peroxide diffusion stage and a plasma stage per sterilization cycle. This revision, which is achieved by a software modification, reduces total processing time from 73 to 52 minutes. The manufacturer believes that the enhanced activity obtained with this system is due in part to the pressure changes that occur during the injection and diffusion phases of the process and to the fact that the process consists of two equal and consecutive half cycles, each with a separate injection of hydrogen peroxide. [856, 884, 885] This system and a smaller version [400, 882] have received FDA 510[k] clearance with limited application for sterilization of medical devices (Table 6). The biological indicator used with this system is *Bacillus atrophaeus* spores[851]. The newest version of the unit, which employs a new vaporization system that removes most of the water from the hydrogen peroxide, has a cycle time from 28-38 minutes (see manufacturer's literature for device dimension restrictions).

Penetration of hydrogen peroxide vapor into long or narrow lumens has been addressed outside the United States by the use of a diffusion enhancer. This is a small, breakable glass ampoule of concentrated hydrogen peroxide (50%) with an elastic connector that is inserted into the device lumen and crushed immediately before sterilization[470, 885]. The diffusion enhancer has been shown to sterilize bronchoscopes contaminated with *Mycobacteria tuberculosis*[886]. At the present time, the diffusion enhancer is not FDA cleared.

Another gas plasma system, which differs from the above in several important ways, including the use of peracetic acid-acetic acid-hydrogen peroxide vapor, was removed from the marketplace because of reports of corneal destruction to patients when ophthalmic surgery instruments had been processed in the sterilizer[887, 888]. In this investigation, exposure of potentially wet ophthalmologic surgical instruments with small bores and brass components to the plasma gas led to degradation of the brass to copper and zinc[888, 889]. The experimenters showed that when rabbit eyes were exposed to the rinsates of the gas plasma-sterilized instruments, corneal decompensation was documented. This toxicity is highly unlikely with the hydrogen peroxide gas plasma process since a toxic, soluble form of copper would not form (LA Feldman, written communication, April 1998).

Mode of Action. This process inactivates microorganisms primarily by the combined use of hydrogen peroxide gas and the generation of free radicals (hydroxyl and hydroproxyl free radicals) during the plasma phase of the cycle.

***Microbicidal Activity*.** This process has the ability to inactivate a broad range of microorganisms, including resistant bacterial spores. Studies have been conducted against vegetative bacteria (including mycobacteria), yeasts, fungi, viruses, and bacterial spores[469, 721, 856, 881-883, 890-893]. Like all sterilization processes, the effectiveness can be altered by lumen length, lumen diameter, inorganic salts, and organic materials[469, 721, 855, 856, 890, 891, 893].

Uses. Materials and devices that cannot tolerate high temperatures and humidity, such as some plastics, electrical devices, and corrosion-susceptible metal alloys, can be sterilized by hydrogen peroxide gas plasma. This method has been compatible with most (>95%) medical devices and materials tested[884, 894, 895].

Peracetic Acid Sterilization

Overview. Peracetic acid is a highly biocidal oxidizer that maintains its efficacy in the presence of organic soil. Peracetic acid removes surface contaminants (primarily protein) on endoscopic tubing[711, 717]. An automated machine using peracetic acid to sterilize medical, surgical, and dental instruments chemically (e.g., endoscopes, arthroscopes) was introduced in 1988. This microprocessor-controlled, low-temperature sterilization method is commonly used in the United States[107]. The sterilant, 35% peracetic acid, and an anticorrosive agent are supplied in a single-dose container. The container is punctured at the time of use, immediately prior to closing the lid and initiating the cycle. The concentrated peracetic acid is diluted to 0.2% with filtered water (0.2 μm) at a temperature of approximately 50°C. The diluted peracetic acid is circulated within the chamber of the machine and

