

Reviewer's report

Title: Modeling Microbial Survival in Buildup Biofilm for Complex Medical Devices

Version: 2 **Date:** 11 November 2008

Reviewer: Lawrence Muscarella

Reviewer's report:

November 10, 2008

A. DISCRETIONARY REVISIONS:

1. Initial comment: This manuscript states, and advances the theme, that GI endoscopes, for example, "pose a unique challenge to infection control." Some might argue, however, that the risk of infections associated with flexible endoscopes in general and GI endoscopes in particular is extremely low. Please have the authors reconcile in more detail these two seemingly dichotomic conclusions (e.g., explain in more detail the authors' comment about "unrecognized transmission").

Authors' response: Detailed text has been added to address this on page 4 in the manuscript.

Final comment: My comment/question was sufficiently addressed.

2. Initial comment: The authors state that: "Development of traditional biofilm in endoscopes has typically been associated with residual moisture remaining in channels that likely originated from water sources, e.g., in AERs and moisture in channels involving such organisms as *Mycobacterium* spp, *Legionella* spp, and *Pseudomonas aeruginosa*." Please have the authors provide one or more references in support of this statement.

Authors' response: Text has been rephrased with appropriate references added (see page 5, manuscript).

Final comment: My comment/question was sufficiently addressed.

3. Initial comment: This study uses vegetative bacteria to gauge the relative effectiveness of two high-level disinfectants. Please have the authors explain why it did not use *Clostridium difficile* (too difficult to grow and cultivate in a laboratory?) or atypical mycobacteria (e.g., *Mycobacterium terrae* is the microorganism of choice that the FDA uses to evaluate the effectiveness of high-level disinfectants). *M. terrae* is more resistant to high-level disinfection than vegetative bacteria and fungi, and atypical bacteria are described by these authors as organisms that form biofilms (TBFs).

Authors' response: *C. difficile* does not replicate under aerobic conditions and could not be used in this model. *Mycobacterium chelonae* was tested, but the data was not included because it was too much data for one manuscript and the results obtained with this mycobacterium species did not change the conclusions reached by testing vegetative bacteria.

Final comment: My comment/question was sufficiently addressed.

B. MINOR ESSENTIAL REVISIONS:

1. Initial comment: The statement that "most reports of infection transmission have been associated with breeches (sic) in the reprocessing protocol" should be modified to state that "virtually every report of infection transmission during flexible endoscopy has been associated with breaches in the reprocessing protocol."

Authors' response: Additional statistics have been added to support this statement and the text was reworded to reflect this (see page 4, manuscript).

Final comment: My comment/question was not addressed. To be clear, the reader can interpret this manuscript to be suggesting that cases of disease transmission and associated infection indeed have been documented despite "good adherence to infection control guidelines." My concern can be resolved by the authors stating in the manuscript that, while biofilms may pose a concern in vitro, there are no reports in the medical literature linking an endoscope, cleaned in accordance with current reprocessing standards and guidelines and not defective in design, to transmission of a biofilm or any other infectious agent. This is an important point not addressed in the manuscript.

2. Initial comment: Please have the authors provide one or more references in independent support of this manuscript's statement that: "Traditional biofilm (TBF) forms on a surface continually bathed in fluid and exposed to microorganisms (e.g. indwelling lines (catheter, IV) and medical implants)."

Authors' response: References have been added (see page 5, manuscript).

Final comment: My comment/question was not completely addressed but can be resolved by addressing the following. The authors of this manuscript place significant weight on the published papers authored by Pajkos et al. (2004) and Vickery et al. (2004). While these papers are important, their findings do not link the organisms that were collected in a laboratory setting to patient infections, which is the requisite linchpin of their conclusions. This and other concerns can be resolved by the authors of this manuscript stating that, to date and notwithstanding this manuscript's findings, there are no papers causally associating disease transmission to a biofilm that: (a) formed on the internal surfaces (e.g., channels) of flexible endoscopes, (b) remained on these surfaces despite staff compliance with current reprocessing guidelines and standards, and (c) were "protected from the disinfectant challenge, particularly from GLUT

