Worst-case Challenge to Sterilization Efficacy

Objective

To characterize the microbial killing efficacy of sterilants utilizing ATS.

Methods

To characterize the microbial killing efficacy of the sterilants to be evaluated under worst-case conditions, 10 uL of ATS containing ~10^8 cfu/ml of the test microorganism is inoculated inside a surrogate lumen carrier (3mm internal diameter, 2 cm long), and dried at room temperature overnight. The inoculated test segment is then assembled into a lumen of 125cm total length and then processed through the sterilization process. The test carriers consist of PVC plastic tubing cut into 2 cm lengths or PTFE endoscopy biopsy channel tubing cut into 2 cm lengths.

Sterility Testing

The central lumen segment is aseptically removed and placed in a tube containing 2 mLs of sterile tryptic soy broth containing 10% fetal bovine serum. This segment is incubated at 35°C for 5 days for *Enterococcus faecalis*; 55°C for 5 days for *Geobacillus stearothermophilus*; and 30°C for 10 days for *M. chelonae*. Because turbidity may be hard to detect for *M. chelonae*, blind subcultures are performed after 10 days of incubation by sub-culturing the broth to blood agar (BA) and incubating the plates at 30°C for 5 days.

To quantify the level of residual viable test organisms post-treatment, the entral segment of the duplicate set of lumen test carriers is aseptically disassembled and placed into a sterile tube containing 2 mLs of sterile tryptic soy broth containing 10% fetal bovine serum. The tube is sonicated in a Branson 1220 sonicator bath for 2 x 5 secs, and then mixed on an S/P Multitube vortexer (American Dade, Miami, FL) on setting "2" for 10 minutes. Serial 1:10 dilutions are prepared in sterile tryptic soy broth and 0.1 mLs plated on tryptic soy agar plates (BA plates for *M. chelonae*) using the spread plate technique. The inoculated plates are incubated at 35°C for 24 - 48 hours for *E. faecalis*, 55°C for 24 - 48 hours for *G. stearothermophilus* and 30°C for 48 - 72 hours for *M. chelonae*. Colonies are enumerated and the viable count per test carrier is calculated.