delay caused by mailing to the test laboratory could potentially cause false-negatives results. Studies revealed, however, that the post-sterilization time and temperature after a 7-day delay had no influence on the test results[219]. Delays (7 days at 27°C and 37°C, 3-day mail delay) did not cause any predictable pattern of inaccurate spore tests [220].

Disinfection of HBV-, HCV-, HIV- or TB-Contaminated Devices

The CDC recommendation for high-level disinfection of HBV-, HCV-, HIV- or TB-contaminated devices is appropriate because experiments have demonstrated the effectiveness of high-level disinfectants to inactivate these and other pathogens that might contaminate semicritical devices [61, 62, 73, 81, 105, 121, 125, 221-238]. Nonetheless, some healthcare facilities have modified their disinfection procedures when endoscopes are used with a patient known or suspected to be infected with HBV, HIV, or *M. tuberculosis* [28, 239]. This is inconsistent with the concept of Standard Precautions that presumes all patients are potentially infected with bloodborne pathogens[228]. Several studies have highlighted the inability to distinguish HBV- or HIV-infected patients from noninfected patients on clinical grounds[240-242]. In addition, mycobacterial infection is unlikely to be clinically apparent in many patients. In most instances, hospitals that altered their disinfection procedure used EtO sterilization on the endoscopic instruments because they believed this practice reduced the risk for infection [28, 239]. EtO is not routinely used for endoscope sterilization because of the lengthy processing time. Endoscopes and other semicritical devices should be managed the same way regardless of whether the patient is known to be infected with HBV, HCV, HIV or *M. tuberculosis*.

An evaluation of a manual disinfection procedure to eliminate HCV from experimentally contaminated endoscopes provided some evidence that cleaning and 2% glutaraldehyde for 20 minutes should prevent transmission [236]. A study that used experimentally contaminated hysteroscopes detected HCV by polymerase chain reaction (PCR) in one (3%) of 34 samples after cleaning with a detergent, but no samples were positive after treatment with a 2% glutaraldehyde solution for 20 minutes [120]. Another study demonstrated complete elimination of HCV (as detected by PCR) from endoscopes used on chronically infected patients after cleaning and disinfection for 3–5 minutes in glutaraldehyde [118]. Similarly, PCR was used to demonstrate complete elimination of HCV after standard disinfection of experimentally contaminated endoscopes [236] and endoscopes used on HCV-antibody–positive patients had no detectable HCV RNA after high-level disinfection [243]. The inhibitory activity of a phenolic and a chlorine compound on HCV showed that the phenolic inhibited the binding and replication of HCV, but the chlorine was ineffective, probably because of its low concentration and its neutralization in the presence of organic matter [244].

Disinfection in the Hemodialysis Unit

Hemodialysis systems include hemodialysis machines, water supply, water-treatment systems, and distribution systems. During hemodialysis, patients have acquired bloodborne viruses and pathogenic bacteria [245-247]. Cleaning and disinfection are important components of infection control in a hemodialysis center. EPA and FDA regulate disinfectants used to reprocess hemodialyzers, hemodialysis machines, and water-treatment systems.

Noncritical surfaces (e.g., dialysis bed or chair, countertops, external surfaces of dialysis machines, and equipment [scissors, hemostats, clamps, blood pressure cuffs, stethoscopes]) should be disinfected with an EPA-registered disinfectant unless the item is visibly contaminated with blood; in that case a tuberculocidal agent (or a disinfectant with specific label claims for HBV and HIV) or a 1:100 dilution of a hypochlorite solution (500–600 ppm free chlorine) should be used [246, 248]. This procedure accomplishes two goals: it removes soil on a regular basis and maintains an environment that is consistent with good patient care. Hemodialyzers are disinfected with peracetic acid, formaldehyde, glutaraldehyde, heat pasteurization with citric acid, and chlorine-containing compounds [249]. Hemodialysis systems usually are disinfected by chlorine-based disinfectants (e.g., sodium hypochlorite), aqueous

formaldehyde, heat pasteurization, ozone, or peracetic acid [250, 251]. All products must be used according to the manufacturers' recommendations. Some dialysis systems use hot-water disinfection to control microbial contamination.

