Inactivation of Cryptosporidium parvum oocyst infectivity by disinfection and sterilization processes

Susan L. Barbee, MS, David J. Weber, MD, MPH, Mark D. Sobsey, PhD, William A. Rutala, PhD, MPH

Chapel Hill, North Carolina

Background: Cryptosporidium parvum is a common cause of self-limited gastroenteritis in the normal host but may cause severe disease in immunocompromised persons. Person-to-person transmission has been well documented in households, child care centers, and hospitals. Because contaminated environmental surfaces and medical devices such as endoscopes may play a role in disease transmission, we studied the susceptibility of C. parvum to chemical agents commonly used for disinfection and evaluated the efficacy of sterilization processes.

Methods: Seven disinfectants were studied at their use dilution using a suspension test. Antimicrobial activity was assessed with the use of a cell infectivity assay.

Results: All sterilization processes tested (steam, ethylene oxide, Sterrad 100) inactivated 3 logs or greater of C. parvum. The only liquid disinfectant/sterilant able to inactivate greater than 3 logs of C. parvum was 6% and 7.5% hydrogen peroxide. Agents that did not completely inactivate C. parvum included hydrogen peroxide at lower concentrations or exposure times, peracetic acid, sodium hypochlorite, a phenolic, a quaternary ammonium compound, 2% glutaraldehyde, and ortho-phthalaldehyde.

Conclusions: Most high-level disinfectants used on endoscopes have limited efficacy against C. parvum. However, the infectivity of C. parvum on dry surfaces decreases rapidly. Therefore, current cleaning and high-level disinfection guidelines are adequate to prevent nosocomial transmission of C. parvum by means of endoscopes. (Gastrointest Endosc 1999;49:605-11.)
ment. Because of this possibility of indirect transmission, we studied the susceptibility of *Cryptosporidium* to surface disinfectants, high-level disinfectants (used on endoscopes), and sterilization processes.

**MATERIAL AND METHODS**

**Oocyst**

Oocysts of *C. parvum* (Iowa isolate) which were isolated from the feces of experimentally infected neonatal calves and purified by differential sucrose gradients as previously described were purchased from Pleasant Hill Farm (Troy, Id.). Oocysts were stored in a phosphate-buffered saline solution (PBS), pH 7.5, containing 1000 U/mL penicillin and 1000 μg/mL streptomycin (Gibco, Grand Island, N.Y.). The oocyst suspension was stored at 4° C and used within 4 months of preparation. Before the experiments, the oocysts were washed twice in PBS then resuspended in sterile, distilled water. Oocyst concentrations were determined by hemocytometer enumeration under phase-contrast microscopy.

**Cell cultures**

Madin-Darby canine kidney (MDCK) cell cultures (ATCC CCL 34, Rockville, Md.) were obtained from the laboratory of M. J. Arrowood (Centers for Disease Control and Prevention, CDC, Atlanta, Ga.). MDCK cells were maintained in Dulbecco's modified Eagle's (DMEM/F12 medium (Sigma, St. Louis, Mo.) supplemented with 10% fetal bovine serum (Sigma), 2 mM l-glutamine (Sigma), and 15 mM HEPES buffer (Sigma). Cell cultures for the *Cryptosporidium* oocyst infectivity assay were propagated in double-chambered Lab Tech Chamber Slides (Nunc, Naperville, Ill.) using serum-free Ultraculture Medium (BioWhittaker, Walkersville, Md.) supplemented with 2 mM l-glutamine and antibiotics (kanamycin 250 μg/mL, gentamycin 50 μg/mL, mycostatin 150 μg/mL) at 37° C in a 5% carbon dioxide atmosphere.

