COMMENTS SUBMITTED BY

Safety and Effectiveness of Health Care Antiseptics;
Topical Antimicrobial Drug Products for Over-the-Counter Human Use;
Proposed Amendment of the Tentative Final Monograph; Reopening of Administrative Record;
Docket No. FDA-2015-N-0101, Regulatory Information No. 0910-AF69

October 28, 2015

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Re: Safety and Effectiveness of Health Care Antiseptics; Topical Antimicrobial Drug Products for Over-the-Counter Human Use; Proposed Amendment of the Tentative Final Monograph; Reopening of Administrative Record; Docket No. FDA-2015-N-0101, Regulatory Information No. 0910-AF69

The American Cleaning Institute (ACI)[1] appreciates this opportunity to provide comments in response to the Food and Drug Administration's (FDA's) proposed amendment (Proposed Rule) to the tentative final monograph for over-the-counter (OTC) antiseptic drug products intended for use by health care professionals in a hospital or other health care situation outside the hospital (Health Care Antiseptics).[2]

We submit these comments to support FDA in its ultimate drafting of a final monograph for Health Care Antiseptics that is based on sound science and policy and that promotes the public health by developing reasonable standards to evaluate the safety and effectiveness of the active ingredients in these products.

I. Executive Summary

The OTC drug review monograph system is an established and recognized mechanism for manufacturers to market OTC drugs that were on the market in 1972. The process relies on public rulemaking to establish final monographs that identify acceptable ingredients, doses, formulations, and labeling for OTC drugs. The OTC drug review is a crucial regulatory pathway for topical antiseptic ingredients that are used in a wide variety of consumer, food handler, and health care products.

Health Care Antiseptics is a category of topical antiseptic products that is critical to public health because of the importance hand hygiene plays in the prevention of infection in health care settings. The clinical benefit of topical antiseptics in health care settings is well established. Health Care Antiseptics prevent between 22,100 and 223,100 cases of hospital-acquired bacterial infections every year, with a corresponding avoidance of $175 million to

[1] ACI is a trade association representing the $30 billion U.S. cleaning products industry. ACI members include the formulators of soaps, detergents, and general cleaning products used in household, commercial, industrial and institutional settings; companies that supply ingredients and finished packaging for these products; and oleochemical producers.

[2] 80 Fed. Reg. 25166 (May 1, 2015)

$4 billion annually in costs for hospital-acquired infections.[3] This estimate does not include additional bacterial infections that may be prevented beyond those assumed, and it does not include costs associated with hospital readmissions, short-term rehabilitation, long-term follow-up care, lost wages, lost productivity or transportation.

The following summarizes our response to the points raised in the Proposed Rule and the attachments provide additional supporting details on these points.

First, FDA should re-evaluate all data relevant to the safety and efficacy of health care antiseptic active ingredients and make affirmative findings that they are generally recognized as safe (GRAS) and generally recognized as effective (GRAE). The active ingredients used in healthcare antiseptic drug products have very favorable benefit/risk ratios demonstrated over many years of extensive use. These products clearly save lives by reducing bacterial transmission which can cause infections in healthcare settings. By reducing Healthcare-Associated Infections (HAI) (e.g., nosocomial infections), these products also reduce health care costs. There is no evidence of adverse health effects in humans as a result of the use of health care topical antiseptic products. In addition, a robust body of research supports the safety of active ingredients used in healthcare topical antiseptic products.

Second, FDA should consider all existing data relevant to the efficacy of health care antiseptic active ingredients to make determinations of generally recognized as effective (GRAE). We do not agree that any particular active ingredient eligible for use under this monograph requires any particular efficacy data. Unlike its Safety Determination, the agency has effectively thrown out all of the existing efficacy data and proposed to require the generation of an entirely new batch of efficacy data for all active ingredients. In its Effectiveness Determination, the agency should conduct an active-by-active assessment to determine where gaps might exist in the existing data. In particular, we note that the agency has proposed *in vitro* testing of 1,150 microorganisms (25 clinical isolates and 25 reference isolates for 23 microorganisms). For the agency to suggest that previous tests of the same or similar strains are no longer valid is arbitrary and the requirement for new repeated tests is unduly burdensome. In addition, the agency's proposal to raise the criteria for *in vivo* effectiveness testing of active ingredients in health care personnel hand wash to a reduction of 2.5 $\log_{10}$ on each hand within 5 minutes after a single wash with a threshold of 70% success rate is inappropriate and should be modified. FDA should adopt an effectiveness criteria for health care personnel hand wash of 2.0 $\log_{10}$ reduction for the average of the two hands within 5 minutes of a single wash and at least a 70% success rate.

Third, FDA should maintain the Category I classifications proposed in the 1994 TFM. The agency has proposed that a number of active ingredients previously classified in the 1994 TFM as Category I (GRAS and/or GRAE) be reclassified as Category III (additional data needed). In particular, alcohol and povidone-iodine were classified as GRAS/E and isopropyl alcohol was classified as GRAS in the 1994 TFM, and in the Proposed Rule they are Category III for several indications. FDA has provided no data to indicate that there is any safety issue associated with

[3] Exponent (2015). *Estimation of Charges Associated with Preventable Healthcare Acquired Infections if Antibacterial Handwash Products Were Unavailable*. Report prepared for the American Cleaning Institute (see Attachment 9).

the use of products containing these actives, or that they are ineffective in their use. In the Proposed Rule and related public announcements the agency recommended that health care personnel continue to use these products consistent with infection control guidelines. In addition, a number of the members of the Nonprescription Drug Advisory Committee spoke out against the re-classification of the Category I active ingredients to Category III during their September 3, 2014 meeting. The current antiseptic products being used in health care facilities have become the standard for care around the world with particular endorsement of the use of alcohol-based hand sanitizers by the U.S. Centers for Disease Control and Prevention and the World Health Organization.

Fourth, should the agency determine after a detailed review of the exiting data that further efficacy data is needed, FDA should continue to use clinical simulation studies with surrogate endpoints to validate the effectiveness of active ingredients in health care antiseptic products. We agree with the agency's conclusion that clinical simulation studies with surrogate endpoints are a practical means to assess the general recognition of effectiveness of active ingredients in health care antiseptic products. With regard to the effectiveness criteria for associated *in vivo* testing, the proposed criteria (2.5 $\log_{10}$ reduction with a 70% success criteria) is a level unattainable even by current FDA-approved products. FDA should retain the effectiveness criteria of active ingredients in health care personnel hand wash products proposed in the 1994 Tentative Final Monograph (2.0 $\log_{10}$ reduction) with a 70% responder rate. FDA should adopt effectiveness criteria for *in vivo* effectiveness testing of active ingredients in surgical hand rubs and scrub of a 1 $\log_{10}$ reduction within one minute after the first application procedure with no return to baseline within six hours. Likewise, FDA should retain the effectiveness criteria proposed for surgical scrubs and patient pre-operative skin preparations identified in the 1994 TFM for single applications only. ACI agrees that measurement of residual efficacy over a period of 5 days for surgical scrubs is unnecessary. Also, it is inappropriate to propose a 30 second contact time for patient pre-operative skin preparations. The proposed 10 minute application period identified in the 1994 TFM is more representative of current clinical application practices.

Fifth, in proposing new safety testing, FDA must consider the following factors: actual risks, existing safety assessments and non-animal alternative test methods. FDA should consider the level of human exposure to each of the antimicrobial active ingredients and assess the potential for harm from those exposures prior to determining the need for additional data. If current product exposures do not present unacceptable risks based on the existing safety data for an individual ingredient, FDA should refrain from requiring efficacy information that is not consistent with normal intended use of these products. In addition, FDA should be flexible in its approach to the evaluation of exposure, using all currently acceptable means, including pharmacokinetic modeling. In instances where further safety evaluation is needed, FDA should recognize and allow the use of alternative toxicity evaluation methods that have been accepted by scientific and regulatory communities. The agency also should consider other authoritative bodies' safety assessments for active ingredients, such as EPA's registration and re-registration review of active ingredients, which routinely assess potential effects from chronic exposures. Overall, where done, such assessments have not suggested potential human safety concerns for the actives of interest to ACI members. Further, FDA should support safety evaluation approaches that avoid or minimize animal testing.

Sixth, we ask FDA to formally recognize antiseptic hand washes that are used in the food industry under 21 C.F.R. 333 as a distinct category that should be subject to its own monograph, and pending that development, confirm that Food Handler topical antiseptic products can continue to be marketed under the current regulatory framework. We further recommend FDA's Center for Drug Evaluation and Research (CDER) consult with FDA's Center for Food Safety and Applied Nutrition (CFSAN) regarding these products.

Seventh, we request that FDA defer further rulemaking with respect to alcohol (ethanol), benzalkonium chloride, benzethonium chloride, chloroxylenol, isopropyl alcohol and povidone-iodine to allow the submission of any necessary new safety and effectiveness data to the record, consistent with the preamble to the Proposed Rule. We will require significant guidance from FDA regarding the studies that the agency may deem necessary for the determination of GRAS/E. We request additional time to allow for engagement with, and feedback from, FDA on the appropriate testing protocols and methods. Moreover, we believe there are a number of open questions that need to be resolved before a decision can be made as to whether any particular study is necessary for an active ingredient. A deferral is consistent with past agency practice. FDA has granted extension requests when a Tentative Final Monograph (TFM) is substantially changed from a previous proposed monograph and the required testing guidelines have been extensively modified.[4] The last step in this Rulemaking was a 1994 TFM. FDA's proposed testing in the Proposed Rule is an extensive modification of the proposal in the 1994 TFM. Further, that TFM did not request submission of safety or efficacy information for a number of antiseptics (i.e., those antiseptics that were classified as Category 1). We require significant guidance from FDA regarding the need for studies that the agency proposes as necessary for the determination of GRAS/E in light of the data already in the record for each of these six active ingredients and new data submitted in response to the agency's current proposal. The types of studies that FDA is requesting would take several years or more to design, execute, analyze and report depending on the active. Additional time is necessary upfront to get agency assessment and approval of the protocols for any new testing that is needed. For these reasons, FDA's timeline for new data submission is unreasonable and unrealistic. We are submitting with these comments initial draft protocols for *in vitro* and *in vivo* efficacy studies that could help start a discussion regarding what effectiveness data are necessary for the various active ingredients.

