Methods

For the endoscope model, we used new polytetrafluoroethylene (PTFE) tubes with a 4.0-mm internal diameter and a 20-cm length (volume, 2.51 mL). First the PTFE tubes were flushed with 2.6 mL from a pool of 4 positive hemocultures containing *S. aureus, P. aeruginosa, E. coli* and *C. dubliniensis.* Then they were flushed with a 50ml syringe containing air and kept at room temperature for 24 hours, simulating a worst-case soil scenario in which the endoscope is reprocessed after 24 hours. Four soiled PTFE tubes were used as positive controls. We also processed one PTFE tube, that wasn’t soiled, as negative control.

After 24 hours, the tubes were submerged in water and flushed with a syringe containing 10ml water. One of the 3 brushes was passed through the lumen according to the manufacturer’s instructions. Each brush was tested on 5 different tubes. Next, the tube was taken out of the water and flushed with a 50ml syringe containing air. The negative control tube was brushed with the PULL THRU brush. Two positive control tubes weren’t submerged in water, two other positive control tube were submerged in water and flushed with water but not brushed.

After that, the outside of the tubes was wiped once with a clot containing ethanol. The tubes were cut into small pieces of approximately 1cm that were collected in sterile containers filled with 10 mL sterile water. These containers were then vortexed for 30 seconds, sonicated for 1 minute, and vortexed again for 30 seconds. All samples and controls were processed for adenosine triphosphate (ATP) measurement and culture at the microbiology laboratory within 1 hour.

ATP measurement was performed in duplicate using the Aquasnap Total test (Hygiena, Watford, UK) according to the manufacturer’s instructions with the SystemSURE Plus luminometer. Additionally, the remaining samples were diluted (1:10,000), and 100 µL was plated on trypticase soy agar (TSA), which was incubated for 7 days at 30°C. The total number of colony-forming units (CFU) was recorded.

Mean, range and 95% confidence intervals (CI) of ATP and culture results were calculated for each brushing technique. All statistical analyses were conducted using SPSS version 25 statistical software (IBM, Armonk, NY).