**Liquid disinfectants/sterilants**

The disinfectants studied were by chemical class, use-dilution (trade name, if applicable; manufacturer): hydrogen peroxide, 6% and 3% (Mallinckrodt [30% hydrogen peroxide], McGaw Park, Ill.); Sporox, hydrogen peroxide, 7.5% (Reckitt-Colman, Montvale, N.J.); alkaline glutaraldehyde, 2.4% (CIDEX, Johnson & Johnson, Arlington, Tex.); phenolic (active ingredient, 9.65% sodium o-phenolphenate, 8.34% sodium p-tertiary-amylphenate), 1:128 (Vespheine Ilse, Calgon-Vestal, St. Louis, Mo.); quaternary ammonium (active ingredients, 8% alkyl [50% C14, 40% C12, 10% C16 dimethyl benzyl ammonium chloride]), 1:128 (TBQ, Calgon-Vestal, St. Louis, Mo.); sodium hypochlorite, 5.25% (Clorox, Oakland, Calif.); ethyl alcohol, 70% (Fisher Scientific, Norcross, Ga.); iodophor (1% available iodine), manufacturers recommended concentration, undiluted (Betadine, Purdue Fredrick Co., Norwalk, Conn.); peracetic acid, 0.35% and 0.2% (Alrich Chemical Co., Milwaukee, Wis.); peracetic acid, 0.2% (Steris Corp.[35% peracetic acid], Mentor, Ohio); and, ortho-phthalaldehyde, manufacturer's recommended concentration-undiluted (CIDEX-OPA, Johnson & Johnson). All products were stored in the dark at room temperature (23° to 25° C) and tested within their specified use-life. For evaluation of the Steris System disinfectant, the contents of the Steris carton were carefully diluted into 6.1 L of sterile, distilled water. The mixture was stirred until all contents were completely dissolved as has previously been described, then the sterilant was immediately used in the assay.

Products were analyzed before use when applicable to determine the current disinfectant concentration and test efficacy of the neutralizer chosen to terminate the reaction at the end of exposure. All disinfectants requiring dilution were diluted in sterile, distilled water (hardness ≤ 0.6 ppm dissolved solids) according to the manufacturer's instructions. The hypochlorite was tested using a pHydrion Testuff Sanitizer Kit (Fisher Scientific, Norcross, Ga.). The chlorine concentration was approximately 5.25%, which was completely neutralized by the addition of an equal volume of 2% sodium thiosulfate. The hydrogen peroxide and Sporox were analyzed with a hydrogen peroxide test kit (Hach, Loveland, Colo., with a sensitivity of 0.2 mg/L [ppm]). The initial concentration of the diluted product (Mallinckrodt) was 6% to 7% or 2% to 3% which was neutralized by an equal volume of 2% sodium thiosulfate. The glutaraldehyde product was tested using glutaraldehyde monitor strips (PyMaH Corp., Flemington, N.J.). Strips are semi-quantitative indicators that allow detection above or below 1% only. The initial concentration of the product was greater than 1% and was neutralized to less than 1% with the addition of an equal volume of 2% glycine. Betadine, an iodophor, was tested using a pHydrion Micro and Lo iodine test kit (Micro Essential Laboratory, Brooklyn, N.Y.) with a sensitivity of greater than 0 mg/L. The available iodine concentration was approximately 1%, which was neutralized by an equal volume of 2% sodium thiosulfate. No assay method was available for the ethyl alcohol, peracetic acid, CIDEX-OPA, Vespheine Ilse, or TBQ. An equal volume of double-strength Letheen Broth (Difco, Detroit, Mich.) was adopted as a neutralizer for these products, except peracetic acid which was neutralized with 1% sodium thiosulfate.

**Sterilization processes**

The ethylene oxide sterilizer (2057 Eagle Series ETO sterilizer/aerator, AMSCO, Erie, Pa.) was a standard sterilizer which used a hydrochlorofluorocarbon carrier gas (Oxyfume 2002, Allied Signal, Morristown, N.J., 10% ethylene oxide, 90% hydrochlorofluorocarbons [27% chlorodifluoromethane, 63% chlorotrifluoroethane]). This sterilizer was operated at 54° C with a 2-hour exposure to ETO followed by overnight aeration (~12 to 14 hours) at 55° C.

The Sterrad 100 system (Advanced Sterilization Products [ASP], Irvine, Calif.), uses a single diffusion stage (hydrogen peroxide vapor) and a plasma stage per sterilization cycle. Approximately 6 mg/L of hydrogen peroxide is delivered to the evacuated chamber and allowed
to diffuse for a fixed period of time. The vapor is excited by a radiofrequency energy of 13.6 MHz creating a secondary, low temperature plasma that forms around the products being sterilized. Hydrogen peroxide is converted into a variety of species, including the free radicals hydroxyl and hydroperoxyl which recombine during the process to form primary oxygen and water by-products, eliminating the need for aeration.41

The steam autoclave system (Vernitron Steam Sterilizer, Carlstadt, N.J.) was operated at 121°C for 18 minutes on a liquid cycle.