II. Intended Uses

We commend FDA for continuing efforts to define use areas for topical antiseptics and the agency's intent to create four monographs for these products: Consumer Antiseptic Hand Wash Products, Health Care Antiseptic Products, Consumer Antiseptic Hand Rub Products, and, presumably, Food Handler Products.

The agency proposes Health Care Antiseptics as being "intended for use by health care professionals in a hospital or other health care situation outside the hospital." We feel this

[4] See, e.g., 43 Fed. Reg. 4637 (Feb. 3, 1978) (granting extension of time for objections to TFM on antibacterial soaps, surgical scrubs, skin cleaners and first-aid preparations).

definition is overly complicated and would prefer the agency refine this definition to: "intended for use in a hospital or other health care situation outside the hospital."

This monograph contains products which may be used by persons other than health care professionals. Therefore, the proposed revision would more clearly articulate the agency's intent for the monograph (the use of topical antiseptics as part of a health care facility's frequent, standardized disinfection procedures and stringent infection control measures critical to preventing the spread of infection) and avoid confusion in practice.

III. Active Ingredients

We have identified six active ingredients that are of interest to our members. These ingredients are used in health care topical antiseptic products with a variety of indications eligible under the proposed rule. Table 1 below identifies the active ingredients and their eligible indications, as presented in the FDA Proposed Rule, and which are the subject of further discussion in this document.

Table 1. Eligibility of Antiseptic Active Ingredients for Health Care Antiseptic Uses Supported by ACI Members

Active Ingredient	Patient preoperative skin preparation	Health care personnel hand wash	Health care personnel hand rub	Surgical hand scrub	Surgical hand rub
Alcohol 60 to 95 percent	[1] Y	[2] N	Y	N	Y
Benzalkonium chloride	Y	Y	Y	Y	N
Benzethonium chloride	Y	Y	N	Y	N
Chloroxylenol	Y	Y	N	Y	N
Isopropyl alcohol 60 to 91.3 percent	Y	N	Y	N	Y
Povidone-iodine 5 to 10 percent	Y	Y	N	Y	N

[1] Y = Eligible for specific use based on Table 3 of the Proposed Rule

[2] N = Ineligible for specific use based on Table 3 of the Proposed Rule

We note that the Proposed Rule indicates a concentration range of 70 to 91.3 percent for isopropyl alcohol. There is evidence that concentrations as low as 50 percent were available for health care personnel hand rub and surgical rub products before the adoption of the monograph process (1972). As such, we recommend that the agency consider levels as low as 60% of isopropyl alcohol eligible for evaluation as healthcare personnel hand rubs, surgical scrubs and patient pre-operative skin preparations.

IV. Safety Determination

A. FDA Should Maintain the Category I Classifications Proposed in the 1994 TFM.

The agency has proposed that a number of active ingredients previously classified in the 1994 TFM as Category I (generally recognized as safe and/or effective (GRAS/E)) be reclassified as Category III (additional data needed). In particular, alcohol and povidone-iodine were classified as GRAS/E and isopropyl alcohol was classified as GRAS in the 1994 TFM, and in the Proposed Rule they are Category III for several indications.

FDA has provided no data to indicate that there is any safety issue associated with the use of these products, or that they are ineffective in their use. In fact, the agency states in the Proposed Rule "We emphasize that our proposal for more safety and effectiveness data for health care antiseptic active ingredients does not mean antiseptic products containing these ingredients are ineffective or unsafe, or that their use should be discontinued." Further, Dr. Janet Woodcock, director of the FDA's Center for Drug Evaluation and Research (CDER), stated in the agency's press release for the Proposed Rule "The FDA recommends that health care personnel continue to use these products consistent with infection control guidelines while additional data are gathered."[5] In particular, since the 1994 TFM, alcohol-based hand rubs have become the standard for care in health care settings. The U.S. Centers for Disease Control and Prevention, and the World Health Organization recommend using alcohol-based hand rubs for routinely decontaminating hands in clinical situations.[6,7]

During the September 3, 2014 meeting Dr. Arrieta of the NDAC noted that "it's not inconsistent for a product to be GRAS at the same time that we try to address some gaps in our knowledge about the product. They have been used for long enough without any serious safety signals. So I think we should continue to do so as we try to address the existing gaps."[8] Likewise, Dr. Margolis of the NDAC stated "Yes, I would agree. I don't know that there was any real data presented today that showed that there was a safety signal that we should be concerned about, which is also part of what concerns me as we get more information about these agents, how we're going to use it after millions of people have been exposed to these products without any real safety signal concerns that were voiced today."[9] In addition, Dr. Kramer of the NDAC stated "I agree, as per our vote, that these additional data would be useful. I think it would be extremely unfortunate if there was a requirement to officially change its title from Category I to Category III, that that led to fear and misunderstanding to the point that people didn't use alcohol-based hand washes in

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[5] http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm445002.htm

[6] CDC Guideline for Hand Hygiene in Health-Care Settings; http://www.cdc.gov/handhygiene/Guidelines.html.

[7] WHO Guidelines on Hand Hygiene in Health Care; http://whqlibdoc.who.int/publications/2009/9789241597906_eng.pdf.

[8] http://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/Drugs/NonprescriptionDrugsAdvisoryCommittee/UCM421121.pdf; Dr. Arrieta at p. 311.

[9] *Id.*

the hospital."[10] She went on to say "So it is totally consistent to say, with new information, we have newer techniques. We need to get more information. We should do these things. But until we get them, we shouldn't make any changes, including not even changing the category." Dr. Gudas of the NDAC was even more definitive in stating that the active ingredients in Category I in the 1994 TFM should remain Category I.[11]

B. FDA's Premises for Requesting Additional Safety Data Are Flawed.

1. FDA Should Consider Exposure-Driven Risk Assessments Before Requiring More Data.

FDA should base its decision to require additional data on more robust analysis of current knowledge about human exposure and risk and this should precede any proposal requiring additional testing. In other words, FDA should analyze all existing hazard data and consider the extent of human or environmental exposure as part of the process for deciding the nature and extent of hazard data required to understand potential safety concerns. Before declaring that a dataset is inadequate to assess the risks associated with an antiseptic active ingredient, FDA should understand the margins of safety using the available data to the extent possible. Data generation based on an understanding of human exposures prevents the irresponsible use of laboratory animals and waste of resources to generate toxicology data that will not further inform potential safety decisions.

We agree that it is necessary to perform a safety assessment using test data and it is appropriate to consider the endpoints cited in the Proposed Rule (human pharmacokinetics, animal pharmacokinetics, carcinogenicity, reproductive toxicity, potential hormonal effects and resistance potential). However, it is not necessary to have study data for every one of those endpoints for the agency to make a determination that an active ingredient is generally recognized as safe (GRAS). In its presentation before the Nonprescription Drugs Advisory Committee (NDAC) on September 3, 2014, FDA noted that there are a number of criteria associated with the GRAS Standard:[12]

- Low incidence of adverse events under directions for use and warnings
- Low potential for harm if abused under conditions of widespread availability
- Significant human marketing experience
- Adequate tests to show proof of safety
- Data publically available
- Applies to all potential formulations of an ingredient

[10] *Id. at p. 319*

[11] *Id.* at p. 345

[12] http://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/Drugs/NonprescriptionDrugsAdvisoryCommittee/UCM414581.pdf; slide 3.

In the Proposed Rule, the agency is inappropriately discounting the low incidence of adverse events, significant human marketing experience and global acceptance of health care antiseptic products as a standard for care in health care settings.

Furthermore, numerous scientific and regulatory bodies have performed exposure-driven risk assessments and have not requested the types of human and animal study data mentioned in FDA's proposal. This is especially noteworthy since those evaluations consider cumulative human exposure from common use products, such as in opinions issued by the European Commission's Scientific Committee on Consumer Safety, rather than focusing on just individual product types.

a) Animal and Human Pharmacokinetic Data Can Provide a Margin of Exposure.

In the Proposed Rule, FDA comments that the lack of pharmacokinetic data prevents FDA from calculating a margin of exposure for the risk assessment. Although the safety evaluation of drugs may rely on correlating findings from animal toxicity studies to humans based on kinetic information in both species, safety evaluations for antiseptic ingredients in health care products are not based on kinetic information under standard international practice. Instead, safety evaluations are based on conservative assumptions of exposure and potential differences between species.[13] Kinetic information is only required when use of these conservative assumptions fails to provide a sufficient margin of exposure. Using these conservative and internationally accepted approaches, other scientific bodies and regulatory authorities have been able to complete the risk assessment for these types of ingredients in formulations with much greater levels of human exposure than these health care antiseptic uses.

Therefore, FDA should not require additional animal testing unless the following conditions are met:

- Use of conservative approaches to calculate the margin of exposure is inadequate.
- The margin of exposure justifies the need for more data, but it is not possible to generate the data by non-animal approaches, such as using physiologically-based pharmacokinetic modeling, or through animal alternative test methods.
- There is perceived need for all active ingredients to have the same type of information.

b) FDA should reconsider the concept of the Maximal Use Trial (MUsT) and its value in determining the safety of health care antiseptic products.