challenge.” (I am unaware of any studies, including the manuscript’s references “2, 9, 11, 12, 18, 19,” that document disease transmission during flexible endoscopy due to a biofilm that formed on the internal surfaces of flexible endoscopes and remained on these surfaces despite staff compliance with current reprocessing guidelines and standards.) Whether a scenario has the potential to result in patient infection is very different from a scenario that has been directly linked to patient infection, and it is this specific distinction that this manuscript, in my opinion, needs to more clearly clarify, to avoid reader misinterpretation.

3. Initial comment: The authors state that: “However the gradual build up of material over repeated use in reprocessed flexible endoscopes forms by a very different kinetic background. The initial stages of formation (surface conditioning from patient secretions, microbial attachment, growth and colonization) are similar to TBF. However medical devices such as gastrointestinal (GI) endoscopes are used repeatedly in a day, with cyclic exposure to high levels of microbes due to contact with the mucosal surface of the gut.” I was unaware of this. Because these are not conclusions resulting from data collected during the study described in this manuscript, please have the authors provide one or more references in support of these statements.

Authors’ response: This is a research project; therefore, no published data exist. The text has been reworded (on the bottom of page 5 to top of page 6) to clarify that this is a hypothesis and the reason for the study.

Final comment: My comment/question was sufficiently addressed.

4. Initial comment: The authors state that: “Repeated use over time can facilitate a buildup biofilm formation (BBF) consisting of layers of dried organic material with embedded microorganisms. This resultant biofilm (BBF) would have a unique composition, microbial proliferation, biofilm formation and survival characteristics compared to TBF.” I was unaware of this, too. Because these are not conclusions resulting from data collected during the study described in this manuscript, please have the authors provide one or more references in support of this statement.

Authors’ response: Response as indicated in #3 (above). Our data is the first to show this.

Final comment: My comment/question was sufficiently addressed.

5. Initial comment: The “results” section of this manuscript states that: “The survival of a range of representative microorganisms (bacteria, fungi and viruses) in TBF and BBF was evaluated to determine the impact of an organic matrix and disinfectant chemistry.” Please have the authors remove “viruses” because they were not used during this study.

Authors’ response: Note, viruses were tested, but data from this testing has not

included in this manuscript, so the word “viruses” has been removed.

Final comment: My comment/question was sufficiently addressed

6. Initial comment: The text states that: “Nelson indicated that in reprocessed flexible endoscopes, residual organic matter and biofilm is likely a result of “multiple cleaning and disinfection cycles over the life of the instrument.”

True, but “Nelson” in this reference also questions whether the results of studies evaluating “biofilm as it relates to endoscope cleaning and disinfection” “can be generalized to clinical practice.” Further, Nelson states that: the “methodologic flaws” of studies that raise the possibility that biofilm might be an issue in GI endoscope reprocessing “make it difficult to determine the clinical relevance” of these published studies’ findings.

Please ask that the authors provide some discussion about the clinical relevance of their results. Specifically, please ask that the authors reconcile these statements by Nelson with the statement of this manuscript, which concludes that “the chance of organisms surviving device reprocessing and being transmitted to another patient is greater once BBF has developed, particularly when GLUT is used as the high-level disinfectant.”

In short, please have the authors clarify the specific clinical implications of this study’s results and clarify in this context what has and has not been published.

Authors’ response: Text has been added on page 20 to address the reviewer’s comments.

Final comment: I do not see my comment/question addressed on page 20 – maybe the authors meant p. 22. But, the comments on p. 22 do not resolve my comment/question.

The revised and improved text states that: “Therefore, the data from our in vitro model suggests that for patient-used endoscopes the chance of organisms surviving device reprocessing and being transmitted to another patient is greater if BBF has developed within the endoscope channels, particularly when GLUT is used as the high-level disinfectant with exposure times of 20 minutes at room temperature. Further clinical studies are warranted to further evaluate this finding.”