Liquid disinfection/sterilization exposure procedure

For each experimental treatment 100 μL of a suspension containing 10⁵ or 10⁶ purified oocysts were aliquoted to 1.5 mL polypropylene, sterile, siliconized micro centrifuge tubes. Oocysts were pelleted, washed twice in sterile, distilled water then resuspended in 0.5 mL of disinfectant pre-equilibrated to the target temperature. Samples were incubated in a thermostatically controlled waterbath at the target temperature for the specified time intervals. At completion of the required contact time with the disinfectant, 0.5 mL of the disinfectant neutralizer was added to stop the reaction. The oocysts were then pelleted by centrifugation, washed a second time with neutralizer, then washed with PBS to remove any residual disinfectant. After being washed, the oocysts were resuspended in 100 μL of Ultraculture medium before inoculation into MDCK cell cultures. In each experiment, all disinfection treatments were performed in triplicate at each concentration. A positive infectivity control consisting of oocyst suspension inoculated onto carriers was added to each experiment along with a negative control consisting of oocyst suspension inoculated onto carriers and left at room temperature in a laminar flow hood for the duration of the sterilization process (Sterrad 2 to 3 hours, ETO 18 to 24 hours).

**Heat (steam sterilization).** Suspensions containing 10⁵ and 10⁶ purified oocysts were dispensed to 1.5 mL polypropylene, sterile, siliconized microtubes. Tubes were wrapped in Kimguard (Kimberly Clark, Roswell, Ga.), then placed into the autoclave with caps open and processed according to standard operational procedure. At the completion of an autoclave run, sample tubes were capped, then immediately transferred to ice to cool down. Tubes were centrifuged and the oocysts were washed twice in PBS. Control oocyst suspensions were kept at room temperature for the duration of the autoclave cycle. Oocysts were resuspended in 100 μL Ultraculture medium and then added to cell cultures.

In vitro infectivity assay

Four-day old MDCK cell monolayers were inoculated with C parvum oocysts from the disinfection and sterilization experiments. The cell cultures were incubated for 48 hours at 37°C. Infected cell monolayers were washed with PBS then fixed with Bouin’s solution (Sigma). The fixed cells were blocked by exposure to 1% bovine serum albumin in PBS for 30 minutes. Cell culture–associated infective stages of C parvum were reacted with a rhodamine-labeled monoclonal antibody, CY3/C3-C3 provided by M. J. Arrowood (CDC) for 90 minutes. Cell layers were rinsed three times with 1.5 mL of PBS to remove unreacted antibodies. The cell layers were observed under an ultraviolet fluorescent microscopy.

Time-related survival

Washed pellets containing 10⁷ oocysts were resuspended in 20 μL of 70% ethanol and transferred to sterile, stainless steel surgical blades and allowed to air dry (approximately 7 minutes). After the oocyst suspension was dried, the inoculated blades were transferred to a sterile petri dish and left under a laminar flow hood at room temperature and ambient humidity (45% relative humidity) for varying time periods. Immediately after incubation, oocysts were recovered from carriers as previously described under “Ethylene Oxide and Sterrad 100.” Recovery controls were included. The oocyst titer after
recovery was determined by correcting initial concentration of test oocysts by recovery of controls. Recovered oocysts were diluted in Ultraculture medium to 10^5 and 10^6 oocysts/100 μL before addition to cell cultures. The oocysts used in these experiments were approximately 3 months old.

**Data analysis**

Microscopic examination of MDCK infected cell was performed to quantitate the extent of infectivity based on the frequency of presence of live parasitic stages in 100 random fields. Each experiment included an infectivity assay of serial 10-fold dilutions of a control oocyst suspension that was scored for the percent positive fields at each dilution. Oocyst titer was calculated using the dilutions at which only some of the 100 fields were infected. The percent positivity was converted to probit value and plotted against the log_{10} concentration of oocysts. A best fit linear regression was calculated as a dose-response. For treated samples, 100 microscopic fields were scored for infectivity, then percent positivity converted to probit value. The observed degree of infectivity of the treated samples was compared with that of the titer control providing an estimate of the infectivity of the treated oocysts exposed to a disinfection or sterilization process.