The Proposed Rule would require a Maximal Use Trial (MUsT) to characterize maximum systemic exposure following health care antiseptic product use during the course of a work day or shift in healthcare settings. Measured levels determined by the MUsT would establish the maximum systemic dose for the active ingredient in the particular antimicrobial product type. The representativeness of the measured systemic active concentration will be dependent upon a number of variables associated with this trial, not the least of which are the number of applications made

[13] *E.g.* http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_s_006.pdf.

per day or shift, the appropriate usage of the product, the concentration of active in the tested product, the sensitivity of the analytical method applied, and the extent to which the experimental protocol matches or approximates the actual usage of the product in the health care setting. Use of the same product in different health care settings (e.g., out-patient clinics or offices vs. emergency rooms or operating rooms) can be expected to have different patterns of use.

Limitations exist in the practical conduct of a MUsT that influence and dictate what may be achieved by a specific protocol. The practical requirements, for instance, of the time needed to collect biological samples, or even to perform washing or application of the product, will dictate how may washes or applications are possible in a given time period regardless of what may be deemed desirable or required to evaluate perceived or empirical usage. The MUsT conditions described in the Proposed Rule will result in assays that are very large and complex, and there is very little precedent to consult in the published literature.

For the following reasons, we believe FDA should reconsider the concept of the Maximal Use Trial and its necessity in the determination of the safety of health care antiseptic product active ingredients.

- The practical aspects of conducting a MUsT dictate what can reasonably be performed in terms of number of product applications, number of subjects, study legs, timing, etc. This is due to the necessity of time for collection of biological samples and rest/recovery periods for the study subjects. Whatever the number of applications that may be desired to match empirical usage data/studies, the practical aspects of conducting a MUsT may preclude performing/evaluating that number of applications in a given timeframe. A practical upper limit of applications exists. It is likely that the numbers of subjects and numbers of applications proposed in the Proposed Rule may not be practically achievable. If the defined, or desired, maximal use is not achievable in a MUsT, one must ask to what extent the data derived from the study are useful. If the resulting data do not meet the needs of the safety and risk assessment process, it is reasonable to question the utility, and expense, of conducting the study at all.

- While data on active level in systemic circulation is arguably important for risk and safety assessment, it is not clear what any observed levels from MUsT may mean even in this context. FDA has provided little guidance on how these data are used. Moreover, FDA has provided no data to indicate that there is any safety issue associated with any of the six active ingredients we have identified.

- While the MUsT will provide information on active levels in systemic circulation, it fundamentally remains a pharmacokinetic study. As such, it is not apparent that results from a MUsT study will provide data that might better be provided by an alternative or otherwise validated and accepted approach.

If MUsT studies are to be executed, field studies of health care facility application frequency would be necessary to determine maximum rates as adequate data do not currently exist. While these studies could take the form of a direct observational study, other avenues may also be

considered, such as the use of automated hand hygiene monitoring data. This data acquisition approach is not subject to behavioral modification interferences by the observer, nor hospital department access restrictions such as ICU and Surgery. Recently, this technology has progressed substantially in its sophistication and data reliability.

We urge FDA to work with industry to reasonably consolidate MUsT requirements and testing between the different monographs (Consumer Hand Wash, Health Care, Consumer Hand Rub, and Food Handler, when proposed) and the different indications within each Proposed Rule to minimize the numbers of trials if a MUsT would be needed.

2. FDA Should Consider the Long Record of Safety and Relative Low Risk of Active Ingredients in Health Care Antiseptic Products.

FDA should weigh the long record of safety of health care antiseptics in considering the risk-benefit of antiseptic active ingredients and the need for additional safety data.

a) Long-term Cohort Studies of Nurses Do Not Signal Adverse Effects

The FDA's proposed evaluation of risks associated with extensive use of antiseptic soaps by health care workers should consider the information available in the Nurses' Health Studies (NHS). The NHS are a series of long-term studies of health outcomes in several large cohorts of nurses.[14] Starting in 1976, the original and the second cohorts (NHS and NHS II, respectively) included approximately 120,000 registered nurses each. By selecting registered nurses for these studies the study authors anticipated that "…because of their nursing education, they would be able to respond with a high degree of accuracy to brief, technically-worded questionnaires and would be motivated to participate in a long term study." Although these studies were initially based exclusively on responses to questionnaires, blood samples (33,000 in 1989-90 and 18,700 samples in 2000-01) were collected "…to identify potential biomarkers, such as hormone levels and genetic markers." Notably, in 1996 and 2004, participants in NHS II were invited to enroll their children (between the ages of 9 and 14) in the Growing Up Today Study (GUTS). GUTS is ongoing and a third cohort of nurses is being assembled to conduct NHS III.

To date, there is no evidence in these studies that suggests that use of topical antiseptic products leads to adverse health outcomes in nurses. Although these studies were not designed to evaluate risks associated with use of antiseptic soaps, the large size of the cohorts, the long-term follow-up, the inclusion of children in GUTS, and the professional training of the study participants leads to the conclusion that these studies are adequate to detect clinically-relevant health outcomes, including those associated with endocrine effects, that might arise from the use of antiseptic soaps. Thus, the Nurses' Health Studies are a valuable source of real-world information for professional healthcare workers that must be considered by the FDA.

b) MedWatch has Not Identified Safety Concerns

[14] http://www.channing.harvard.edu/nhs/

FDA closely monitors the safety of drug products through MedWatch, which is an adverse event reporting program that allows stakeholders, students, health professionals (FDA Form 3500), and consumers (FDA Form 3500B) to voluntarily report safety-related problems to FDA. Mandatory reports are required for severe adverse events (FDA Form 3500A). Initiated in 2000, FDA keeps a comprehensive online database of these complaints.[15] A search of this database fails to find safety-related complaints related to products in the proposed rule.

In the event that safety issues are detected through the monitoring program, FDA releases "safety alerts," which address the safety concern and make recommendations to minimize risk. The safety alert may include a recommendation to recall the product. To date, no safety alerts have been released in response to concerns related to antiseptic skin products.

3. There Is No Evidence of Higher Systemic Exposure.

FDA calls for additional safety data in the Proposed Rule because "systemic exposure is higher than previously thought, and new information is available about the potential risks from systemic absorption and long-term exposure"[16] to antiseptic active ingredients. FDA offers no definitive evidence to support the statement that a "higher than previously thought" exposure is associated with the use of these ingredients. To substantiate this statement, FDA must document the level of systemic exposure from Health Care Antiseptic products that was used in its prior safety assessment and how this differs from any new information the agency has received. Further, any such data do not appear in the (historic) public docket for the health care antiseptic rulemaking (FDA-1975-N-0012) and are not available to stakeholders. FDA should also clarify that the new information provides either *in vitro* or dose dependent data, not "risk," as we are unaware of FDA's current thinking on specifics regarding risk assessment.

C. FDA Should Reconsider the Requirement for Carcinogenicity Studies.

We agree that a carcinogenicity assessment of Health Care Antiseptic active ingredients is critical. For the majority of the active ingredients listed in FDA's proposal, a good quality systemic carcinogenicity data set exists, along with *in vitro* genetic toxicology studies. It is unclear why FDA is concerned enough to propose a second carcinogenicity study on these ingredients. While there are ADME differences between oral, inhalation and dermal exposure, in the absence of tumors in an oral or inhalation study, and provided that good quality *in vitro* genetic toxicity data are available, it is difficult to envisage which modes of action would cause concern for these ingredients when applied by the dermal route. Under international standards, "[s]ince carcinogenicity studies are time consuming and resource intensive they should only be performed when human exposure warrants the need for information from life-time studies in animals in order to assess carcinogenic potential."[17]

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[15] MedWatch, The FDA Safety Information and Adverse Event Reporting Program. http://www.fda.gov/Safety/MedWatch/default.htm.

[16] 80 Fed. Reg at 25167

[17] International Conference on Harmonization - Safety, Guideline for Industry: The Need for Long-term

We are not aware of any chemical that provides negative *in vitro* genetic toxicity data and negative oral carcinogenicity data, but is positive by the dermal route. In addition, it is highly unlikely that intermittent dermal exposure would result in systemic exposures higher than those obtained following oral exposure. We therefore strongly advocate that, rather than establishing "studies to be performed," FDA rephrases the proposal to focus on "health effects to be addressed in the safety assessment." This will allow the use of a more integrated and data-based approach to risk assessment rather than the 'check-the-box' approach currently presented.

The potential requirement of additional studies by the dermal route of exposure when a carcinogenicity study by the oral route exists needs justification because it is highly unlikely that systemic exposure would be higher than that resulting from oral exposure. For example, the existence of factors which could be considered "of concern" may include: (1) previous demonstration of carcinogenic potential in the product class that is considered relevant to humans; (2) structure-activity relationship suggesting carcinogenic risk; (3) evidence of pre-neoplastic lesions in repeated dose toxicity studies; and (4) long-term tissue retention of parent compound or metabolite(s) resulting in local tissue reactions or other pathophysiological responses.

D. FDA Should Take a Flexible Approach on Measuring Hormonal Effects.

Any potential for hormonal effects has been, and can be, addressed by the interpretation of repeat-dose or developmental and reproductive toxicity testing (DART) data. FDA defines a "hormonally active compound" as a "substance that interferes with the production, release, transport, metabolism, binding, activity, or elimination of natural hormones, which results in a deviation from normal homeostasis, development, or reproduction."[18] Results from *in vitro* high throughput screening fail to satisfy this definition. Despite varying modes of action, actual adverse effects from endocrine disrupting chemicals are typically manifested as: (1) alterations in development; (2) reproductive impairment; and/or (3) reduction in growth. These types of effects can be noted in traditional DART studies of antiseptic active ingredients.