My comment/question would be resolved by the authors either removing the clause “particularly when GLUT is used as the high-level disinfectant with exposure times of 20 minutes at room temperature” or, alternatively, providing references that support this statement and that document a higher risk of infection (as this clause and manuscript strongly intimates) associated with glutaraldehyde solutions compared to other agents, such as the manuscript’s discussed hydrogen peroxide product. Papers showing an increased risk of infection associated with glutaraldehyde have not been published. Please clarify this for the reader.

7. Initial comment: Please have the authors clarify whether there is a difference between the labeling of the two tested disinfectants in the U.S. and Canada.

Authors' response: The text has been reworded on page 7 under Methods: 2. Test Disinfectants.

Final comment: My comment/question appears to have been sufficiently addressed.

8. Initial comment: Please have the authors validate that their detailed method of recovering microorganisms (i.e., sampling technique) from their test samples ("pegs") (e.g., rins(ing) in sPBS, 3 times for 20 seconds," etc.) is sound, consistent, and reliable. These validation data will verify whether the efficiency of recovery is 100%. If it is less than 100%, then a correction factor would have to be applied (e.g., a multiplication factor) to each of the collected data points, to compensate for inefficient recovery. (Without performing this validation test and providing these validation data, the reliability and efficiency of this recovery technique is unknown. Might it only recover 50% sometimes and, sporadically, 80% of the organisms another time?)

Alternatively, please ask the authors to provide a previously published reference that validates the soundness of this detailed sampling technique. Maybe references #13 and #14?

Authors' response: Error bars included on graphs indicate that reproducibility was excellent. Also, refer to bottom of page 11 and top of page 12 of the manuscript for further information on reproducibility.

Also: Page 8-9, Methods: 4.1 Recovery, we have added text to indicate that this recovery method is recommended for MBEC pegs by references 13, and 14.

Final comment: My comment/question was not addressed. The authors' point is well taken that the provided graphs indicate reproducibility. But, such reproducible data do not ensure or demonstrate the validity of an employed test method – in this case, a sampling technique. An invalid process or system that contains error can still yield reproducible, although wrong, data. For example, a ship's guidance system can be programmed to hit an enemy target using a high powered missile. This guidance system's software program, however, can be flawed and contain error such that one missile after another fired from the ship reproducibly and consistently hits the wrong target, an ally target. While the guidance system's outcome is reproducible – always hitting the ally's, instead of the enemy's, target – it is clearly flawed and in error.

My comment/question would be resolved by the authors if they were to mention in the manuscript's discussion section that validated tests were not performed to confirm a recovery efficiency of nearly 100%, that the manuscript's methodology assumes (without having shown) a 100% recovery in accordance with references 13 and 14.

9. Initial comment: The authors state that: “The results of our data demonstrate the ability of organisms to replicate in enzymatic detergent at the manufacturer’s recommended use-dilution when held at room temperature overnight (normal contact time recommended by the manufacturer for cleaning is at least 5 minutes). Therefore the practice (although not recommended) of leaving patient-used scopes in enzymatic detergent overnight or over the weekend can serve to increase rather than reduce microbial load and protein buildup, thereby hindering efficacy of the disinfection process.”

Please ask that the authors clarify whether they are aware of any reports of disease transmission directly attributed to the immersion of an endoscope in an enzymatic detergent overnight, or over the weekend. I am unaware of any such reports.

Authors’ response: No, we are not aware of any published reports of disease transmission related to immersion of an endoscope in an enzymatic detergent overnight or over the weekend. However, this practice is similar to “wet” overnight storage of flexible endoscopes which is recognized as poor practice by APIC guidelines (Ref 12).

Final comment: My comment/question appears to have been sufficiently addressed. I would note, however, that discussions of “immersion of an endoscope in an enzymatic detergent overnight or over the weekend” is not similar to “wet’ overnight storage of flexible endoscopes.” The former refers to a non-terminal step that is followed by disinfection and drying, whereas the latter refers to the storage of endoscopes that once were dry but that might have become wet during storage and that will be used wet on patients without undergoing any reprocessing measures, to prevent bacterial colonization and disease transmission.