### RESULTS

**Die-off of *C parvum* with time**

When *Cryptosporidium* oocyst air-dried onto surgical blades were exposed to room temperature (i.e., 23° to 25° C) and a relative humidity of 45%, the following log_{10} decreases in infective *C parvum* oocysts was observed: 30 minutes, 2.9; 60 minutes, 3.8; and 90 minutes, greater than 4.0.

**Effect of liquid chemical disinfectants on *C parvum* infectivity**

The infectivity of *C parvum* oocysts was not decreased compared with controls when exposed to several disinfectants, including TBQ, Betadine, ethanol, Alrich peracetic acid (0.35%, 20° C, 20 min), Steris peracetic acid (0.2%, 23° to 25° C, 12 min), and CIDEX-OPA (Table 1). A detectable infectivity decrease of less than 1 log was observed with exposure to Clorox, Vespene IIse, and CIDEX. A greater than 1 log_{10} decrease in infectivity was observed for Alrich peracetic acid (0.2%, 50° C, 12 min), Steris peracetic acid (0.2%, 48° to 50° C, 12 min), and 3% hydrogen peroxide. Only 6% hydrogen peroxide (20° C, 20 min) reduced oocyst infectivity greater than 3 log_{10}, which was the detection limit of the assay. Inactivation by hydrogen peroxide was both concentration and time dependent. Hydrogen peroxide at a 6% concentration was more effective than at a 3% concentration for both 20- and 10-minute exposures. Hydrogen peroxide exposure at both 3% and 6% was more effective at 20 minutes than after 10 minutes.

**Effect of sterilization processes on *C parvum* infectivity**

Complete inactivation (> 3 log_{10}) of *C parvum* was observed after sterilization using a standard gravity displacement steam sterilizer, ethylene oxide, or the Sterrad 100 (Table 2). The latter two methods were effective even if the carrier inoculated with *C parvum* was placed in the center of a 40 cm tube of diameter of 1 or 3 mm.

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**Table 1. Susceptibility of *Cryptosporidium parvum* to liquid chemical disinfectants/sterilants**

<table>
<thead>
<tr>
<th>Product</th>
<th>Chemical class</th>
<th>Concentration</th>
<th>Temperature (°C)</th>
<th>Time (min)</th>
<th>Log_{10} infectivity reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBQ</td>
<td>Quaternary ammonium</td>
<td>1:128</td>
<td>20</td>
<td>10</td>
<td>No change</td>
</tr>
<tr>
<td>Clorox</td>
<td>Hypochlorite</td>
<td>5.25%</td>
<td>20</td>
<td>10</td>
<td>&lt; 1.0</td>
</tr>
<tr>
<td>Vespene IIse</td>
<td>Phenolic</td>
<td>1:128</td>
<td>20</td>
<td>10</td>
<td>0.6</td>
</tr>
<tr>
<td>Betadine</td>
<td>Iodophor</td>
<td>Undiluted (1% I₂)</td>
<td>20</td>
<td>20</td>
<td>No change</td>
</tr>
<tr>
<td>Ethyl alcohol</td>
<td>Alcohol</td>
<td>70%</td>
<td>20</td>
<td>10</td>
<td>No change</td>
</tr>
<tr>
<td>Hydrogen peroxide</td>
<td>Hydrogen peroxide</td>
<td>6%</td>
<td>20</td>
<td>20</td>
<td>&gt; 3.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3%</td>
<td>20</td>
<td>20</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6%</td>
<td>20</td>
<td>10</td>
<td>2.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3%</td>
<td>20</td>
<td>10</td>
<td>1.8</td>
</tr>
<tr>
<td>Sporox</td>
<td>Hydrogen peroxide</td>
<td>7.5%</td>
<td>20</td>
<td>20</td>
<td>&gt; 3.0</td>
</tr>
<tr>
<td>CIDEX</td>
<td>Glutaraldehyde</td>
<td>2.4%</td>
<td>25</td>
<td>45</td>
<td>0.3</td>
</tr>
<tr>
<td>Peracetic acid</td>
<td>Alrich</td>
<td>0.35%</td>
<td>20</td>
<td>20</td>
<td>No change</td>
</tr>
<tr>
<td>Peracetic acid</td>
<td>Steris</td>
<td>0.2%</td>
<td>23-25</td>
<td>12</td>
<td>No change</td>
</tr>
<tr>
<td>Cidex-OPA</td>
<td>Ortho-phthalaldehyde</td>
<td>Undiluted</td>
<td>20</td>
<td>20</td>
<td>No change</td>
</tr>
</tbody>
</table>