pumped through the channels of the endoscope for 12 minutes, decontaminating exterior surfaces, lumens, and accessories. Interchangeable trays are available to permit the processing of up to three rigid endoscopes or one flexible endoscope. Connectors are available for most types of flexible endoscopes for the irrigation of all channels by directed flow. Rigid endoscopes are placed within a lidded container, and the sterilant fills the lumens either by immersion in the circulating sterilant or by use of channel connectors to direct flow into the lumen(s) (see below for the importance of channel connectors). The peracetic acid is discarded via the sewer and the instrument rinsed four times with filtered water. Concern has been raised that filtered water may be inadequate to maintain sterility[896]. Limited data have shown that low-level bacterial contamination may follow the use of filtered water in an AER but no data has been published on AERs using the peracetic acid system[161]. Clean filtered air is passed through the chamber of the machine and endoscope channels to remove excess water[719]. As with any sterilization process, the system can only sterilize surfaces that can be contacted by the sterilant. For example, bronchoscopy-related infections occurred when bronchoscopes were processed using the wrong connector[155, 725]. Investigation of these incidents revealed that bronchoscopes were inadequately reprocessed when inappropriate channel connectors were used and when there were inconsistencies between the reprocessing instructions provided by the manufacturer of the bronchoscope and the manufacturer of the automatic endoscope reprocessor[155]. The importance of channel connectors to achieve sterilization was also shown for rigid lumen devices[137, 856].

The manufacturers suggest the use of biological monitors (*G. stearothermophilus* spore strips) both at the time of installation and routinely to ensure effectiveness of the process. The manufacturer's clip must be used to hold the strip in the designated spot in the machine as a broader clamp will not allow the sterilant to reach the spores trapped under it[897]. One investigator reported a 3% failure rate when the appropriate clips were used to hold the spore strip within the machine[718]. The use of biological monitors designed to monitor either steam sterilization or ETO for a liquid chemical sterilizer has been questioned for several reasons including spore wash-off from the filter paper strips which may cause less valid monitoring[898-901]. The processor is equipped with a conductivity probe that will automatically abort the cycle if the buffer system is not detected in a fresh container of the peracetic acid solution. A chemical monitoring strip that detects that the active ingredient is >1500 ppm is available for routine use as an additional process control.

Mode of Action. Only limited information is available regarding the mechanism of action of peracetic acid, but it is thought to function as other oxidizing agents, i.e., it denatures proteins, disrupts cell wall permeability, and oxidizes sulfhydral and sulfur bonds in proteins, enzymes, and other metabolites[654, 726].

Microbicidal Activity. Peracetic acid will inactivate gram-positive and gram-negative bacteria, fungi, and yeasts in <5 minutes at <100 ppm. In the presence of organic matter, 200-500 ppm is required. For viruses, the dosage range is wide (12-2250 ppm), with poliovirus inactivated in yeast extract in 15 minutes with 1500 to 2250 ppm. Bacterial spores in suspension are inactivated in 15 seconds to 30 minutes with 500 to 10,000 ppm (0.05 to 1%)[654].

Simulated-use trials have demonstrated microbicidal activity [111, 718-722] and three clinical trials have demonstrated both microbial killing and no clinical failures leading to infection[90, 723, 724]. Alfa and co-workers, who compared the peracetic acid system with ETO, demonstrated the high efficacy of the system. Only the peracetic acid system was able to completely kill 6-$\log_{10}$ of *Mycobacterium chelonae*, *Enterococcus faecalis*, and *B. atrophaeus* spores with both an organic and inorganic challenge[722]. Like other sterilization processes, the efficacy of the process can be diminished by soil challenges [902] and test conditions[856].

Uses. This automated machine is used to chemically sterilize medical (e.g., GI endoscopes) and surgical (e.g., flexible endoscopes) instruments in the United States. Lumened endoscopes must be connected to an appropriate channel connector to ensure that the sterilant has direct contact with the contaminated lumen. [137, 856, 903] Olympus America has not listed this system as a compatible product for

use in reprocessing Olympus bronchoscopes and gastrointestinal endoscopes (Olympus America, January 30, 2002, written communication).

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 except the peracetic acid immersion system (Table 11)[721]. Complete killing (or removal) of 6-$\log_{10}$ of *Enterococcus faecalis, Mycobacterium chelonei*, and *Bacillus atrophaeus* spores in the presence of salt and serum and lumen test objects was observed only for the peracetic acid immersion system.

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].

Bioburden of Surgical Devices

In general, used medical devices are contaminated with a relatively low bioburden of organisms[179, 911, 912]. Nystrom evaluated medical instruments used in general surgical, gynecological, orthopedic, and ear-nose-throat operations and found that 62% of the instruments were contaminated with $<10^1$ organisms after use, 82% with $<10^2$, and 91% with $<10^3$. After being washed in an instrument washer, more than 98% of the instruments had $<10^1$ organisms, and none $>10^2$ organisms[911]. Other investigators have published similar findings[179, 912]. For example, after a standard cleaning procedure, 72% of 50 surgical instruments contained $<10^1$ organisms, 86% $<10^2$, and only 6% had $>3 \times 10^2$[912]. In another study of rigid-lumen medical devices, the bioburden on both the inner and outer surface of the lumen ranged from 10^1 to 10^4 organisms per device. After cleaning, 83% of the devices had a bioburden $\leq 10^2$ organisms[179]. In all of these studies, the contaminating microflora consisted mainly of vegetative bacteria, usually of low pathogenicity (e.g., coagulase-negative *Staphylococcus*)[179, 911, 912].