C. MAJOR COMPULSORY REVISIONS:

1. Initial comment: The authors’ disclosure under the heading: “Competing interests” that “There are no competing interests for either of the authors.”

This manuscript evaluates the effectiveness of an accelerated hydrogen peroxide high-level disinfectant in comparison to 2% (alkaline) glutaraldehyde (Metricide#). Several published papers and reports indicate that one of the authors (MA) of this manuscript is a consultant for, and has been for several years financially associated with, the Steris Corporation, which markets this accelerated hydrogen peroxide high-level disinfectant – once known as PerCept (Virox) – under the name Resert#.

(To avoid reader confusion, please ask that the authors clarify whether Percept (previously marketed by Virox) is now marketed by the Steris Corporation as Resert#, and, if so, please ask the authors to replace the former name throughout the manuscript’s text with the latter name (see:

<http://www.steris.com/healthcare/view_product_page.cfm?productid=3804).)

Each of the following three references discloses the financial association of at least one of the authors of this manuscript (MA) with the Steris Corporation: (1) Alfa M, et al. Automated washing with the Reliance Endoscope Processing System and its equivalence to optimal manual cleaning. *AM J Infect Control* 2006;34:561-70; (2) Alfa MJ. System 1: Sterile processing system. *Liquid chemical sterilization anthology*. March 4, 2004. Pages 1-33; and (3) Davies P. Germ Watch: Clinic Infections Put a Sterilizer Of Lab Devices Under Microscope --- Maker of Widely Used System Defends Its Effectiveness After Bacterial Outbreaks --- Word of a Probe by the FDA. *The Wall Street Journal* 24 December 2004; page A1.

Indeed, it is possible that MA is no longer financially associated with the Steris Corporation or any other company. Please request that this be clarified and that each author disclose in this journal's "competing interests" section any direct or indirect financial associations with any companies that could pose a potential conflict of interest with this submitted manuscript's assumptions, test methods, and results.

It is important to disclosure any such existing financial associations, because this manuscript concludes that:

(a) "high-level disinfection using glutaraldehyde (Metricide) was less effective than AHP (the accelerated hydrogen peroxide high-level disinfectant) for killing microorganism in either TBF or BBF";

(b) "in-use studies of GI endoscope disinfection have shown HLD (high-level disinfection) alternatives to aldehyde chemistry (such as oxidants, e.g., peracetic acid ...) have a superior ability to reduce bacterial loads";

(c) Metricide, Cidex, and other 2% glutaraldehyde solutions are "not (a) very effective method to ensure microorganisms are eradicated"; and

(d) "rapidly escalating numbers of surviving microorganism can result when a cross-linking agent such as GLUT is used as the HLD."

I understand that authors pay a processing fee to this journal for the publication of submitted manuscripts. Please ask the authors to clarify their funding source for this fee, were this manuscript to be accepted and published in this journal.

Authors' response: The conflict of interest for both authors is as originally stated. The text has been reworded in Methods: 2. Test Disinfectants (page 7) to clarify the HLD conditions used and how these relate to currently acceptable practice and the manufacturer's label instructions.

Final comment: My comment/question was not addressed and remains an outstanding, unresolved issue. This journal's standards and policies for the proper management of potential conflicts of interest would presumably require that the authors openly and definitively disclose in the manuscript or cover letter

whether or not either is directly or indirectly financially associated with a company that markets, or plans to market, a hydrogen peroxide-based high-level disinfectant, similar to the one discussed in the manuscript (e.g., PreCept, marketed by Virox; or Resert, marketed by Steris), for reprocessing GI endoscopes and other types of semi-critical instruments. My understanding is not consistent with the manuscript's statement that "There are no competing interests for either MJA or RH." Please have the authors clarify whether or not either is associated with a company, like Steris, that markets a hydrogen-peroxide high-level disinfectant.

