**SUPPLEMENTAL METHODS: SARS-CoV-2 viral, serologic, and genomic testing**

Nasal and rectal swabs collected from zoo animals were placed in sterile transport media and processed using Quick-RNA Viral Kits (Zymo Research, [www.zymoresearch.com](http://www.zymoresearch.com), Cat# R1034/R1035). Extracted genomic material was tested by TGen’s in-house developed and validated SARS-CoV-2 real-time RT-PCR (rRT-PCR) assay, which targets the nucleocapsid gene (N2 amplicon length: 77 basepairs) and spike gene (S4 amplicon length: 78 basepairs) in the viral genome (sequences available upon request).37 Samples were run on the CFX96 real-time PCR instrument (Bio-Rad) with positive (N gene and S gene plasmid transcription product) and negative (distilled water) controls, plus an internal amplification control (RNase P, as published by the Centers for Disease Control and Prevention)21 included as separate reaction mixtures. Samples with cycle threshold (Ct) values <38 for both N2 and S4 rRT-PCR targets were considered positive; genomic sequencing of positive samples was performed on Illumina instruments using standard protocols.14,37 Serum was tested using a SARS-CoV-2 Surrogate Virus Neutralization Test (GenScript, https://www.genscript.com/covid-19-detection-svnt.html) following manufacturer instructions.37

Presumptive positive samples were submitted to the US Department of Agriculture (USDA) National Veterinary Services Laboratories (NVSL) for confirmation and tested using a probe-based real-time RT PCR for SARS-CoV-2 using 2019 nCoV\_N1 and 2019 nCoV\_N2 markers). Beta-actin is used as an inhibition control and to ensure sample quality.

Additional testing methodologies, including histopathology, immunohistochemistry, and RT-PCR on FFPE tissues were performed at CDC’s Infectious Disease Pathology Branch (IDPB) given the fatal outcome of the squirrel monkey case.. RNA was extracted from formalin-fixed, paraffin-embedded lung, trachea, and colon tissue samples and evaluated by conventional RT-PCR using primers targeting 150bp of the SARS-CoV-2 nucleocapsid gene, as previously described.4 Briefly, the assays was performed using the OneStep RT-PCR Kit (Qiagen, Valencia, CA, USA) and 5 ml of RNA template, according to the manufacturer’s instructions. The thermocycling conditions for the assay were as follows: 1 cycle at 50°C for 30 minutes, 1 cycle at 95°C for 15 minutes, then 40 cycles of incubation at 94°C, 56°C and 72°C for 1 minute each, followed by 1 cycle of final extension at 72°C for 10 minutes. In each run, RNA extracted from FFPE SARS-CoV-2 cultured cells and no template water controls were used as positive and negative controls, respectively. The PCR amplicons were identified by gel electrophoresis.

Immunohistochemistry was performed on 4um sections of FFPE colon and liver tissue using a rabbit polyclonal anti-Clostridium spp. antibody (Meridian Life Science, Memphis, TN) in combination with alkaline phosphatase polymer detection system and Fast Red chromogen for visualization.16 Positive and negative controls run in parallel included tissue from a previously confirmed *Clostridium* infection, and normal rabbit serum in place of the primary antibody, respectively.