Appendix 1. Supplemental Methods: SARS-CoV-2 Environmental Contamination and Childbirth

We conducted this study at Oregon Health & Science University (OHSU), Portland, Oregon with University of Oregon's Biology & the Built Environment Center (UO-BioBE), Eugene, Oregon (March 2020 to June 2020). The births occurred in a neutral pressure room (VB #1), negative pressure room (VB#2), and positive pressure rooms (CB #1 and #2). The minimum code required air exchanges are 15 for cesarean delivery rooms, 12 for negative pressure L&D rooms and 4 for neutral pressure L&D rooms. All L&D rooms are private and if cesarean delivery is needed they are moved to operating room. Patients and visitors are required to wear a mask and perform routine hand hygiene. All clinicians follow standard donning and doffing workflow when entering and leaving rooms of COVID+ patients. Clinicians are instructed to not touch face shields or masks with their hands after donning.

Three sampling techniques were used. Surface swabs were collected from unoccupied, cleaned rooms and again immediately after birth. Face shields (outward facing surface) were also swabbed after birth from health care workers present during delivery. Sterile swabs pre-moistened with viral transport media (VTM) touched each surface for 20 seconds (surface sampling). For passive air sampling, sterile 60mm x 15 mm Petri dishes are opened and both halves placed on a surface with the sterile interior exposed to the room air. No media is used. Airborne microbes accumulate on the both halves of the dish and after a period of time, the dish is closed and sealed with paraffin for transport to the lab for processing. For active air sampling we used a calibrated mechanical SKC pump at 12.5L/min with glass impingers (SKC Inc. Catalog # 225-9595) to pull indoor air through filter media (PBS) at a known rate. Passive and active air sampling occurred for one-hour minimum. Trained research personnel wearing PPE conducted the sampling. Research personnel performing the RT-PCR and majority of sample collection were confirmed COVID negative throughout study. Two other personnel were not tested but were asymptomatic.

In the OHSU COVID-19 BSL-2+ laboratory, samples were vortexed and settled for five minutes. An aliquot of 200 µL was placed into a sterile micro centrifuge tube containing 600 µL of a lysis/preservative buffer (DNA/RNA Shield, Zymo Research catalog # R-1100-250). Total RNA was extracted from all samples using Zymo Quick DNA/RNA Viral MagBead kit (Zymo Research catalog # R2141) and stored at -80°C until further analysis was performed.

Samples were run in triplicate and SARS-CoV-2 abundance determined using qRT-PCR targeting a 157 bp segment of the SARS-CoV-2 spike glycoprotein gene. An artificial gene standard (IDT custom order) with known copy number was used to create a dilution series and standard curve to determine viral gene copy number in each sample with a limit of detection of 11.1 viral gene copies. We removed any amplification observed in 'no template' controls from samples accounting for interrun variability.

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The authors provided this information as a supplement to their article.