2. Initial comment: The authors state that: "The implication of these findings is that organisms remain trapped or embedded due to repetitive GLUT cycles that cross-link organic material forming a protective layer shielding them from the HLD. Once microbial survival within the BBF is established then "grow out" occurs quickly once reintroduced into a moist environment. This scenario supports findings by other researchers describing the kill ability of glutaraldehyde in biofilm but inability to remove bioburden with subsequent impairment of cleaning [17]."

Please have the authors clarify in the manuscript's discussion whether they are suggesting, per this study's results and conclusions, that that Metricide, Cidex, or another 2% glutaraldehyde solution has been independently documented to be associated with a higher risk of infection than the evaluated AHP product.

Please have the authors clarify in the manuscript's discussion whether they are aware of any cases as intimated in which organic material was identified to have formed a protective layer that shielded from a solution of 2% glutaraldehyde (or, for that matter, any other type of high-level disinfectant) pathogens (either that did or did not "grow out" in a moist environment) and was causally (directly) associated with healthcare-acquired infections following a procedure that used a GI endoscope reprocessed in accordance with current standards and practices.

If such papers have been published, please have the authors discuss them. My research indicates that no such papers have been published. If true, please request that the authors modify the text of their manuscript so that the reader does not erroneously conclude that studies showing that this detailed "scenario" (i.e., organic material forming o the internal channels of GI endoscope and infecting patients despite strict adherence to published reprocessing guidelines) have been published.

Contrary to the intimations of this manuscript's statement that Metricide, Cidex, and other 2% glutaraldehyde solutions are "not (a) very effective method to ensure microorganisms are eradicated," there are no published papers in the peer-reviewed medical literature that demonstrate, first, that any one type of FDA-cleared high-level disinfectant (e.g., 2% glutaraldehyde) or sterilant poses an increased risk of healthcare-acquired infection compared to another marketed high-level disinfectant or sterilant (e.g., accelerated hydrogen peroxide high-level disinfectant, peracetic acid) (assuming each is used in accordance with its

labeling and/or published guidelines), or, second, that 2% glutaraldehyde or any other disinfectant or sterilant on the U.S. market used in accordance with its labeling is “not very effective” for eradicating microorganisms. Please ask that the authors point these two points out, for perspective, in their manuscript.

Authors’ response: We have not stated that glutaraldehyde solutions are not effective in eradicating microorganisms. Indeed the wording used is: “The results of the TBF and BBF models in this study indicate that HLD is effective at killing bioburden within young biofilm but not mature biofilm and highlight the value in studying biofilm formation in reprocessed scopes over extended periods of time.” Using the BBF model in this study we present data showing a higher rate of survival when glutaraldehyde was used compared to AHP. Final conclusions have been modified to clarify this on pages 21 to 22 of the manuscript.

Final comment: Respectfully, I would disagree with the authors’ response. The reader is left with the false impression that published papers have demonstrated that hydrogen peroxide-based high-level disinfectants has been shown to be more effective than and associated with a decreased risk of healthcare-acquired infection compared to 2% glutaraldehyde. My comment/question can be resolved by the authors stating in the text that no published studies have shown any one high-level disinfectant to be associated with a lower risk of infection than another. Refer to my “initial comment,” above.

The authors’ modification of the manuscript on page 21 and 22 does not resolve my questions, but, rather, supports them, stating that: “Therefore, the data from our in vitro model suggests that for patient-used endoscopes the chance of organisms surviving device reprocessing and being transmitted to another patient is greater if BBF has developed within the endoscope channels, particularly when GLUT is used as the high-level disinfectant.”

My confusion is further advanced by, contrary to the authors’ response that “We have not stated that glutaraldehyde solutions are not effective in eradicating microorganisms,” the manuscript’s assertion that 2% glutaraldehyde solutions are “not (a) very effective method to ensure microorganisms are eradicated.”