At its high point, 82% of U.S. chronic hemodialysis centers were reprocessing (i.e., reusing) dialyzers for the same patient using high-level disinfection [249]. However, one of the large dialysis organizations has decided to phase out reuse and, by 2002 the percentage of dialysis facilities reprocessing hemodialyzers had decreased to 63% [252]. The two commonly used disinfectants to reprocess dialyzers were peracetic acid and formaldehyde; 72% used peracetic acid and 20% used formaldehyde to disinfect hemodialyzers. Another 4% of the facilities used either glutaraldehyde or heat pasteurization in combination with citric acid [252]. Infection-control recommendations, including disinfection and sterilization and the use of dedicated machines for hepatitis B surface antigen (HBsAg)-positive patients, in the hemodialysis setting were detailed in two reviews [245, 246]. The Association for the Advancement of Medical Instrumentation(AAMI) has published recommendations for the reuse of hemodialyzers[253].

Inactivation of *Clostridium difficile*

The source of health-care–associated acquisition of *Clostridium difficile* in nonepidemic settings has not been determined. The environment and carriage on the hands of health-care personnel have been considered possible sources of infection [66, 254]. Carpeted rooms occupied by a patient with *C. difficile* were more heavily contaminated with *C. difficile* than were noncarpeted rooms [255]. Because *C. difficile* spore-production can increase when exposed to nonchlorine-based cleaning agents and the spores are more resistant than vegetative cells to commonly used surface disinfectants[256], some investigators have recommended use of dilute solutions of hypochlorite (1,600 ppm available chlorine) for routine environmental disinfection of rooms of patients with *C. difficile*-associated diarrhea or colitis [257], to reduce the incidence of *C. difficile* diarrhea [258], or in units with high *C. difficile* rates. [259] Stool samples of patients with symptomatic *C. difficile* colitis contain spores of the organism, as demonstrated by ethanol treatment of the stool to reduce the overgrowth of fecal flora when isolating *C. difficile* in the laboratory[260, 261]. *C. difficile*-associated diarrhea rates were shown to have decreased markedly in a bone-marrow transplant unit (from 8.6 to 3.3 cases per 1,000 patient-days) during a period of bleach disinfection (1:10 dilution) of environmental surfaces compared with cleaning with a quaternary ammonium compound. Because no EPA-registered products exist that are specific for inactivating *C. difficile* spores, use of diluted hypochlorite should be considered in units with high *C. difficile* rates. Acidified bleach and regular bleach (5000 ppm chlorine) can inactivate 10^6 *C. difficile* spores in $\leq$10 minutes [262]. However, studies have shown that asymptomatic patients constitute an important reservoir within the health-care facility and that person-to-person transmission is the principal means of transmission between patients. Thus, combined use of hand washing, barrier precautions, and meticulous environmental cleaning with an EPA-registered disinfectant (e.g., germicidal detergent) should effectively prevent spread of the organism [263].

Contaminated medical devices, such as colonoscopes and thermometers,can be vehicles for transmission of *C. difficile* spores [264]. For this reason, investigators have studied commonly used disinfectants and exposure times to assess whether current practices can place patients at risk. Data demonstrate that 2% glutaraldehyde [79, 265-267] and peracetic acid [267, 268] reliably kill *C. difficile* spores using exposure times of 5–20 minutes. *ortho*-Phthalaldehyde and $\geq$0.2% peracetic acid (WA Rutala, personal communication, April 2006) also can inactivate $\geq 10^4$ *C. difficile* spores in 10–12 minutes at 20°C [268]. Sodium dichloroisocyanurate at a concentration of 1000 ppm available chlorine achieved lower $\log_{10}$ reduction factors against *C. difficile* spores at 10 min, ranging from 0.7 to 1.5, than 0.26% peracetic acid with $\log_{10}$ reduction factors ranging from 2.7 to 6.0[268].

OSHA Bloodborne Pathogen Standard

In December 1991, OSHA promulgated a standard entitled "Occupational Exposure to

Bloodborne Pathogens" to eliminate or minimize occupational exposure to bloodborne pathogens [214]. One component of this requirement is that all equipment and environmental and working surfaces be cleaned and decontaminated with an appropriate disinfectant after contact with blood or other potentially infectious materials. Even though the OSHA standard does not specify the type of disinfectant or procedure, the OSHA original compliance document [269] suggested that a germicide must be tuberculocidal to kill the HBV. To follow the OSHA compliance document a tuberculocidal disinfectant (e.g., phenolic, and chlorine) would be needed to clean a blood spill. However, in February 1997, OSHA amended its policy and stated that EPA-registered disinfectants labeled as effective against HIV and HBV would be considered as appropriate disinfectants ". . . provided such surfaces have not become contaminated with agent(s) or volumes of or concentrations of agent(s) for which higher level disinfection is recommended." When bloodborne pathogens other than HBV or HIV are of concern, OSHA continues to require use of EPA-registered tuberculocidal disinfectants or hypochlorite solution (diluted 1:10 or 1:100 with water) [215, 228]. Studies demonstrate that, in the presence of large blood spills, a 1:10 final dilution of EPA-registered hypochlorite solution initially should be used to inactivate bloodborne viruses [63, 235] to minimize risk for infection to health-care personnel from percutaneous injury during cleanup.

