Reply to Kerkhove et al and Oh

To the Editor—We appreciate the valuable comments of Kerkhove et al [1] and Oh [2] on our recent study [3]. The purpose of our immunofluorescence assay was to confirm the presence of replicating virus in cultures of the air and in surface samples. As Oh points out, compared with cells infected with the clinical isolate, fewer cells infected with the environmental sample showed positive viral antigen detection results after prolonged incubation. This finding can be explained by the difference in the number of viable virus particles in the inocula used; the clinical isolate, Middle East respiratory syndrome coronavirus (MERSCoV)/Korea/Korea National Institute of Health (KNIH)/002_05_2015, was previously adapted in Vero cells, with viral yields of $>1.4 \times 10^5$ plaque-forming units/mL, whereas the environmental isolates were used immediately after their collection from the air and environmental surfaces. Characterizing the replication capabilities of the clinical and environmental isolates would require additional studies directly comparing their replication kinetics in cells infected with each isolate at the same multiplicity of infection.

Regarding the concern about the sequence differences between the spike genes of the 19 environmental isolates, we found that their similarities ranged from 97% to 100% (Supplementary Figure 2) when we analyzed the entire readouts from Sanger sequencing of the reverse-transcription products with the Basic Local Alignment Search Tool alogrithm.

Common limitations of DNA sequencing by the Sanger method include poor quality of the first 15–40 bases of sequences owing to primer binding, as well as deterioration of sequencing traces after 700–900 bases. All the incomplete matches between our environmental samples (subjects) and the MERS-CoV/Korea/KNIH/002_05_2015 clinical isolate (nucleotide 22 300 to approximately 22 628) were restricted to the first 15–40 bases, where poor primer binding occurs. In fact, recent complete sequencing of the spike genes of the environmental isolates yielded 100% homology with the target region (nucleotides 22 300 to approximately 22 628) (data not shown). Characterizing genotypic variation would require additional studies comparing full-genome sequences of the environmental isolates and clinical isolate.

We reported that viable MERS-CoV could be detected in the air and on surfaces further than the spread of wet droplets, and this finding is consistent with those of other researchers. An earlier study conducted in Korea detected MERS-CoV by means of reverse-transcription polymerase chain reaction at the entrance of air-ventilating equipment [4]. In addition, several studies performed at Pyungtaek St Mary Hospital (the first epidemiologic hotspot) and Samsung Medical Center (the second epidemiologic hotspot) in South Korea during the MERS outbreak consistently obtained positive MERS-CoV polymerase chain reaction results with environmental swab samples from the entrance of air-ventilating equipment [5, 6]. Hence, it seems reasonable to conclude that some small droplets or droplet nuclei can travel >1–2 m under certain conditions of airflow. However, we appreciate the comment of Kerkhove et al [1] that the presence of cultivable MERS-CoV in the air does not provide evidence of airborne transmission. We believe that the mode of transmission depends not only on particle size but also on the amount of virus present in each particle. With respect to Oh's last comment [2], we collected large amounts of room air, but we were unable to measure the amount of MERS-CoV in the samples because they had to be amplified in the cell culture system.

Finally, we would like to emphasize certain details of the air sampling procedure. We placed the air sampler ≥3–4 m from the patients in hospital A, and ≥2–3 m away from the patient in hospital B. No coughing or sneezing was observed, and all 3 patients stayed in their rooms during the sampling process. The patient in hospital B was bedridden at the time, but the isolation rooms occupied by other patients shared a common corridor with his room. Therefore, it is not impossible that other MERS-infected patients left their rooms before the sampling, but we are sure that none passed along the corridor during sampling. In addition, the 2 patients in hospital A were kept in rooms with ≥12 air changes per hour and received mechanical ventilation during the environmental sampling; they also routinely underwent both open (owing to thick sputum) and closed endotracheal suction. Therefore the air sampling in hospital A was performed in the intervals (30–60 minutes after the suction) between the endotracheal suction procedures. Because we did not wish to disturb the routine clinical procedures in the MERS isolation wards, our findings reflect the real clinical situation. They imply that the possible presence of some culturable MERS-CoV particles in the air of intensive care units with MERS-infected patients should not be dismissed out of hand until the results of further studies are available.

Notes

Financial support. This work was supported by a grant from the Korea Health Technology R&D Project through the Korea Health Industry Development Institute, funded by the Ministry of Health & Welfare, Republic of Korea (grant no. HI15C2774). It was also supported by a National Research Foundation of Korea (NRF-No. 2014K1A4A7A01074646) grant funded by the Korean government (Ministry of Science, ICT, and Future Planning) and by a GSTEP MERS grant funded by Gyeonggi-do.

Potential conflicts of interest. All authors: No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

Sung-Han Kim,[1,a] Minki Sung,[2] and Ji-Young Min[3,a]

[1]Department of Infectious Diseases, Asan Medical Center, University of Ulsan College of Medicine, [2]Department of Architectural Engineering, Sejong University, Seoul, and [3]Respiratory Viruses Research Laboratory, Institut Pasteur Korea, Seongnam-si, Gyeonggi, Republic of Korea

References

1. Van Kerkhove MD, Peiris M, Malik MR, Embarek PB. Interpreting results from environmental contamination studies of Middle East respiratory syndrome coronavirus. Clin Infect Dis **2016**; 63:1142.
2. Oh MD. Transmissibility of Middle East respiratory syndrome by the airborne route. Clin Infect Dis **2016**; 63:1143.
3. Kim SH, Chang SY, Sung M, et al. Extensive viable Middle East respiratory syndrome (MERS) coronavirus contamination in air and surrounding environment in MERS isolation wards. Clin Infect Dis **2016**; 63:363–9.
4. Bin SY, Heo JY, Song MS, et al. Environmental contamination and viral shedding in MERS patients during MERS-CoV outbreak in South Korea. Clin Infect Dis **2016**; 62:755–60.
5. Ki M. Epidemiology of MERS in the Republic of Korea. Presented at the MERS International Symposium; 15–16 December 2015; Seoul, South Korea.
6. Chun BC. How can we explain the super-spreading events by CCTV analysis? Presented at the 2016 Annual Meeting of the Korean Society for Chemotherapy & the Korean Society of Infectious Diseases; 21–22 April 2016; Seoul, South Korea.

[a]S.-H. K. and J.-Y. M. contributed equally to this work.
Correspondence: J.-Y. Min, Respiratory Viruses Research Laboratory, Institut Pasteur Korea, Seongnam-si, Gyeonggi, Republic of Korea (jiyoung.min@ip-korea.org).

Clinical Infectious Diseases® 2016;63(8):1143–4
 DOI: 10.1093/cid/ciw480