The Proposed Rule states that "data are also needed to assess whether antiseptic active ingredients have hormonal effects that could produce developmental or reproductive toxicity."[19] We agree that any toxicological risk assessment should consider whether, under conditions of use, an ingredient could cause adverse effects as a result of its ability to interfere with endocrine homeostasis. The Proposed Rule also correctly states that general and reproductive toxicology studies are generally adequate to identify potential hormonal effects. We welcome the apparently flexible approach to determining risks to endocrine-sensitive tissues on a case-by-case basis. However, FDA should emphasize that a repeat-dose or reproductive and developmental toxicity study will provide the point of departure (e.g., NOAEL, BMDL10) for an ingredient that acts by

Rodent Carcinogenicity Studies of Pharmaceuticals, at 1 (March 1996). http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm074911.pdf.

[18] 80 Fed. Reg. at 25182

[19] 80 Fed. Reg. at 25182

an endocrine mode of action. These animal studies form the highest tier of endocrine testing strategies.[20] Therefore, where data from these studies exist, there is rarely a need to go back and generate *in vitro* data to inform the risk assessment. FDA should not require further testing for endocrine modulation, where the adverse outcomes associated with endocrine modes of action have already been adequately addressed in existing *in vivo* tests.

DART studies for Health Care Antiseptic active ingredients should not be required to resemble studies for new drug application (NDA) approval because monograph products must follow acceptable ingredients, doses, formulations, and labeling set forth in an OTC monograph. FDA also should remain cognizant that in other countries these types of ingredients have other intended uses (e.g., industrial uses) and will have study designs more appropriate for those uses that should be considered in FDA assessments before requiring further testing.[21]

E. FDA Should Work With Stakeholders on Approach for Measuring Antimicrobial Resistance.

1. There Is No Evidence of Real-World Antibacterial Resistance from Use of Health Care Antiseptic Products.

Antimicrobial resistance is an issue demonstrated to occur due to the indiscriminate use of antibiotics, but not due to use of topical antiseptic products. Recently, a group of experts participated in a workshop to evaluate the interconnection between microbial resistance to biocides and antibiotics.[22] They found that even though mutant strains resistant to antibiotics have been identified to have transient resistance, the observed level of resistance to biocides was lower than predicted because the concentration required for the expression of resistance was toxic to bacteria. When molecular mechanisms were evaluated in three different scenarios, the conclusion was that biocides show very low correlation coefficients with antibiotic resistance.

While antimicrobial resistance has been demonstrated in laboratory settings, it has not been demonstrated in real world scenarios, as reflected by data from current monitoring programs. Recent studies have reinforced the evidence that resistance and cross-resistance associated with biocides and antiseptics is a laboratory phenomenon, observed only when tests are conducted under conditions that are not clinically relevant.[23]

Studies about the mechanism of antiseptic action are important as a research tool, but would be an unrealistic requirement for a GRAS determination. Identifying cellular targets of

[20] *See, e.g.*, EPA, Endocrine Disruptor Screening Program, *available at* http://www.epa.gov/endo/.

[21] *See, e.g.*, OECD, Guidelines for Testing of Chemicals Section 4: Health Effects, Test Nos. 414 (Prenatal Development Toxicity Study), 416 (Two-Generation Reproduction Toxicity Study), 443 (Extended One-Generation Reproductive Toxicity Study).

[22] Oggioni MR, Furi L, Coelho JR, Maillard J-Y, Martinez JL, Recent advances in the potential interconnection between antimicrobial resistance to biocides and antibiotics. *Expert Rev. Anti Infect. Ther.* 2013; 11(4), 363-366

[23] Condell O, Iversen C, Cooney S, Power KA, Walsh C, Burgess C and Fanning S, Efficacy of Biocides Used in the Modern Food Industry To Control *Salmonella enterica*, and Links between Biocide Tolerance and Resistance to Clinically Relevant Antimicrobial Compounds. *Applied and Environmental Microbiology.* 2012; 78, 3087-3097.

antimicrobial activity is not a simple or straightforward undertaking. Data characterizing the potential for transferring a resistance determinant to other bacteria is also an unrealistic requirement for GRAS determination. Currently, it is unclear which methods could be used to determine the transfer of resistance. Furthermore, transfer of resistance by exposure to an antiseptic active is a theoretical risk.

There is little credible evidence that antiseptic products play any role in antibiotic resistance in human disease. While some *in vitro* lab studies have been successful in forcing the expression of resistance in some bacteria to antiseptic active ingredients, real world data from community studies using actual product formulations, show no correlation between the use of such products and antibiotic resistance.[24,25,26,27,28, 29,30,31,32] Further evidence of real world data showing no antimicrobial resistance development after the continued use of consumer products containing antimicrobial active compounds can be extracted from oral care clinical studies. These provide *in vivo* data, under well controlled conditions, on exposure to antimicrobial-containing formulations over prolonged periods of time (e.g., 6 months to 5 years). A considerable number of studies related to oral care available in the scientific literature have been reviewed by Gilbert *et al.* (2007) and Sreenivasan (2002).[33,34]

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[24] Rutala WA, Weber DJ, Barbee SL, Gergen MF and Sobsey MD, Evaluation of antibiotic resistance bacteria in home kitchens and bathrooms. *Infection Control and Hospital Epidemiology.* 2000; 21, 132.

[25] Aiello AE, Marshall B, Levy SB, la-Latta P and Larson E, Relationship between triclosan and susceptibilities of bacteria isolated from hands in the community. *Antimicrob. Agents Chemother.* 2004; 48, 2973-2979.

[26] Marshall BM, Robleto E, Dumont T, Levy SB The frequency of antibiotic-resistant bacteria in homes differing in their use of surface antibacterial agents *Current Microbiology*, 65, pp. 407-415. Aiello A.E., Marshall B., Levy S.B., Della-Latta P., Lin S.X. and Larson E. (2005) Antibacterial cleaning products and drug resistance. *Emerg Infect Dis.* 2012; 11(10): 1565-1570.

[27] Aiello AE, Marshall B, Levy SB, Della-Latta P, Lin SX, Larson E, Antibacterial cleaning products and drug resistance. *Emerg Infect Dis.* 2005 11(10): 1565-1570.

[28] Cole EC, Addison RM, Rubino JR, Leese KE, Dulaney PD, Newell MS, Wilkins J, Gaber DJ, Wineinger, T. and Criger, D.A., Investigation of antibiotic and antibacterial agent cross-resistance in target bacteria from homes of antibacterial product users and nonusers. *J. Appl. Microbiol.* 2003; 95, 664-676

[29] Cole EC, Addison RM, Dulaney PD, Leese KE, Madanat HM and Guffey AM, Investigation of antibiotic and antibacterial susceptibility and resistance in *Staphylococcus* from the skin of users and non-users of antibacterial wash products in home environments. *Int. J. Microbiol. Res.* 2011; 3, 90-96.

[30] Marshall BM, Robleto E, Dumont T and Levy SB, The frequency of antibiotic-resistant bacteria in homes differing in their use of surface antibacterial agents *Current Microbiology;* 2012; 65, pp. 407-415.

[31] Rutala WA, Weber DJ, Barbee SL, Gergen, MF and Sobsey MD, Evaluation of antibiotic resistance bacteria in home kitchens and bathrooms. *Infection Control and Hospital Epidemiology.* 2000; 21, 132.

[32] Weber DJ and Rutala WA, Use of germicides in the home and the healthcare setting: is there a relationship between germicide use and antibiotic resistance? *Infection Control and Hospital Epidemiology.* 2006; 27, 1107-1119.

[33] Gilbert P, McBain A and Sreenivasan P, Common therapeutic approaches for the control of oral biofilms: microbiological safety and efficacy. *Clin Microbiol Infect*. 2007; 13 (Suppl. 4): 17–24.

[34] Sreenivasan P and Gaffar A. Antiplaque biocides and bacterial resistance: a review. *J Clin Periodontol*. 2002; 29(11): 965-974.

2. A Survey of the Scientific Literature is an Acceptable Approach for Generating Information to Provide to FDA to Evaluate the Potential Impact of Antiseptic Active Ingredients on the Development of Resistance.

In a meeting with FDA on March 20, 2015, we stated: *We propose to conduct ingredient-specific reviews of the literature pertaining to antiseptic resistance and antibiotic cross-resistance as a substitute for studies proposed by the Agency to assess the development of cross-resistance to antibiotics.* The agency responded "A literature search is an acceptable approach to addressing this issue." In addition, "FDA suggested that Industry submit as much information and data as can be provided." We concur with the agency's recommendation. The requirements for information and data should be able to be satisfied without generation of new laboratory data.

V. Active Ingredient Effectiveness Determination

A. *In Vitro* Studies

In the proposed rule, the agency has essentially reiterated requirements for *in vitro* demonstration of antimicrobial activity of *active ingredients* proposed in the 1994 TFM; namely, a determination of the *in vitro* spectrum of antimicrobial activity, minimum inhibitory concentration (MIC) testing against 25 fresh clinical isolates and 25 laboratory isolates for a list of 23microorganisms and time-kill testing against the same list of microorganisms. The agency has provided the option of assessing the minimum bactericidal concentration (MBC) as an alternate to testing the MIC to demonstrate the broad spectrum activity of the active ingredient.

In addition, we note the proposed rule specifies the microorganisms identified in the 1994 TFM for antimicrobial activity testing. This list does not include pathogens that are relevant to current health care settings such as Methicillin-resistant *Staphylococcus* species (MRSA) or Vancomycin-resistant *Enterococcus* species (VRE). We are aware that the agency has been advising entities with potential new drug applications for antimicrobial products to conduct *in vitro* testing against microorganisms that are of current concern in health care settings. We believe the list of microorganisms in Table 2, which includes those identified in the Proposed Rule in addition to organisms relevant to current health care settings, represents a suitable list of candidates from which to select for *in vitro* effectiveness testing.