Emerging Pathogens (*Cryptosporidium, Helicobacter pylori, Escherichia coli* O157:H7, Rotavirus, Human Papilloma Virus, Norovirus, Severe Acute Respiratory Syndrome [SARS] Coronavirus)

Emerging pathogens are of growing concern to the general public and infection-control professionals. Relevant pathogens include *Cryptosporidium parvum, Helicobacter pylori, E. coli* O157:H7, HIV, HCV, rotavirus, norovirus, severe acute respiratory syndrome (SARS) coronavirus, multidrug-resistant *M. tuberculosis*, and nontuberculous mycobacteria (e.g., *M. chelonae*). The susceptibility of each of these pathogens to chemical disinfectants and sterilants has been studied. With the exceptions discussed below, all of these emerging pathogens are susceptible to currently available chemical disinfectants and sterilants [270].

Cryptosporidium is resistant to chlorine at concentrations used in potable water. *C. parvum* is not completely inactivated by most disinfectants used in healthcare including ethyl alcohol [271], glutaraldehyde [271, 272], 5.25% hypochlorite [271], peracetic acid [271], ortho-phthalaldehyde [271], phenol [271, 272], povidone-iodine [271, 272], and quaternary ammonium compounds[271]. The only chemical disinfectants and sterilants able to inactivate greater than 3 $\log_{10}$ of *C. parvum* were 6% and 7.5% hydrogen peroxide [271]. Sterilization methods will fully inactivate *C. parvum,* including steam [271], EtO [271, 273], and hydrogen peroxide gas plasma[271]. Although most disinfectants are ineffective against *C. parvum*, current cleaning and disinfection practices appear satisfactory to prevent healthcare-associated transmission. For example, endoscopes are unlikely to be an important vehicle for transmitting *C. parvum* because the results of bacterial studies indicate mechanical cleaning will remove approximately 10^4 organisms, and drying results in rapid loss of *C. parvum* viability (e.g., 30 minutes, 2.9 $\log_{10}$ decrease; and 60 minutes, 3.8 $\log_{10}$ decrease) [271].

Chlorine at ~1 ppm has been found capable of eliminating approximately 4 $\log_{10}$ of *E. coli* O157:H7 within 1 minute in a suspension test[64]. Electrolyzed oxidizing water at 23°C was effective in 10 minutes in producing a 5-$\log_{10}$ decrease in *E. coli* O157:H7 inoculated onto kitchen cutting boards[274]. The following disinfectants eliminated >5 $\log_{10}$ of *E. coli* O157:H7 within 30 seconds: a quaternary ammonium compound, a phenolic, a hypochlorite (1:10 dilution of 5.25% bleach), and ethanol[53]. Disinfectants including chlorine compounds can reduce *E. coli* O157:H7 experimentally inoculated onto alfalfa seeds or sprouts [275, 276] or beef carcass surfaces[277].

Data are limited on the susceptibility of *H. pylori* to disinfectants. Using a suspension test, one study assessed the effectiveness of a variety of disinfectants against nine strains of *H. pylori* [60]. Ethanol (80%) and glutaraldehyde (0.5%) killed all strains within 15 seconds; chlorhexidine gluconate (0.05%, 1.0%), benzalkonium chloride (0.025%, 0.1%), alkyldiaminoethylglycine hydrochloride (0.1%), povidone-iodine (0.1%), and sodium hypochlorite (150 ppm) killed all strains within 30 seconds. Both ethanol

(80%) and glutaraldehyde (0.5%) retained similar bactericidal activity in the presence of organic matter; the other disinfectants showed reduced bactericidal activity. In particular, the bactericidal activity of povidone-iodine (0.1%) and sodium hypochlorite (150 ppm) markedly decreased in the presence of dried yeast solution with killing times increased to 5 - 10 minutes and 5 - 30 minutes, respectively.

