**Supplementary material**

**Title: Evaluation of a New Technology for Terminal Sterilization of Flexible Endoscopes Using Hydrogen Peroxide Gas Plasma**

***Detailed description of the device***

The hydrogen peroxide gas plasma sterilizer (Steroscope, Ideate Medical, St. Louis, MS) is designed for terminal sterilization of a broad range of flexible endoscopes after manual cleaning and drying with filtered forced air for a minimum of 10 minutes. The sterilizer can accommodate endoscopes with as many as 8 internal channels with lumen dimensions of inner diameter of 1.0 mm or larger and a length of 3580 mm or shorter and inner diameter of 1.2 mm or larger and a length of 4095 mm or shorter. The device uses a pressure differential in each internal endoscope channel to rapidly diffuse hydrogen peroxide gas plasma and achieves the required efficacy concentration in all internal channels up to 4 m long in less than 20 seconds. Each cycle requires a total of 44 minutes and includes 2 injections of hydrogen peroxide gas plasma 16 minutes apart. The relatively low hydrogen peroxide concentration and short exposure time minimizes the potential for damage to the endoscope.

During sterilization, the endoscope is placed inside a sterilization container that interfaces with the sterilizer and subsequently provides a sterile storage container for the endoscope for up to 6 months after processing. Prior to sterilization, the water-resistant cap must be removed from video endoscopes and a leak test is performed to ensure that there is no leak, which could cause fluid invasion of the endoscope. During hydrogen peroxide gas plasma transfer, a single-use channel connector seals to provide a fluidic path for the hydrogen peroxide gas plasma to flow through the internal channels of the endoscope, while also contacting and sterilizing the mated connector interface.

Per the manufacturer, testing demonstrated 0 positive wires per 39 replicates when stainless steel wires were inoculated with 106 *Geobacillus stearothermophilus* spores with no soil and placed in a duodenoscope elevator channel with an average hydrogen peroxide concentration of 19.98 mg/L. The FDA approval letter for the technology indicates that it was effective in eliminating *G. stearothermophilus* with a half cycle and eliminated *G. stearothermophilus* in simulated use testing with a full cycle in the presence of a soil load. The manufacturer also indicated that the technology was used to sterilize an Olympus duodenoscope (TJF-Q160F) 125 times with no evidence of damage to the device.

***Detailed methods used to assess efficacy of the sterilizer***

***Method 1. Inoculated steel wires***

The test organisms were inoculated onto 6.5-cm length steel wires that were placed inside the elevator channel. For inoculation, the wires were placed in a sterile petri dish and 10 to 12 drops containing 20 µL of test organism suspension were applied and allowed to dry. The organism inoculation process was repeated a second time. The wires were then placed inside the elevator channel using a sterile tweezer. Evaluations were completed in triplicate with no soil and with 5% fetal calf serum or RPMI medium as soil.

***Method 2. Inoculation directly into the elevator recess and instrument channel***

The test organisms were inoculated directly into the elevator recess and the instrument channel as described by Molloy-Simard et al.1 The elevator mechanism was inoculated using 10 µL of the test organism suspension. The organisms were pipetted into the hinge and the recessed space associated with the hinge. The mechanism was then moved up and down at least 3 times to allow spreading of the inoculum and penetration in the hinge at the distal end of the endoscope. The instrument channel was inoculated with 10 µL of inoculum followed by flushing with ~100 mL of sterile water to allow the inoculum to reach the center of the channel by gravity. The endoscope was allowed to dry for at least 2 hours before placing it inside the sterilizer. All tests were completed 3 times with 5% fetal calf serum as the soil.

***Method 3. Inoculation into the instrument channel followed by use of a brush to spread the inoculum throughout the lumen***

The test organisms were inoculated into the instrument channel followed by use of a brush to spread the inoculum throughout the lumen. A sterile cleaning brush was inserted into the biopsy port and pushed in until it reached the distal tip of the endoscope. With the brush in place, the distal tip of the duodenoscope was immersed in 20 mL of test organism suspension. The elevator wire mechanism of the endoscope was articulated through its full range of motion 3 times. The brush was then withdrawn completely from the biopsy port. This process was repeated 3 times. The endoscope was allowed to dry at room temperature for at least 2 hours before placing it inside the sterilizer. Testing was completed with 5% fetal calf serum or ATS-2015 as the soil. The number of tests ranged from 1 to 5 (N=5 tests for *B. atrophaeus* with 5% FCS; N=1 test for *C. difficile* with 5% FCS; and N=2 tests for other conditions).

***Prior studies demonstrating that hydrogen peroxide vapor and hydrogen peroxide-ozone technologies can achieve sterilization of endoscopes***

Two prior studies have demonstrated that hydrogen peroxide vapor and hydrogen peroxide-ozone technologies can achieve sterilization of endoscopes. Omidbakhsh et al.2 demonstrated that hydrogen peroxide vapor delivered using the STERRAD 100NX Sterilization System (Advanced Sterilization Products Inc., Irvine, CA) was effective for sterilization of colonoscopes and a duodenoscope when an experimental cycle was used. The experimental cycle created turbulence inside the chamber such that vaporized hydrogen peroxide molecules could penetrate long and narrow lumens.2 Material compatibility testing suggested that a graphite-based inert lubricant but not a molybdenum disulphide lubricant was compatible with vaporized hydrogen peroxide. Molloy-Simard et al.1 demonstrated that an FDA-approved hydrogen peroxide-ozone sterilizer was effective for terminal sterilization of duodenoscopes. A half cycle of sterilization resulted in a 6 log10 or greater reduction of *G. stearothermophilus* spores and a full cycle achieved a 6 log10 reduction of *G. stearothermophilus* spores in the presence of inorganic and organic soils.

**References**

1. Molloy-Simard V, Lemyre JL, Martel K, Catalone BJ. Elevating the standard of endoscope processing: Terminal sterilization of duodenoscopes using a hydrogen peroxide-ozone sterilizer. *Am J Infect Control* 2019;47:243-250.
2. Omidbakhsh N, Manohar S, Vu R, Nowruzi K. Flexible gastrointestinal endoscope processing challenges, current issues and future perspectives. *J Hosp Infect* 2021;110:133-138.