An evaluation of the microbial load on used critical medical devices such as spinal anesthesia needles and angiographic catheters and sheaths demonstrated that mesophilic microorganisms were detected at levels of 10^1 to 10^2 in only two of five needles. The bioburden on used angiographic

catheters and sheath introducers exceeded 10^3 CFUs on 14% (3 of 21) and 21% (6 of 28), respectively[907].

Effect of Cleaning on Sterilization Efficacy

The effect of salt and serum on the efficacy of low-temperature sterilization technologies has raised concern regarding the margin of safety of these technologies. Experiments have shown that salts have the greatest impact on protecting microorganisms from killing[426, 469]. However, other studies have suggested that these concerns may not be clinically relevant. One study evaluated the relative rate of removal of inorganic salts, organic soil, and microorganisms from medical devices to better understand the dynamics of the cleaning process[426]. These tests were conducted by inoculating Alfa soil (tissue-culture media and 10% fetal bovine serum) [469] containing 10^6 *G. stearothermophilus* spores onto the surface of a stainless-steel scalpel blade. After drying for 30 minutes at 35°C followed by 30 minutes at room temperature, the samples were placed in water at room temperature. The blades were removed at specified times, and the concentration of total protein and chloride ion was measured. The results showed that soaking in deionized water for 60 seconds resulted in a >95% release rate of chloride ion from NaCl solution in 20 seconds, Alfa soil in 30 seconds, and fetal bovine serum in 120 seconds. Thus, contact with water for short periods, even in the presence of protein, rapidly leads to dissolution of salt crystals and complete inactivation of spores by a low-temperature sterilization process (Table 10). Based on these experimental data, cleaning procedures would eliminate the detrimental effect of high salt content on a low-temperature sterilization process.

These articles [426, 469, 721] assessing low-temperature sterilization technology reinforce the importance of meticulous cleaning before sterilization. These data support the critical need for healthcare facilities to develop rigid protocols for cleaning contaminated objects before sterilization[472]. Sterilization of instruments and medical devices is compromised if the process is not preceded by meticulous cleaning.

The cleaning of any narrow-lumen medical device used in patient care presents a major challenge to reprocessing areas. While attention has been focused on flexible endoscopes, cleaning issues related to other narrow-lumen medical devices such as sphinctertomes have been investigated[913]. This study compared manual cleaning with that of automated cleaning with a narrow-lumen cleaner and found that only retro-flushing with the narrow lumen cleaner provided adequate cleaning of the three channels. If reprocessing was delayed for more than 24 hours, retro-flush cleaning was no longer effective and ETO sterilization failure was detected when devices were held for 7 days [913]. In another study involving simulated-use cleaning of laparoscopic devices, Alfa found that minimally the use of retro-flushing should be used during cleaning of non-ported laparoscopic devices[914].

Other Sterilization Methods

Ionizing Radiation. Sterilization by ionizing radiation, primarily by cobalt 60 gamma rays or electron accelerators, is a low-temperature sterilization method that has been used for a number of medical products (e.g., tissue for transplantation, pharmaceuticals, medical devices). There are no FDA-cleared ionizing radiation sterilization processes for use in healthcare facilities. Because of high sterilization costs, this method is an unfavorable alternative to ETO and plasma sterilization in healthcare facilities but is suitable for large-scale sterilization. Some deleterious effects on patient-care equipment associated with gamma radiation include induced oxidation in polyethylene [915] and delamination and cracking in polyethylene knee bearings[916]. Several reviews [917, 918] dealing with the sources, effects, and application of ionizing radiation may be referred to for more detail.

Dry-Heat Sterilizers. This method should be used only for materials that might be damaged by moist heat or that are impenetrable to moist heat (e.g., powders, petroleum products, sharp instruments). The advantages for dry heat include the following: it is nontoxic and does not harm the environment; a dry heat cabinet is easy to install and has relatively low operating costs; it penetrates materials; and it is noncorrosive for metal and sharp instruments. The disadvantages for dry heat are the slow rate of heat penetration and microbial killing makes this a time-consuming method. In addition, the high temperatures