Table 2. Proposed list of candidate organisms for *in vitro* effectiveness testing

1 *Acinetobacter baumannii*
2 *Bacteroides fragilis*
3 *Haemophilus influenza*
4 Enterobacter species
5 *Escherichia coli*
6 Klebsiella species
7 *Klebsiella pneumonia*
8 *Pseudomonas aeruginosa*
9 *Proteus mirabilis*
10 *Serratia marcescens*
11 *Staphylococcus aureus*
12 *Staphylococcus epidermis*
13 *Staphylococcus hominis (warnerii)*
14 *Staphylococcus haemolyticus*
15 *Staphylococcus saprophyticus*
16 *Micrococcus luteus*
17 *Streptococcus pyogenes*
18 *Enterococcus faecalis*
19 *Enterococcus faecium*
20 *Streptococcus pneumonia*
21 *Candida* species
22 *Candida albicans*
23 *Burkholderia cepacia*
24 Methicillin-resistant *Staphylococcus aureus* (MRSA)
25 Methicillin-resistant *Staphylococcus epidermidis* (MRSE)
26 Vancomycin-resistant *Enterococcus* (VRE*; E. faecali)*
27 Vancomycin-resistant *Enterococcus* (VRE *E. faecium)*

We do not see this proposed list of candidate organisms for *in vitro* effectiveness testing as being static and hope that in further discussions with the agency we can identify an up-to-date list of organisms relevant to clinical health care settings.

1. FDA Should Endorse Standard Methods for Time-Kill Testing.

In Advice from FDA to ACI based on a meeting on March 20, 2015, the agency indicated that the ASTM protocol E2783-11 "Standard Test Method for Assessment of Antimicrobial Activity for Water Miscible Compounds Using a Time-Kill Procedure" is acceptable for conducting Time-Kill studies of active ingredients in *consumer antiseptic products*. We believe the same protocol is suitable for conducting Time-Kill studies of active ingredients in *health care antiseptic products*. We have included in our comments a draft Time-Kill test protocol which is currently under development in cooperation with BioScience Laboratories, Inc. (see Attachment 1). The draft protocol details what we believe is a suitable approach for Time-Kill testing of six active ingredients used in health care antiseptic products described in the proposed rule that are of

interest to our members, namely alcohol, benzalkonium chloride, benzethonium chloride, chloroxylenol, isopropyl alcohol and povidone iodine.

2. FDA Should Use a More Specific and Updated, Health Care Targeted List of Microorganisms and Strains/Isolates for Any Necessary MIC/MBC Testing of Health Care Antiseptic Active Ingredients.

The agency has proposed that broad spectrum MIC or MBC testing is necessary to demonstrate the antimicrobial activity of active ingredients used in health care antiseptic products under this monograph. There may be value in some such testing. However, testing 50 isolates/strains for 27 microorganisms is excessive and unwarranted. Given the decades of safe and effective use of health care antiseptic products and the experience of countless health care facilities, the rejection of previous data submitted to the agency is inappropriate.

We propose two recommendations to offer as a more targeted approach to acquiring *in vitro* effectiveness data, when necessary, particularly with respect to MIC/MBC testing. First, we recommend that the agency select among microorganisms from an abbreviated list derived from Table 2 above. We note that in several cases different strains of the same species are identified; therefore, combining into a single identified strain will not diminish the data collected. We propose the following suggested list of current clinically relevant organisms for MIC/MBC testing (Table 3).

Table 3. Proposed list of MIC/MBC test organisms

1. *Acinetobacter baumannii*
2. *Bacteroides fragilis*
3. *Haemophilus influenza*
4. *Enterobacter* species
5. *Escherichia coli*
6. *Klebsiella* species to include *Klebsiella pneumoniae*
7. *Pseudomonas aeruginosa*
8. *Proteus mirabilis*
9. *Serratia marcescens*
10. *Staphylococcus aureus,* including Methicillin-resistant *Staphylococcus aureus (MRSA)*
11. *Staphylococcus epidermis,* including Methicillin-resistant *S. epidermidis (MRSE)*
12. *Staphylococcus hominis (warnerii)*
13. *Staphylococcus haemolyticus*
14. *Staphylococcus saprophyticus*
15. *Micrococcus luteus*
16. *Streptococcus pyogenes*
17. *Enterococcus faecalis,* including Vancomycin-resistant *Enterococcus (VRE; E. faecalis)*
18. *Enterococcus faecium,* including Vancomycin-resistant *Enterococcus (VRE E. faecium)*
19. *Streptococcus pneumoniae*
20. *Burkholderia cepacia*
21. *Candida* species, including *Candida albicans*

Second, we note that there is a precedent for the agency to accept a reduced MIC/MBC data set in the approval of NDAs for topical antiseptic products. We note that this was the case for Avagard health care personnel hand wash (NDA 21-074) and ChloraPrep patient preoperative and preinjection skin preparation (NDA 21-555). In both cases, the agency accepted data from 5 of the 25 ATCC strains and 5 of 25 fresh clinical isolates for a total of 10 strains for each of 21 microorganisms. We believe a similar approach would be appropriate for any of the health care antiseptic active ingredients for which new *in vitro* test data is deemed necessary by the agency.

In addition, we offer a draft test protocol based on the standardized test method *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard—Ninth Edition (M07-A9)* from the Clinical and Laboratory Standards Institute (CLSI) (see Attachment 2). This protocol, which is currently under development in cooperation with BioScience Laboratories, Inc., details what we believe is a suitable approach for MIC/MBC testing of six active ingredients used in health care antiseptic products described in the proposed rule that are of interest to our members, namely ethyl alcohol, benzalkonium chloride, benzethonium chloride, chloroxylenol, isopropyl alcohol and povidone iodine.

B. *In Vivo* Studies

We agree with the agency's proposal and the recommendations of the NDAC at its March 23, 2005 meeting that the use of bacterial log reductions as a means of demonstrating that active ingredients are GRAE for use in health care antiseptic drug products is appropriate.

1. Health Care Personnel Hand Wash

a) FDA should adopt ASTM method E1174-13 for effectiveness testing.

We recommend that FDA adopt ASTM method E1174-13 *Standard Test Method for Evaluation of the Effectiveness of Health Care Personnel Handwash Formulations* as a standardized test method to demonstrate that an active ingredient is GRAE for use in health care personnel hand wash products. In addition, we offer a draft test protocol based on E1174 (see Attachment 3). This protocol, which is currently under development in cooperation with BioScience Laboratories, Inc., details what we believe is a suitable approach for *in vivo* effectiveness testing of four active ingredients used in health care antiseptic products described in the proposed rule that are of interest to our members, namely benzalkonium chloride, benzethonium chloride, chloroxylenol, and povidone iodine. We propose that E1174 be conducted under conditions described in the protocol described below and request that the agency confirm that these test conditions are suitable.

- A single use wash would be applied.
- A physiological saline solution would be used as the vehicle control.
- An FDA-approved NDA hand wash product containing 4% chlorhexidine gluconate (CHG), Hibiclens®, is proposed as the active control.

- ASTM E1054-08 *Standard Test Methods for Evaluation of Inactivators of Antimicrobial Agents* would be used as a suitable method to validate the neutralizer to be used in the *in vivo* efficacy studies.
- Two pivotal studies should be conducted.

b) FDA should adopt an effectiveness criteria for health care personnel hand wash of 2.0 $\log_{10}$ reduction with a 70% success rate.

The agency's proposal to raise the effectiveness criteria for health care personnel hand wash to a reduction of 2.5 $\log_{10}$ on each hand within 5 minutes after a single wash coupled with the agency's proposal for a threshold of 70% success rate for a particular active is inappropriate. An analysis of effectiveness data shows that the typical positive control for health care personnel hand wash, Hibiclens® (4% CHG), a healthcare personnel handwash product approved by FDA under the New Drug Application (NDA) process as safe and effective, fails to meet the proposed effectiveness criteria.

The net effect of imposing the 2.5 $\log_{10}$ reduction rate would be to remove most, if not all, Health Care Personnel Hand Washes from the market, including those previously approved through the NDA process. These are products that have served the health care industry and more specifically health care workers and facilities of all kinds over the last 20+ years. This would introduce an undue hygiene risk into the health care field. As will be demonstrated by the two following analyses, these efficacy criteria are not obtainable and represent an unprecedented and largely unachievable performance hurdle.

In one meta-analysis, data acquired from 20 health care personnel hand wash studies recently conducted at BioScience Laboratories, Inc. on behalf of GOJO Industries, Inc., using Hibiclens® as a positive control product were analyzed (see Attachment 4, Tables 1 and 2). This analysis examined the performance necessary to achieve the 2.5 $\log_{10}$ reduction rate with a 95% confidence interval and a 70% responder rate. It found that it would be necessary for *any* product to have a lower bound of the 95% confidence interval at a 3.10 $\log_{10}$ reduction or greater in order to meet the proposed 2.5 $\log_{10}$ reduction rate. Within these analyses the following points are clear:

- For the 20 studies examined, the $\log_{10}$ reduction average was 2.827 for Hibiclens®,
- For two of the 20 studies, Hibiclens® was not able to meet the proposed 2.5 $\log_{10}$ reduction rate at the 95% confidence interval, and
- No study was able to achieve the lower bound of the 95% confidence interval at $\log_{10}$ 3.10 reduction or greater required to pass the 2.5 $\log_{10}$ reduction at the 70% responder rate.

In a related meta-analysis of 13 health care personnel hand wash studies recently conducted at BioScience Laboratories, Inc. on behalf of GOJO Industries, Inc., only 4 of the 13 studies (31%) passed the 70% responder rate at 2.5 $\log_{10}$ reduction on the first wash with Hibiclens® as a positive control product (see Attachment 4, Tables 3, 4 and 5).