Immersing biopsy forceps in formalin before obtaining a specimen does not affect the ability to culture *H. pylori* from the biopsy specimen [278]. The following methods are ineffective for eliminating *H. pylori* from endoscopes: cleaning with soap and water [119, 279], immersion in 70% ethanol for 3 minutes[280], instillation of 70% ethanol[126], instillation of 30 ml of 83% methanol[279], and instillation of 0.2% Hyamine solution[281]. The differing results with regard to the efficacy of ethyl alcohol against *Helicobacter* are unexplained. Cleaning followed by use of 2% alkaline glutaraldehyde (or automated peracetic acid) has been demonstrated by culture to be effective in eliminating *H. pylori* [119, 279, 282]. Epidemiologic investigations of patients who had undergone endoscopy with endoscopes mechanically washed and disinfected with 2.0%–2.3% glutaraldehyde have revealed no evidence of person-to-person transmission of *H. pylori* [126, 283]. Disinfection of experimentally contaminated endoscopes using 2% glutaraldehyde (10-minute, 20-minute, 45-minute exposure times) or the peracetic acid system (with and without active peracetic acid) has been demonstrated to be effective in eliminating *H. pylori* [119]. *H. pylori* DNA has been detected by PCR in fluid flushed from endoscope channels after cleaning and disinfection with 2% glutaraldehyde [284]. The clinical significance of this finding is unclear. *In vitro* experiments have demonstrated a >3.5-$\log_{10}$ reduction in *H. pylori* after exposure to 0.5 mg/L of free chlorine for 80 seconds[285].

An outbreak of healthcare-associated rotavirus gastroenteritis on a pediatric unit has been reported [286]. Person to person through the hands of health-care workers was proposed as the mechanism of transmission. Prolonged survival of rotavirus on environmental surfaces (90 minutes to >10 days at room temperature) and hands (>4 hours) has been demonstrated. Rotavirus suspended in feces can survive longer [287, 288]. Vectors have included hands, fomites, air, water, and food [288, 289]. Products with demonstrated efficacy (>3 $\log_{10}$ reduction in virus) against rotavirus within 1 minute include: 95% ethanol, 70% isopropanol, some phenolics, 2% glutaraldehyde, 0.35% peracetic acid, and some quaternary ammonium compounds [59, 290-293]. In a human challenge study, a disinfectant spray (0.1% ortho-phenylphenol and 79% ethanol), sodium hypochlorite (800 ppm free chlorine), and a phenol-based product (14.7% phenol diluted 1:256 in tapwater) when sprayed onto contaminated stainless steel disks**,** were effective in interrupting transfer of a human rotavirus from stainless steel disk to fingerpads of volunteers after an exposure time of 3- 10 minutes. A quaternary ammonium product (7.05% quaternary ammonium compound diluted 1:128 in tapwater) and tapwater allowed transfer of virus [52].

No data exist on the inactivation of HPV by alcohol or other disinfectants because in vitro replication of complete virions has not been achieved. Similarly, little is known about inactivation of noroviruses (members of the family *Caliciviridae* and important causes of gastroenteritis in humans) because they cannot be grown in tissue culture. Improper disinfection of environmental surfaces contaminated by feces or vomitus of infected patients is believed to play a role in the spread of noroviruses in some settings [294-296]. Prolonged survival of a norovirus surrogate (i.e., feline calicivirus virus [FCV], a closely related cultivable virus) has been demonstrated (e.g., at room temperature, FCV in a dried state survived for 21–18 days) [297]. Inactivation studies with FCV have shown the effectiveness of chlorine, glutaraldehyde, and iodine-based products whereas the quaternary ammonium compound, detergent, and ethanol failed to inactivate the virus completely. [297] An evaluation of the effectiveness of several disinfectants against the feline calicivirus found that bleach diluted to 1000 ppm of available chlorine reduced infectivity of FCV by 4.5 logs in 1 minute. Other effective ($\log_{10}$ reduction factor of >4 in virus) disinfectants included accelerated hydrogen peroxide, 5,000 ppm (3 min); chlorine dioxide, 1,000 ppm chlorine (1 min); a mixture of four quaternary ammonium compounds, 2,470 ppm (10 min); 79% ethanol with 0.1% quaternary ammonium compound (3 min); and 75% ethanol (10 min) [298]. A quaternary ammonium compound exhibited activity against feline calicivirus supensions dried on hard surface carriers in 10 minutes [299]. Seventy percent ethanol and 70% 1-propanol reduced FCV by a 3–4-$\log_{10}$

reduction in 30 seconds [300].