3. Initial comment: The authors state that: “Repeated use over time can facilitate a buildup biofilm formation (BBF) consisting of layers of dried organic material with embedded microorganisms.” The authors also state that: “Biofilm formation has been suggested in reprocessed flexible endoscopes in spite of adequate reprocessing, [2, 9, 11, 12, 18, 19]” and that the study’s test organisms “are difficult pathogens to eliminate (in) by the endoscopic disinfection process.”

In the context of these and other statements in this manuscript, please have the authors clarify for the reader whether any published clinical (as opposed to laboratory, simulated) studies document disease transmission causally due to a biofilm that formed on the internal surfaces (e.g., channels) of flexible endoscopes, remained on these surfaces despite staff compliance with current reprocessing guidelines and standards, and were “protected from the disinfectant

challenge, particularly from GLUT challenge.”

If no such reports have been published, please ask the authors include this finding in this manuscript. (I am unaware of any studies, including references “2, 9, 11, 12, 18, 19,” that document disease transmission during flexible endoscopy due to a biofilm that formed on the internal surfaces of flexible endoscopes and remained on these surfaces despite staff compliance with current reprocessing guidelines and standards.)

Authors’ response: Issues related to the clinical impact of our findings have been added. Please refer to questions 1 and 2 in this section and pages 20, 21, 22 of the manuscript.

My comment: My comment/question was not addressed. The text continues to suggest a conclusion that is not supported by published data. My comment/question can be resolved by the authors stating in the manuscript that, the authors’ findings notwithstanding, lacking are published clinical (as opposed to laboratory, simulated) studies that document disease transmission causally due to a biofilm that formed on the internal surfaces (e.g., channels) of flexible endoscopes and remained on these surfaces despite staff compliance with current reprocessing guidelines and standards, which routinely include using 2% glutaraldehyde solutions to high-level disinfect endoscopes.

4. Initial comment: Please have the authors clarify whether the pegs (samples) were immersed in both high-level disinfectants in accordance with manufacturer’s instructions. The text states that: “HLD (high-level disinfection) was achieved using glutaraldehyde and accelerated hydrogen peroxide (for 20 minute exposure times at RT [room temperature] as per manufacturer’s recommendations).” This statement is confusing, because the labeling of Metricide, at least in the U.S., indicates a 45 minute immersion time, not a 20 minute immersion time as this statement implies. Please have the authors correct this error, assuming the labeling of Metricide in both the U.S. and Canada indicates a 45 minute immersion time at (25o C).

For the findings of this study arguably to be valid and to compare objectively the effectiveness of glutaraldehyde with AHP, it is arguably requisite that both high-level disinfectants be used at the specific FDA-cleared immersion times and temperatures indicated on their labeling. Please have the authors explain in the manuscript why the solution of Metricide they used during testing was used to achieve high-level disinfection during a 20 minute exposure at room temperature as Figure 1’s legend indicates, not for “45 minutes at 25o C,” “as per manufacturer’s recommendation.” Does the labeling of Metricide in Canada indicate a 20 minute immersion claim?

Because these authors performed their tests using 2% glutaraldehyde at 20 minutes instead of 45 minutes, it can be argued that this study’s specific testing methodology reduced the potential effectiveness of 2% glutaraldehyde by shortening its immersion time (the reasons for which this manuscript does not

discuss), posing an unfair testing environment that is biased in favor of AHP. If these test results are only germane to disinfectants used in Canada, and not the U.S., then please have the manuscript explain this for the reader.

Also, please have the authors clarify the immersion time and temperature listed on the FDA-cleared labeling of the AHP product used during this study (e.g., Resert). Is it 20 minutes at room temperature as this manuscript suggests?

Authors' response: We have included further information on page 20 and 21 of the manuscript for clarification.

Final comment: My comment/question was sufficiently addressed.

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Declaration of competing interests:

I am employed by Custom Ultrasonics, Inc., a manufacturer of washer-disinfectors for reprocessing flexible endoscopes. Neither myself nor my employer is financially associated with any types of high-level disinfectants, whether glutaraldehyde-based or hydrogen peroxide-based.