In a second meta-analysis, data acquired from 13 health care personnel hand wash studies conducted by Hill Top Research and an additional 13 studies conducted by Henkel using Hibiclens® as a positive control product were analyzed (see Attachment 5). Similar performance was observed for Hibiclens® as a positive control, namely a mean $\log_{10}$ reduction of 2.5065 (standard deviation 0.3318) and 2.5165 (st. dev. 0.3284), respectively. Consequently, only 15% and 13% of the studies with Hibiclens® as a positive control meet the proposed effectiveness criterion of 2.5 $\log_{10}$ reduction.

The results from these meta-analyses are clear. The 70% responder rate at 2.5 $\log_{10}$ reduction in the proposed rule is unachievable. However, these meta-analyses go on to examine an alternative effectiveness criterion, namely a 70% responder rate at 2.0 $\log_{10}$ reduction. For the 13 GOJO studies examined, all pass these alternate criteria (Attachment 4). For the Hill Top and Henkel studies (13 each), 100% and 92% pass a 70% responder rate at 2.0 $\log_{10}$ reduction, respectively.

Health care personnel hand wash products have been historically successful in the health care field in providing high quality interventions of hand-transmission of disease or infection, for the last 20+ years. These products have utilized the active ingredients efficiently to achieve both the current performance hurdle and in-use utility. There is no evidence that the current Health Care Personnel Hand Washes are deficient in efficacy. Nor is there evidence that the elevation of the log reduction hurdle in either of these product groups will result in greater positive human health impact; and may in fact result in greater negative skin health interactions due to reformulation to higher concentrations of active ingredient.

As can be seen in Table 4 (below), at the typical standard deviations found in health care personnel hand wash studies, the 2.5 $\log_{10}$ reduction performance standard coupled with the 70% pass criteria will require average log reductions exceeding the performance of CHG washes, the effectiveness of which was established through the new drug application (NDA) process.

Table 4. Criteria 2.5 log reduction, 70% pass criteria

Std Dev	**Subject/arm**	**Average Log reduction needed for 70% subject pass**
0.37	24	2.84
0.37	30	2.83
0.45	24	2.93
0.45	30	2.90

This pass criteria net effect, coupled with the CHG performance data included here, provides substantial evidence that the *in vivo* efficacy threshold should be a 2.0 $\log_{10}$ reduction at the first application for the health care antiseptic hand washes.

In addition, the proposed effectiveness criteria call for comparing each hand. It is more appropriate that the average of the two hands be calculated and compared against a 2.0 $\log_{10}$ mean

reduction as a single subject data point. The fact that inoculum may be transferred between hands during the inoculation and spreading procedure precludes the treatments of the individual hands as single events. The results must be combined to determine an overall subject test result (log_{10} reduction). The ASTM standard methods have been reviewed and vetted including the statistical treatments to assure proper design and experimental variable treatment. The justification and data substantiation to require this change has not been made.

Based on the data in Table 5, we recommend that FDA adopt an effectiveness criteria for health care personnel hand wash of 2.0 log_{10} reduction for the average of the two hands within 5 minutes of a single wash and at least a 70% success rate.

Table 5. Criteria 2.0 log reduction, 70% pass criteria

Std Dev	**Subject/arm**	**Average Log reduction needed for 70% subject pass**
0.37	24	2.55
0.37	30	2.50
0.45	24	2.62
0.45	30	2.60

2. Health Care Personnel Hand Rub

We recommend that FDA adopt ASTM method E2755-15 *Standard Test Method for Determining the Bacteria-Eliminating Effectiveness of Healthcare Personnel Hand Rub Formulations Using Hands of Adults* as a test method to demonstrate that an active ingredient is GRAE for use in health care personnel hand rub products. We propose that E2755 be conducted under conditions described in the protocol in Attachment 6 and request that the agency confirm that these test conditions are suitable.

- A single use wash would be applied.
- A physiological saline solution would be used as the control.
- We believe that Avagard™, the only NDA-approved health care personnel hand rub, is the most suitable choice for an active control. However, pilot studies must be conducted to confirm it is appropriate, as data previously presented indicates that this control may not meet the proposed FDA criteria.[35]
- ASTM E1054-08 *Standard Test Methods for Evaluation of Inactivators of Antimicrobial Agents* would be used as a suitable method to validate the neutralizer to be used in the *in vivo* efficacy studies.
- Two pivotal studies should be conducted.

[35] Briefing information package from GOJO Industries to FDA for July 30, 2015 meeting. Available in Docket No. FDA-2015-N-0101

3. Surgical Hand Scrub and Hand Rub

We recommend FDA adopt ASTM method E1115-11 *Standard Test Method for Evaluation of Surgical Hand Scrub Formulations* as a test method to demonstrate an active ingredient is GRAE for use in surgical hand scrub and rub products. We propose that E1115 be conducted as described in the protocol in Attachment 7 and request the agency confirm these test conditions are suitable.

a) A single recommended application procedure, wash or rub would be applied.

b) A physiological saline solution would be used as the control.

c) An FDA-approved NDA hand wash product containing 4% chlorhexidine gluconate, Hibiclens®, is proposed as the active control for surgical hand scrub actives; and an FDA-approved 1% chlorhexidine gluconate and 61% ethyl alcohol hand rub, Avagard™, would be used as the active control for surgical hand rub actives. However, pilot studies must be conducted to confirm these controls are appropriates, as data previously presented to the agency indicates that they may not meet the proposed FDA criteria.

d) ASTM E1054-08 *Standard Test Methods for Evaluation of Inactivators of Antimicrobial Agents* would be used as a suitable method to validate the neutralizer to be used in the in vivo efficacy studies.

e) Two pivotal studies should be conducted.

Regarding the effectiveness criteria proposed for surgical hand scrubs and rubs, FDA has not established a scientific basis for proposing revised criteria which significantly exceeds performance criteria established for previously approved NDA products. Furthermore, in the 2005 NDAC meeting, the NDAC voted to maintain the proposed performance criteria identified in the 1994 TFM, namely, a 1 $\log_{10}$ reduction of existing flora with no return to baseline in 6 hours after an initial application of a surgical scrub. The NDAC indicated that there was not sufficient evidence provided within the context of that meeting to substantiate a change in these performance criteria.

ACI proposes the agency adopt effectiveness criteria for *in vivo* effectiveness testing of active ingredients in surgical hand rubs and scrub of a 1 $\log_{10}$ reduction within one (1) minute after the first application procedure with no return to baseline within six (6) hours. In addition, it is appropriate that the active ingredient meet the mean log reduction criteria using a 70% responder rate with demonstrated statistically significant activity over vehicle in the study using the 95% confidence interval. Any *in vivo* effectiveness study of this kind should include a positive control to validate the results and the positive control should also meet the effectiveness criteria.

ACI believes a 1 $\log_{10}$ reduction with no return to baseline after 6 hours are acceptable performance criteria based on the performance of FDA-approved (NDA) products such as those containing chlorhexidine gluconate. Based on our statistical analysis of healthcare personnel handwash products to meet the propose effectiveness criteria (above), it is anticipated that arbitrarily increasing the effectiveness criteria for surgical scrubs and rubs to levels above a 2 $\log_{10}$

reduction after a single application, as described in the Proposed Rule, will also significantly impact the ability to validate a study using existing CHG NDA approved products as a test control.

4. Patient Preoperative Skin Preparations

We believe that testing consistent with ASTM method E1173-15 *Standard Test Method for Evaluation of Preoperative, Precatheterization, or Preinjection Skin Preparations* would be appropriate to demonstrate that an active ingredient is GRAE for use in patient preoperative skin preparation products, if generation of new data is needed. The agency's proposal to require efficacy testing at both a wet site (groin) and a dry site (abdomen) for pivotal studies establishing the GRAE status of Patient Preoperative Skin Preparation active ingredients is appropriate. Should the agency find that the data and information found in the administrative record or provided in response to the Proposed Rule is not sufficient to support a finding of GRAE for active ingredients in patient preoperative skin preparation products, ASTM E1173 should be deemed suitable as a method to gather such data. A copy of the E1173-15 Standard Test Method is provided as Attachment 8. In addition, the agency should apply the performance criteria published in the 1994 TFM for patient preoperative skin preparation products, which includes a 10 minute contact time. Although 30 seconds is appropriate for preinjection site products, this criterion is not practical and does not apply to clinical application of patient preoperative skin preparations.

In the 1994 TFM, the agency proposed the following for preinjection skin preparation:[36]

> *As discussed in section I.E., comment 10, the agency is proposing the indication "for the preparation of the skin prior to an injection" for OTC alcohol and isopropyl alcohol drug products. The agency is further proposing that products labeled for such use demonstrate effectiveness by testing according to the same procedure used to demonstrate the effectiveness of patient preoperative skin preparation drug products not labeled for this use. Based on this intended use of alcohol drug products, the agency is proposing a 1-log_{10} reduction in the microbial flora per square centimeter of a dry skin test site within 30 seconds of product use as the effectiveness criteria for these products.*

The agency should adopt the effectiveness criteria proposed in the 1994 TFM for active ingredients used in patient *preinjection* skin preparations, but may not be able to achieve the immediate and persistent efficacy criteria required for Preoperative Skin Preparation. The agency should recognize ASTM E1173 as an appropriate test method for evaluating the effectiveness of patient preinjection skin preparations at an appropriate dry skin site (e.g., median cubital region of the arm). We reserve comment on the log_{10} reduction requirements proposed in the Proposed Rule as more information is needed to determine the performance of an FDA-approved active control relative to these requirements.

In addition, active ingredients demonstrated to meet the proposed efficacy requirements for patient *preoperative* skin preparation should not be required to undergo further testing to

[36] 59 FR 31402 at 31432

demonstrate efficacy as a *preinjection* skin preparation, as the former is a more stringent requirement.