CDC announced that a previously unrecognized human virus from the coronavirus family is the leading hypothesis for the cause of a described syndrome of SARS [301]. Two coronaviruses that are known to infect humans cause one third of common colds and can cause gastroenteritis. The virucidal efficacy of chemical germicides against coronavirus has been investigated. A study of disinfectants against coronavirus 229E found several that were effective after a 1-minute contact time; these included sodium hypochlorite (at a free chlorine concentration of 1,000 ppm and 5,000 ppm), 70% ethyl alcohol, and povidone-iodine (1% iodine) [186]. In another study, 70% ethanol, 50% isopropanol, 0.05% benzalkonium chloride, 50 ppm iodine in iodophor, 0.23% sodium chlorite, 1% cresol soap and 0.7% formaldehyde inactivated >3 logs of two animal coronaviruses (mouse hepatitis virus, canine coronavirus) after a 10-minute exposure time [302]. The activity of povidone-iodine has been demonstrated against human coronaviruses 229E and OC43 [303]. A study also showed complete inactivation of the SARS coronavirus by 70% ethanol and povidone-iodine with an exposure times of 1 minute and 2.5% glutaraldehyde with an exposure time of 5 minute [304]. Because the SARS coronavirus is stable in feces and urine at room temperature for at least 1–2 days (WHO, 2003; http://www.who.int/csr/sars/survival_2003_05_04/en/index.html), surfaces might be a possible source of contamination and lead to infection with the SARS coronavirus and should be disinfected. Until more precise information is available, environments in which SARS patients are housed should be considered heavily contaminated, and rooms and equipment should be thoroughly disinfected daily and after the patient is discharged. EPA-registered disinfectants or 1:100 dilution of household bleach and water should be used for surface disinfection and disinfection on noncritical patient-care equipment. High-level disinfection and sterilization of semicritical and critical medical devices, respectively, does not need to be altered for patients with known or suspected SARS.

Free-living amoeba can be pathogenic and can harbor agents of pneumonia such as *Legionella pneumophila*. Limited studies have shown that 2% glutaraldehyde and peracetic acid do not completely inactivate *Acanthamoeba polyphaga* in a 20-minute exposure time for high-level disinfection. If amoeba are found to contaminate instruments and facilitate infection, longer immersion times or other disinfectants may need to be considered [305].

Inactivation of Bioterrorist Agents

Publications have highlighted concerns about the potential for biological terrorism[306, 307]. CDC has categorized several agents as “high priority” because they can be easily disseminated or transmitted from person to person, cause high mortality, and are likely to cause public panic and social disruption [308]. These agents include *Bacillus anthracis* (the cause of anthrax), *Yersinia pestis* (plague), variola major (smallpox), *Clostridium botulinum* toxin (botulism), *Francisella tularensis* (tularemia), filoviruses (Ebola hemorrhagic fever, Marburg hemorrhagic fever); and arenaviruses (Lassa [Lassa fever], Junin [Argentine hemorrhagic fever]), and related viruses[308].

A few comments can be made regarding the role of sterilization and disinfection of potential agents of bioterrorism[309]. First, the susceptibility of these agents to germicides *in vitro* is similar to that of other related pathogens. For example, variola is similar to vaccinia [72, 310, 311] and *B. anthracis* is similar to *B. atrophaeus* (formerly *B. subtilis)*[312, 313]. *B. subtilis* spores, for instance, proved as resistant as, if not more resistant than, *B. anthracis* spores (>6 $\log_{10}$ reduction of *B. anthracis* spores in 5 minutes with acidified bleach [5,250 ppm chlorine])[313]. Thus, one can extrapolate from the larger database available on the susceptibility of genetically similar organisms[314]. Second, many of the potential bioterrorist agents are stable enough in the environment that contaminated environmental surfaces or fomites could lead to transmission of agents such as *B. anthracis*, *F. tularensis*, variola major, *C. botulinum* toxin, and *C. burnetti* [315]. Third, data suggest that current disinfection and sterilization practices are appropriate for managing patient-care equipment and environmental surfaces when potentially contaminated patients are evaluated and/or admitted in a health-care facility after exposure to a bioterrorist agent. For example,