

## **Reviewer's report**

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

**Version:** 1 **Date:** 9 October 2008

**Reviewer:** Lawrence Muscarella

### **Reviewer's report:**

Discretionary revisions:

1. 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”).

2. 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.

3. 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).

Minor essential revisions:

1. 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.”

2. 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).”

3. 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.

4. 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.

5. 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.

6. 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."

7. Please have the authors clarify whether there is a difference between the

labeling of the two tested disinfectants in the U.S. and Canada.

8. 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?

9. 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.

Major compulsory revisions:

1. 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](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 disclose 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.

2. 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 on 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.

3. 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.)

4. 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?

**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 not financially associated with any type of disinfectant. I am employed by Custom Ultrasonics, Inc., a manufacturer of automated endoscope reprocessing systems. The advancement of the AHP product discussed in this article could, if very indirectly, financially benefit me, because this reusable hydrogen peroxide product which could be popular in the U.S., could be used in the devices manufactured by my employer. But, the content of my review of this manuscript would appear to render this point moot. My employer's automated reprocessing devices do at times use 2% glutaraldehyde, which is discussed in this article.