VI. Final Formulation Testing for Monograph Compliance

The proposed rule invites further comment on the final formulation testing and labeling conditions proposed in the 1994 TFM. We submit that running two full scale pivotal studies on an OTC Monograph product does not fit with the typical framework of the monograph system. Because the active ingredient(s) will have been demonstrated as GRAS/E, testing of final product should be limited to demonstrating that the product formulation has not inhibited the activity of the active ingredient. As such, there should be efficacy testing to confirm that the formulation has not reduced the efficacy of the monograph active. Some details to our proposal for final formulation testing are presented below.

A. Active Control

It is appropriate that GRAS/E active ingredients would serve as the active control for any efficacy studies required for final formulations. For example, alcohol at the concentration and application instructions evaluated in the pivotal studies to help establish GRAS/E status would become the active control for efficacy studies involving alcohol-based final formulations. This would be more appropriate than using an FDA-approved (NDA) product for the active control, particularly for alcohol-based hand sanitizer products where the only FDA-approved drug is a dual-active product.

B. *In Vitro* Efficacy Criteria

We agree with the agency's comments that the *in vitro* testing requirements proposed in the 1994 TFM were overly burdensome, particularly the requirement for MIC testing which does not necessarily provide useful information for health care topical antiseptic products which may be used full strength (i.e., used as is, without dilution). Therefore, we recommend MIC/MBC testing not be required for final formulations. A more appropriate method for demonstration of broad spectrum activity of formulations is the *in vitro* Time-Kill study. Thus, we propose that the only *in vitro* testing required for final formulations should be a Time-Kill study against an appropriate list of relevant microorganisms. We do not see this list of relevant organisms for *in vitro* effectiveness testing as being static and hope that in further discussions with the agency we can assure that the list of organisms relevant to clinical health care settings is identified and can be updated by manufacturers for *in vitro* final formulation efficacy testing as relevant organisms change in healthcare settings.

This test should be performed on final formulations only, undiluted and at a single in use concentration and contact time appropriate for the indication (e.g., 30 seconds).

C. *In Vivo* Efficacy Criteria

As stated above, running two full scale pivotal studies on an OTC Monograph product does not fit with the typical framework of the monograph system. However, because product

formulation can influence the performance of the active ingredient, we believe it may be appropriate that there to be an *in vivo* testing requirement for final products. We are not prepared to make recommendations pertaining to a specific study design at this point, but rather we request that the agency engage with industry to determine an appropriate method, study design and efficacy targets for each indication and as indicated in the use directions.

VII. Regulatory Issues

A. FDA's Regulatory Impact Analysis Fails to Address Key Considerations.

FDA's regulatory impact analysis (RIA) does not account for all costs associated with the proposed regulatory alternative and it overestimates the benefits of the Proposed Rule. In particular, we note that the Summary of Costs and Benefits fails to account for the costs that would be associated with a loss of availability of health care antiseptics in health care settings. In the attached report entitled *Estimation of Charges Associated with Preventable Healthcare Acquired Infections if Antibacterial Handwash Products Were Unavailable* (see Attachment 9), the number of prevented cases of hospital-acquired *bacterial* infections are estimated to be between 22,100 and 223,100 and the potential additional burden of hospital-acquired infections avoided by the use of healthcare antiseptics as $175 million to $4 billion annually in the United States. However, this estimate is conservative as it only looked at bacterial infections that may be prevented, and it did not include costs associated with hospital readmissions, short-term rehabilitation, long-term follow-up care, lost wages, lost productivity and transportation.

In addition, we note that FDA has overestimated the benefits associated with the proposed rule. The agency estimates a potential benefit of 20.6 billion 1-mL exposures to these chemicals avoided if, hypothetically, no antiseptic active ingredient is demonstrated to be GRAS/E for use in health care antiseptics. If the active ingredients in Health Care Antiseptic Products are safe, then there is no benefit realized by avoiding exposures to them. Moreover, this scenario could play out by virtue of the unnecessary burden proposed by the agency, not by virtue of the actual safety and efficacy of those active ingredients. In that, even if those exposures are being avoided it means that Health Care Antiseptics are not being used which would likely result in additional avoidable hospital acquired infections. The agency should correct its flawed economic analysis.

B. More Time Is Needed to Develop and Perform Safety and Efficacy Studies.

Although we believe that the proposed requirements for additional safety and efficacy studies is unjustified, in particular for those active ingredients proposed as Category I in the 1994 TFM, we note that the studies FDA proposed could take several years to design, execute, analyze, and report. FDA's timelines for new data submission therefore are unreasonable and unrealistic. If FDA decides to adopt these proposed testing requirements, we request that FDA defer rulemaking, so that we can work with the agency to confirm the appropriate data requirements and study protocols. We expect it would take five years or more to complete the safety and efficacy studies for the six active ingredients of interest to our members (Table 1 above).

C. FDA Should Formally Recognize the Food Handler Category as Distinct Monograph Category.

The Proposed Rule acknowledges that FDA identified in the 1994 TFM a new category of antiseptics for use by the food industry (i.e., Food Handler Products) but does not address how those products will be regulated.[37] It is clear that FDA recognizes that Food Handler Products do not fit within the definition of Consumer Antiseptics or Health Care Antiseptics. Therefore, unless Food Handler Products are explicitly recognized in a further amendment to the TFM, they could be effectively removed from the market without ever being given a fair hearing on their GRAS/E status and their importance in maintaining public health.

As such, we ask FDA to publish a formal notice clarifying the status of Food Handler Products. Until FDA publishes a Food Handler monograph, we recommend that FDA confirm that Food Handler topical antiseptic products can continue to be marketed under the current regulatory framework (1994 TFM).

D. FDA Should Include Separate Indications for Patient Preoperative Skin Preparations and Patient Pre-injection Skin Preparations.

The Proposed Rule includes a specific indication for patient preoperative skin preparation and includes within that indication products used for preinjection skin preparation[38]. Because these two product types serve a different purpose, have different use and exposure patterns, and different efficacy requirements, we propose that the indication for Patient Preoperative Skin Preparation drug products be separated into two categories for the purpose of defining GRAS/E active ingredients. We propose the following two categories: 1) Patient Preoperative Skin Preparation and 2) Patient Preinjection Skin Preparations. Surgical incision demands persistent activity due to the invasive nature of cutting through skin's natural barrier over a larger area, the procedure duration (which can be hours) and the time the incision point will be open and subsequently heal. As such, persistence may be an important attribute of Patient Preoperative Skin Preparations. In contrast, an injection is a procedure lasting only seconds and poses a relatively low risk of infection. The injection site heals quickly, so there is no need for persistent antimicrobial activity. If preinjection prep products are required to meet the same requirements as preoperative prep, this would effectively clear the market of available cost effective solutions for those who need them. Therefore, the efficacy requirements for these indications should be different. In addition, requiring injection site preps to pass the same persistent efficacy standards as a surgical site preps would provide no clinical benefit and only serve to increase the cost of providing injections and impose an unnecessarily cost burden our healthcare system.

In addition, the active ingredients associated with these two product types may have different safety profiles because they have different use and exposure profiles. Patient Preoperative Skin Preparations are not likely to have a duration and exposure as a result of patient use that would be considered chronic. This has direct implications regarding the safety

[37] 80 Fed. Reg. at 25168
[38] 80 FR 25166 to 25169

requirement for the associated active ingredients for these indications. According to ICH Guidelines, "[p]harmaceuticals administered infrequently or for short duration of exposure (e.g., anesthetics and radio-labelled imaging agents) do not need carcinogenicity studies unless there is cause for concern."[39]

As a consequence, carcinogenicity data and other chronic safety data that FDA requested for active ingredients used in Patient Preoperative Skin Preparations and Patient Preinjection Skin Preparations should be eliminated.

VIII. Conclusions

We respectfully request that FDA consider the recommendations outlined in these comments, which address the scientific weaknesses and practical implications of the Proposed Rule. In summary, our recommendations are:

- FDA should re-evaluate all data relevant to the safety and efficacy of health care antiseptic active ingredients and make affirmative findings that they are generally recognized as safe (GRAS) and generally recognized as effective (GRAE).

- FDA should maintain the Category I classifications proposed in the 1994 TFM.

- FDA should consider all existing data relevant to the efficacy of health care antiseptic active ingredients and indicate where specific data gaps exist, if any, for each active ingredient.

- FDA should accept clinical simulation studies with surrogate endpoints as a practical means to assess the general recognition of effectiveness (GRAE) of active ingredients in health care antiseptic products, when such data are necessary. FDA should recognize ASTM methods E1174, E2755, and E1115 as appropriate to support *in vivo* efficacy testing for active ingredients in respective health care antiseptic products.

- FDA should consider the level of human exposure to each of the antimicrobial active ingredients and assess the potential for harm from those exposures prior to determining the need for additional safety data. In assessing exposure to health care antiseptic product active ingredients, FDA should allow alternatives methods to a Maximal Use Trial (MUsT), including physiologically-based pharmacokinetic (PBPK) models and potentially other animal or human studies. FDA should provide additional guidance how a MUsT study may be conducted in a reasonable manner.

[39] ICH guideline, ''Guideline on the Need for Carcinogenicity Studies of Pharmaceuticals S1A,'' November 1995, available at: http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Safety/S1A/Step4/S1A_Guideline.pdf.

- FDA should formally recognize and acknowledge a separate subcategory for Food Handler Products before the Consumer Antiseptics Rule or Health Care Antiseptics Rule is finalized.

- FDA should set an alternative timeline for the finalization of the monograph and engage with stakeholders to develop appropriate efficacy and safety data requirements and detailed protocols to generate these data.

We would be happy to provide copies of any of the cited studies and reports upon request. We are willing to meet with you to review them in detail. Please contact me if you have any questions on these comments.

