Microbicidal Activity of Low-Temperature Sterilization Technologies

Guideline for Disinfection and Sterilization in Healthcare Facilities (2008)

WHAT TO KNOW

Microbicidal Activity of Low-Temperature Sterilization Technologies from the Guideline for Disinfection and Sterilization in Healthcare Facilities (2008).

Microbicidal Activity of Low-Temperature Sterilization Technologies

Sterilization processes used in the United States must be cleared by FDA, and they require that sterilizer microbicidal performance be tested under simulated-use conditions[904]. FDA requires that the test article be inoculated with 10^6 colony-forming units of the most resistant test organism and prepared with organic and inorganic test loads as would occur after actual use. FDA requires manufacturers to use organic soil (e.g., 5% fetal calf serum), dried onto the device with the inoculum, to represent soil remaining on the device following marginal cleaning. However, 5% fetal calf serum as a measure of marginal cleaning has not been validated by measurements of protein load on devices following use and the level of protein removal by various cleaning methods. The inocula must be placed in various locations of the test articles, including those least favorable to penetration and contact with the sterilant (e.g., lumens). Cleaning before sterilization is not allowed in the demonstration of sterilization efficacy[904]. Several studies have evaluated the relative microbicidal efficacy of these low-temperature sterilization technologies (Table 11). These studies have either tested the activity of a sterilization process against specific microorganisms[892, 905, 906], evaluated the microbicidal activity of a singular technology [711, 719, 724, 855, 879, 882-884, 890, 891, 907] or evaluated the comparative effectiveness of several sterilization technologies[271, 426, 469, 721, 722, 856, 908, 909]. Several test methodologies use stainless steel or porcelain carriers that are inoculated with a test organism. Commonly used test organisms include vegetative bacteria, mycobacteria, and spores of *Bacillus* species. The available data demonstrate that low-temperature sterilization technologies are able to provide a 6-$\log_{10}$ reduction of microbes when inoculated onto carriers in the absence of salt and serum. However, tests can be constructed such that all of the available sterilization technologies are unable to reliably achieve complete inactivation of a microbial load.[425, 426, 469, 721, 856, 909] For example, almost all of the sterilization processes will fail to reliably inactivate the microbial load in the presence of salt and serum[469, 721, 909].

The effect of salts and serums on the sterilization process were studied initially in the 1950s and 1960s[424, 910]. These studies showed that a high concentration of crystalline-type materials and a low protein content provided greater protection to spores than did serum with a high protein content[426]. A study by Doyle and Ernst demonstrated resistance of spores by crystalline material applied not only to low-temperature sterilization technology but also to steam and dry heat[425]. These studies showed that occlusion of *Bacillus atrophaeus* spores in calcium carbonate crystals dramatically increased the time required for inactivation as follows: 10 seconds to 150 minutes for steam (121°C), 3.5 hours to 50 hours for dry heat (121°C), 30 seconds to >2 weeks for ETO (54°C). Investigators have corroborated and extended these findings[469, 470, 721, 855, 908, 909]. While soils containing both organic and inorganic materials impair microbial killing, soils that contain a high inorganic salt-to-protein ratio favor crystal formation and impair sterilization by occlusion of organisms[425, 426, 881].

Alfa and colleagues demonstrated a 6-$\log_{10}$ reduction of the microbial inoculum of porcelain penicylinders using a variety of vegetative and spore-forming organisms (Table 11)[469]. However, if the bacterial inoculum was in tissue-culture medium supplemented with 10% serum, only the ETO 12/88 and ETO-HCFC sterilization mixtures could sterilize 95% to 97% of the penicylinder carriers. The plasma and 100%ETO sterilizer demonstrated significantly reduced activity (Table 11). For all sterilizers evaluated using penicylinder carriers (i.e., ETO 12/88, 100% ETO, hydrogen peroxide gas plasma), there was a 3- to 6-$\log_{10}$ reduction of inoculated bacteria even in the presence of serum and salt. For each sterilizer evaluated, the ability to inactivate microorganisms in the presence of salt and serum was reduced even further when the inoculum was placed in a narrow-lumen test object (3 mm diameter by 125 cm long). Although there was a 2- to 4-$\log_{10}$ reduction in microbial kill, less than

50% of the lumen test objects were sterile when processed using any of the sterilization methods evaluated
system (Table 11)[721]. Complete killing (or removal) of 6-$\log_{10}$ of *Enterococcus faecalis, Mycobacterium chelo*
in the presence of salt and serum and lumen test objects was observed only for the peracetic acid immersion

With respect to the results by Alfa and coworkers[469], Jacobs showed that the use of the tissue culture media created a technique-induced sterilization failure[426]. Jacobs et al. showed that microorganisms mixed with tissue culture media, used as a surrogate body fluid, formed physical crystals that protected the microorganisms used as a challenge. If the carriers were exposed for 60 sec to nonflowing water, the salts dissolved and the protective effect disappeared. Since any device would be exposed to water for a short period of time during the washing procedure, these protective effects would have little clinical relevance[426].

Narrow lumens provide a challenge to some low-temperature sterilization processes. For example, Rutala and colleagues showed that, as lumen size decreased, increased failures occurred with some low-temperature sterilization technologies. However, some low-temperature processes such as ETO-HCFC and the hydrogen peroxide gas plasma process remained effective even when challenged by a lumen as small as 1 mm in the absence of salt and serum[856].

The importance of allowing the sterilant to come into contact with the inoculated carrier is demonstrated by comparing the results of two investigators who studied the peracetic acid immersion system. Alfa and coworkers demonstrated excellent activity of the peracetic acid immersion system against three test organisms using a narrow-lumen device. In these experiments, the lumen test object was connected to channel irrigators, which ensured that the sterilant had direct contact with the contaminated carriers[722]. This effectiveness was achieved through a combination of organism wash-off and peracetic acid sterilant killing the test organisms[722]. The data reported by Rutala et al. demonstrated failure of the peracetic acid immersion system to eliminate *Geobacillus stearothermophilus* spores from a carrier placed in a lumen test object. In these experiments, the lumen test unit was not connected to channel irrigators. The authors attributed the failure of the peracetic acid immersion system to eliminate the high levels of spores from the center of the test unit to the inability of the peracetic acid to diffuse into the center of 40-cm long, 3-mm diameter tubes. This may be caused by an air lock or air bubbles formed in the lumen, impeding the flow of the sterilant through the long and narrow lumen and limiting complete access to the *Bacillus* spores[137, 856]. Experiments using a channel connector specifically designed for 1-, 2-, and 3-mm lumen test units with the peracetic acid immersion system were completely effective in eliminating an inoculum of 10^6 *Geobacillus stearothermophilus* spores[7]. The restricted diffusion environment that exists in the test conditions would not exist with flexible scopes processed in the peracetic acid immersion system, because the scopes are connected to channel irrigators to ensure that the sterilant has direct contact with contaminated surfaces. Alfa and associates attributed the efficacy of the peracetic acid immersion system to the ability of the liquid chemical process to dissolve salts and remove protein and bacteria due to the flushing action of the fluid[722].

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