Simulated-Use Testing: Laparoscopic Instruments

Target Devices

May use either or both ported laparoscopic devices and non-ported devices. Both ported and non-ported devices should be thoroughly cleaned (recommended with a retroflush device accompanied by sonication), packaged and steam sterilized before simulated-use testing.

Inoculation

The inoculum consists of ATS soil that contains *Enterococcus faecalis* (ATCC 29212) and *Geobacillus stearothermophilus* (ATCC 12980) at ~10^6 cfu/mL. Soil laparoscopic devices using a retro-flush adaptor to inject ATS upwards from the distal end of the device until soil is seen exiting from either the handle or the luer port at the handle. This ensures that the entire inner lumen is exposed to soil and organisms. Allow the inoculated laparoscopic devices to sit at room temperature for 1 hour before cleaning. Allow all excess soil to drain out of the laparoscopic devices after the soiling stage. Include *positive controls* (soiled, but un-cleaned) and *negative controls* (unsoiled, un-cleaned) in all experiments.

Methods for evaluating residual soil and organisms

To determine the level of residual organisms and soil, both indirect and direct (in situ) test methods may be used.

Sample collection for indirect quantitative testing

Take samples from the devices by attaching a sterile retro-flush adaptor to the distal end of the laparoscopic device and use a sterile syringe to flush 10mLs of sterile reverse osmosis (RO) water up and down in the lumen for three times prior to withdrawing the sample and placing it in a sterile tube. Evaluate this sample quantitatively for protein, carbohydrate, hemoglobin and viable counts by the methods previously described by Alfa and Nemes.

In situ Bradford's test for residual protein

1. Flush the laparoscopic device with air or held vertically to allow excess fluid to drain. This is done to ensure that there is no residual fluid and/or soil within the channel.
2. Use a sterile retro-flush adaptor to instill undiluted Bradford reagent (Sigma Chemical Co., St Louis, MO, USA) directly into the lumen until it is full. Hold the device horizontally for 20 min at room temperature to allow color development. Bradford reagent will turn blue in the presence of protein.
3. Drain the Bradford reagent after 20 min from the device by using a separate sterile retro-flush adaptor and syringe connected to the distal end to aspirate fluid from the lumen. Use a separate sterile retro-flush adaptor for each device.
4. Score the fluid visually for any visible blue coloration and determine the absorbance by using a spectrophotometer equipped with a 595 nm filter.

In situ TMB-one test for residual hemoglobin

1. Flush the laparoscopic device with air or hold it vertically to allow excess fluid to drain. This is done to ensure that there is no residual fluid and/or soil within the channel.
2. Use a sterile retro-flush adaptor to instill undiluted TMB-One reagent (Biotecx Laboratories, Houston, TX, USA) into the lumen until it is full. Hold the device horizontally for 20 min at room temperature to allow color development. TMB-One reagent will turn green in the presence of hemoglobin.
3. Drain the TMB-One reagent after 20 minutes from the device by using a sterile retro-flush adaptor and sterile syringe connected to the distal end to aspirate fluid from the lumen. Use a separate sterile retro-flush adaptor for each device.
4. Score the fluid visually for any visible green coloration and determine the absorbance by a spectrophotometer equipped with a 495 nm filter. TMB-One testing is to be performed only on non-ported laparoscopic devices.

Study utilizing the above methods:
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To determine if our soil challenge was realistic, clinical samples were collected. Soil levels in patient-used laparoscopic accessory devices were evaluated using the same sampling technique described in *Materials and Methods*. There was an average of 5560 mg/lumen protein, 1679 mg/lumen of hemoglobin and 295 mg/lumen carbohydrate.