Data represent mean values of triplicate samples using both 10^5 and 10^6 oocysts per experimental treatment, except Betadine, ethyl alcohol, and Cidex which represent a mean of six samples at 10^6 oocysts per experimental treatment.
Cryptosporidium species are small coccidian pathogens of the intestinal tract, and the species C. parvum is the important human pathogen that is also found in many other mammals. Infection is initiated by the ingestion of the environmentally stable thick-walled oocysts. The most common symptom of infection is watery diarrhea but other symptoms are also common including abdominal pain, low-grade fever, and vomiting. Infection is generally confined to the GI tract although in immunocompromised persons there may rarely be involvement of the respiratory or biliary tracts.

A review by Guerrant of 78 reports of more than 131,000 patients with diarrhea and more than 6000 control subjects without diarrhea found Cryptosporidium infection in 2.1% to 6.1% of immunocompetent persons in industrialized and developing countries, respectively, versus 0.2% to 1.5% in asymptomatic control subjects. Guerrant also noted that 22 reports of nearly 2000 HIV-infected persons showed Cryptosporidium infection in 14% to 24% of HIV-infected persons with diarrhea versus 0% to 5% infection of asymptomatic HIV-infected persons. Seropidemiologic surveys suggest approximately 25% of young adults in the United States have had infection with Cryptosporidium.

Endoscopy is widely used for both diagnosis and therapy of GI disorders. Failure to appropriately clean and disinfect endoscopes has been associated with person-to-person transmission of infectious agents via a contaminated endoscope. Data regarding the susceptibility of GI pathogens to disinfectants used for surface decontamination as well as disinfectants used for high-level disinfection of endoscopes are important to minimize the risk of disease transmission via contaminated surfaces in endoscopy suites and/or via endoscopes.

We have used an in vitro cell culture infectivity assay to evaluate chemical disinfection efficacy against C. parvum. This assay provides for a quantitative estimate of inactivation and has a high degree of correlation with animal infectivity assays (M. J. Arrowood, personal communication). Previous investigations of the activity of disinfectants against C. parvum have the important flaws that either the concentration used or the duration of exposure were not consistent with current use practices or the assay method did not measure infectivity.

Our data demonstrated that no chemical agent currently used as a surface disinfectant or antiseptic (i.e., TBQ, Clorox, Vesphene IIse, betadine, ethyl alcohol) is capable of inactivating ≥ 3 log10 of Cryptosporidium after a contact time of 20 minutes or less. Thus, with the use of currently available agents, surface disinfection cannot be ensured. With the exception of 6% and 7.5% hydrogen peroxide at a contact time of 20 minutes, no chemical (i.e., CIDEX, peracetic acid, CIDEX-OPA) that may be used as a high-level disinfectant on endoscopes was capable of inactivating ≥ 3 log10 of Cryptosporidium when used at the recommended contact time. The glutaraldehyde product produced a 0.3 log10 decrease in C. parvum oocyst infectivity. Peracetic acid when used at 48° to 50° C, the operating temperature of the Steris System 1, was able to inactivate almost 2 log10 of C. parvum. However, our data demonstrated that the elevated temperature was the important factor in inactivation of C. parvum.

Because C. parvum is a common cause of gastroenteritis, endoscopes are likely to be contaminated with this agent. Our data demonstrate that steam sterilizers, ethylene oxide sterilizers, or the Sterrad 100 were effective in completely inactivating C. parvum on endoscopes. However, most endoscopes either undergo high-level disinfection with 2% glutaraldehyde (or other high-level disinfectants) or are treated in a chemical sterilization process with peracetic acid (i.e., Steris System 1). Immersion in a 2% glutaraldehyde solutions for 20 minutes at room temperature has been recommended for the disinfection of endo-

