'-GGGAGCCTTGAATACACCAAAA-3'	None
CDC	CDC-N3-R	2019-nCoV_N3 Reverse Primer	5'-TGTAGCACGATTGCAGCATTG-3'	None
CDC	CDC-N3-P	2019-nCoV_N3 Probe	5'-FAM-AYCACATTGGCACCCGCAATCCTG-BHQ1-3'	FAM/BHQ1
CDC	RP-F	RNAse P Forward Primer	5'-AGATTTGGACCTGCGAGCG-3'	None
CDC	RP-R	RNAse P Reverse Primer	5'-GAGCGGCTGTCTCCACAAGT-3'	None
CDC	RP-P	RNAse P Probe	5'-FAM–TTCTGACCTGAAGGCTCTGCGCG–BHQ1-3'	FAM/BHQ1
NIID	NIID-N1-F	N_Sarbeco_Forward Primer	5'-CACATTGGCACCCGCAATC-3'	None
NIID	NIID-N1-R	N_Sarbeco_Reverse Primer	5'-GAGGAACGAGAAGAGGCTTG-3'	None
NIID	NIID-N1-P	N_Sarbeco_Probe	5'-FAM-ACTTCCTCAAGGAACAACATTGCCA-TAMRA-3'	FAM/TAMRA
NIID	NIID-N2-F	NIID_2019-nCOV_N_Forward Primer	5'-AAATTTTGGGGACCAGGAAC-3'	None
NIID	NIID-N2-R	NIID_2019-nCOV_N_Reverse Primer	5'-TGGCAGCTGTGTAGGTCAAC-3'	None
NIID	NIID-N2-P	NIID_2019-nCOV_N_Probe	5'-FAM-ATGTCGCGCATTGGCATGGA-TAMRA-3'	FAM/TAMRA
YCH	YCH-N1-F	YCH_N1 Forward Primer	5'-CACATTGGCACCCGCAATC-3'	None
YCH	YCH-N1-R	YCH_N1 Reverse Primer	5'-GAGGAACGAGAAGAGGCTTG-3'	None

YCH	YCH-N1-P	YCH_N1 Probe	5’-FAM/ACTTCCTCA/ZEN/AGGAACAACATTGCCA-IBFQ-3'	FAM/ZEN/IBFQ
YCH	YCH-N2-F	YCH_N1 Forward Primer	5’-AAATTTTGGGGACCAGGAAC-3'	None
YCH	YCH-N2-R	YCH_N1 Reverse Primer	5’-TGGCAGCTGTGTAGGTCAAC-3'	None
YCH	YCH-N2-P	YCH_N1 Probe	5’-FAM/ATGTCGCGC/ZEN/ATTGGCATGGA-IBFQ-3'	FAM/ZEN/IBFQ

NIID, National Institute of Infectious Diseases; YCH, Yamanashi Central Hospital; CDC, Centers for Disease Control and Prevention

FAM, 6-carboxyfluorescein; BHQ1, Black Hole Quencher 1; IBFQ, Iowa Black Fluorescent Quencher

Supplemental Table 2. Time course of patient symptoms, environmental cleaning, and sampling

Date	Symptoms	RT-PCR of SARS-CoV-2	Description
Before admission		Positive	Patient traveled on Diamond Princess cruise ship.
Day 1	Fever, mild cough, and pneumonia		Patient was admitted to the hospital and entered patient Room A.
Day 2	Respiratory failure		
Day 3			Patient left Room A and entered Room B.
Day 4			Patient received supplemental oxygen in Room B. Room A was cleaned.
Day 8			Five environmental samples were collected from Room A via swabbing and analyzed by RT-PCR.
Day 11		Positive in sputum	
Day 17		Negative in nasopharyngeal swab	
Day 21			Patient left Room B. Room B was cleaned. Ten environmental samples were collected from Room B via swabbing and analyzed by RT-PCR.
Day 22		Negative in nasopharyngeal swab	
Day 29		Negative in nasopharyngeal swab	
Day 35	Overall status had improved		