### Table 2.

Susceptibility of Cryptosporidium parvum to sterilization processes

<table>
<thead>
<tr>
<th>Sterilization process</th>
<th>Level or concentration</th>
<th>Carriers</th>
<th>Log10 reduction/ infectivity*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moist heat (under pressure)</td>
<td>250°C (121°C) for 18 min</td>
<td>Microtubes</td>
<td>&gt; 3</td>
</tr>
<tr>
<td>Ethylene oxide</td>
<td>450-500 mg/L at 55-60°C</td>
<td>Stainless steel blades in 1 mm lumen test unit</td>
<td>&gt; 3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Stainless steel blades in 3 mm lumen test unit</td>
<td>&gt; 3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Stainless steel blades in petri dish</td>
<td>&gt; 3</td>
</tr>
<tr>
<td>Sterrad 100</td>
<td>Hydrogen peroxide plasma</td>
<td>Stainless steel blades in 1 mm lumen test unit</td>
<td>&gt; 3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Stainless steel blades in 3 mm lumen test unit</td>
<td>&gt; 3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Stainless steel blades in petri dish</td>
<td>&gt; 3</td>
</tr>
</tbody>
</table>

*Data represent mean of triplicate samples at both 10^5 and 10^6 oocysts per experimental treatment.
scopes by several professional organizations. Immersion at 25°C for 45 minutes has been required by the Food and Drug Administration as the label claim for one 2% glutaraldehyde. Our data suggest that immersion in 2% glutaraldehyde is incapable of inactivating \( C.\ parvum \).

The environmental survival of \( C.\ parvum \) has been investigated. Robertson et al. used a vital dye test to evaluate \( C.\ parvum \) oocyst survival on surfaces at room temperature and observed 97% loss of viability by 2 hours and 100% by 4 hours. Our data confirm this loss of infectivity with time. We found a greater than 2.9 log reduction in oocysts infectivity within 30 minutes. However, it should be noted that \( C.\ parvum \) in diarrheal stool smeared on a wooden surface survived for up to 72 hours.

We believe that endoscopes likely do not represent an important vehicle for the cross-transmission of \( C.\ parvum \) because mechanical cleaning removes approximately 10⁴ organisms, and our data and that of other investigators has demonstrated that \( C.\ parvum \) viability is reduced over time on dry environmental surfaces. Meticulous cleaning and high-level disinfection or sterilization is crucial to preventing the possibility of person-to-person transmission of \( C.\ parvum \) via endoscopes. Our data suggest that commonly used disinfectants would be incapable of inactivating infectious \( C.\ parvum \) oocysts present on environmental surfaces. To prevent the possibility of healthcare workers acquiring \( Cryptosporidium \) or person-to-person transmission via surface contamination, endoscopy personnel should use standard precautions as defined by the Centers for Disease Control and Prevention which include avoiding eating, smoking, or apply lip balm in areas that may be contaminated with stool or blood and handwashing after contact with stool and after endoscopic procedures. Mechanical cleaning of potentially contaminated environmental surfaces may also be important.

Our data demonstrate that the most common sterilization processes (steam sterilization and ethylene oxide) effectively inactivated high numbers of \( C.\ parvum \) oocysts with greater than 3 logs₁₀ reduction. The Sterrad 100 which uses a plasma phase of hydrogen peroxide was also highly effective. However, one should note that our control data demonstrated that \( C.\ parvum \) viability decreases with time and with exposure to high temperature. Thus, time and/or temperature may play the critical role in the inactivation of \( C.\ parvum \) by ethylene oxide sterilization and the Sterrad 100 system. The vacuum cycle included in these sterilization processes may also damage \( C.\ parvum \) oocysts, but we did not assess the separate role of exposure to a vacuum.

Our data represent the most complete set of experiments to assess the infectivity of \( C.\ parvum \) to disinfection and sterilization processes used in healthcare. One must use caution in comparing our data with those of other studies that used different chemicals, exposure times, concentrations and assay techniques, especially assays that did not assess oocyst viability. In conclusion, we believe that our data do not warrant any change in the current recommendations for the cleaning and disinfection of endoscopes.

**ACKNOWLEDGMENTS**

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**DISCLOSURE STATEMENT**

Dr. Rutala has been a consultant to Clorox, Reckitt & Coleman, and Johnson & Johnson, and Drs. Weber and Sobsey have been consultants to Clorox. This research was not supported by any of these companies.

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