Sincerely,

Paul C. DeLeo
Associate Vice President, Environmental Safety

ATTACHMENT 1: AN IN-VITRO TIME-KILL EVALUATION OF SIX TEST MATERIALS WHEN CHALLENGED WITH TWENTY-EIGHT BACTERIAL AND YEAST SPECIES

DRAFT PROTOCOL #150940-201

BIOSCIENCES LABORATORIES, INC.

October 26, 2015

DRAFT PROTOCOL #150940-201

AN IN-VITRO TIME-KILL EVALUATION OF SIX TEST MATERIALS WHEN CHALLENGED WITH TWENTY-EIGHT BACTERIAL AND YEAST SPECIES

Prepared for:

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CONFIDENTIAL

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1.0 TITLE: AN IN-VITRO TIME-KILL EVALUATION OF SIX TEST MATERIALS WHEN CHALLENGED WITH TWENTY-EIGHT BACTERIAL AND YEAST SPECIES

2.0 SPONSOR: AMERICAN CLEANING INSTITUTE
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3.0 TESTING FACILITY: BIOSCIENCE LABORATORIES, INC.
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4.0 PURPOSE:

This study will use an In-Vitro Time-Kill Method to evaluate the broad-spectrum antimicrobial properties of six test materials when challenged with suspensions of 28 bacterial and yeast species. This procedure is based upon the methodology described in ASTM E2783-11, *Standard Test Method for Assessment of Antimicrobial Activity for Water Miscible Compounds Using a Time-Kill Procedure*. All testing will be performed in accordance with Good Laboratory Practices, as specified in 21 CFR Part 58, with two exceptions. The characterization of the identity, strength, purity, composition, stability, and solubility of all test materials remains the responsibility of the Study Sponsor and will not be performed by the Testing Facility (GLP 58.105 and GLP 58.113). Verification of the antibiotic resistance of purportedly drug-resistant American Type Culture Collection (ATCC) strains will be performed by another facility; these procedures are exempt from this GLP statement.

5.0 SCOPE:

An In-Vitro Time-Kill evaluation of six test materials will be performed versus suspensions of the 23 microorganism species listed in the Tentative Final Monograph, *Federal Register*, 17 June 1994, vol. 59:116, p. 31444, as well as *Burkholderia cepacia*, *Enterococcus faecalis* VRE, *Enterococcus faecium* VRE, *Staphylococcus aureus* MRSA, and *Staphylococcus epidermidis* MRSE. The percent and $\log_{10}$ reduction in the microbial population of each challenge strain will be determined following exposure to each test material at room temperature (20 to 30 °C) for 15 seconds only, or for 30 seconds and 60 seconds (reference Section 6.0). Testing of each challenge species versus each test material at each of the appropriate time exposures will be performed in triplicate; all agar-plating will be performed in duplicate.

6.0 TEST MATERIALS:

All test materials will be provided to the Testing Facility by the Study Sponsor, complete with the appropriate documentation. If the product names, lot numbers and expiration dates are not documented below, the information will be presented in the Final Report. Responsibility for determination of the identity, strength, purity, composition, solubility, and stability of the test materials, as well as responsibility for retention of all test materials, rests with the Study Sponsor.

Test Material #1: Benzalkonium Chloride
Lot Number: _____
Manufacture Date: _____
Expiration Date: _____
Exposure Time(s): 30 seconds and 60 seconds

Test Material #2: Benzethonium Chloride
Lot Number: _____
Manufacture Date: _____
Expiration Date: _____
Exposure Time(s): 30 seconds and 60 seconds

Test Material #3: Chloroxylenol
Lot Number: _____
Manufacture Date: _____
Expiration Date: _____
Exposure Time(s): 30 seconds and 60 seconds

Test Material #4: Povidone Iodine
Lot Number: _____
Manufacture Date: _____
Expiration Date: _____
Exposure Time(s): 30 seconds and 60 seconds

Test Material #5: Ethanol
Lot Number: _____
Manufacture Date: _____
Expiration Date: _____
Exposure Time(s): 15 seconds

Test Material #6: Isopropyl Alcohol
Lot Number: _____
Manufacture Date: _____
Expiration Date: _____
Exposure Time(s): 15 seconds

7.0 CHALLENGE BACTERIAL SPECIES:

The challenge bacterial species (American Type Culture Collection [ATCC] strains) to be evaluated are designated below:

7.1 *Acinetobacter baumannii* (ATCC #19606)
7.2 *Bacteroides fragilis* (ATCC #25285)
7.3 *Burkholderia cepacia* (ATCC #25416)
7.4 *Candida albicans* (ATCC #10231)\
7.5 *Candida tropicalis* (ATCC #750)
7.6 *Enterobacter aerogenes* (ATCC #13048)
7.7 *Enterococcus faecalis* (ATCC #29212)
7.8 *Enterococcus faecalis* MDR, VRE (ATCC #51575)
7.9 *Enterococcus faecium* VRE (ATCC #700221)
7.10 *Escherichia coli* (ATCC #11229)
7.11 *Escherichia coli* (ATCC #25922)
7.12 *Haemophilus influenzae* (ATCC #19418)
7.13 *Klebsiella pneumoniae* (ATCC #10031)
7.14 *Micrococcus luteus* (ATCC #7468)
7.15 *Pseudomonas aeruginosa* (ATCC #15442)
7.16 *Pseudomonas aeruginosa* (ATCC #27853)
VRE = Vancomycin-Resistant *Enterococcus*
MDR = Multi-Drug Resistant

7.17 *Proteus mirabilis* (ATCC #7002)
7.18 *Serratia marcescens* (ATCC #14756)
7.19 *Staphylococcus aureus* (ATCC #6538)
7.20 *Staphylococcus aureus* (ATCC #29213)
7.21 *Staphylococcus aureus* MRSA (ATCC #43300)
7.22 *Staphylococcus epidermidis* (ATCC 12228)
7.23 *Staphylococcus epidermidis* MRSE (ATCC #51625)
7.24 *Staphylococcus haemolyticus* (ATCC #29970)
7.25 *Staphylococcus hominis* (ATCC #27845)
7.26 *Staphylococcus saprophyticus* (ATCC #15305)
7.27 *Streptococcus pneumoniae* (ATCC #49619)
7.28 *Streptococcus pyogenes* (ATCC #12344)

MRSA = Methicillin-Resistant *Staphylococcus aureus*
MRSE = Methicillin-Resistant *Staphylococcus epidermidis*

8.0 EQUIPMENT:

8.1 Incubator, Temperature Range 35 ± 2 °C
8.2 Incubator, Temperature Range 30 ± 2 °C
8.3 Incubator Thermometers
8.4 Refrigerators, Temperature Range 2 - 8 °C
8.5 Refrigerator Thermometers
8.6 Water Bath, 47 ± 2 °C
8.7 Water Bath Thermometer
8.8 Vortex Mixers
8.9 Laminar Biological Flow hood
8.10 Steam Autoclaves
8.11 GasPak™ Anaerobic System (Anaerobic Jars)
8.12 Continuously Adjustable Pipetters, 100 µL - 1000 µL Capacity
8.13 Continuously Adjustable Pipetters, 20 µL - 200 µL Capacity
8.14 Microman® Positive Displacement Pipetters, 10 µL - 100 µL Capacity
8.15 Microman® Positive Displacement Pipetters, 100 µL - 1000 µL Capacity
8.16 Portable Pipetters
8.17 Calibrated Minute/Second Timers
8.18 Centrifuge

9.0 SUPPLIES:

9.1 Glass Beakers, Sterilized
9.2 Dry Anaerobic Indicator Strips, or equivalent
9.3 GasPak™ EZ Anaerobe Container System Sachets, or equivalent
9.4 GasPak™ EZ CO_2 Container System Sachets, or equivalent
9.5 Hand-Tally Counters
9.6 Inoculating Loops
9.7 Sterile Disposable Centrifuge Tubes, 15 mL
9.8 Sterile Disposable Pipettes
9.9 Sterile Disposable T-Spreaders
9.10 Sterile Disposable Petri Plates
9.11 Sterile Disposable Specimen Containers, 2 ounce and 4 ounce
9.12 Sterile 1.0 mL and 0.1 mL Positive Displacement Tips
9.13 Sterile Syringes
9.14 Sterile Universal 1.0 and 0.2 mL Pipette Tips
9.15 Test Tubes, Sterilized

10.0 MEDIA:

10.1 Tryptic Soy Broth (TSB)
10.2 Tryptic Soy Agar (TSA)
10.3 Tryptic Soy Agar with product neutralizers, including 0.5% Tween 80 and 0.07% lecithin (TSA+)
10.4 Tryptic Soy Agar with 5% sheep blood (SBA)
10.5 Sabouraud Dextrose Broth (SDB)
10.6 Sabouraud Dextrose Agar (SDA)
10.7 Sabouraud Dextrose Agar with product neutralizers, including 0.5% Tween 80 and 0.07% lecithin (SDA+)
10.8 Brain-Heart Infusion Broth (BHIB)
10.9 Brain-Heart Infusion Agar (BHIA)
10.10 Brain-Heart Infusion Agar with product neutralizers, including 0.5% Tween 80 and 0.07% lecithin (BHIA+)
10.11 Schaedler's Broth (SB)
10.12 Schaedler's Agar with 0.5% lysed horse blood (SA-B)
10.13 Chocolate Agar with Enrichment (CAE)
10.14 Neutralizing Formulation – Butterfield's Phosphate Buffer solution with product neutralizers (BBP++)
10.15 0.9% Sodium Chloride Irrigation, USP (SCI)

11.0 INOCULUM PREPARATION:

Haemophilus influenzae (ATCC #19418) and *Streptococcus pneumoniae* (ATCC #49619)

11.1 Approximately 48 hours prior to testing, inocula from lyophilized vials or cryogenic cultures will be suspended in 0.9% Sodium Chloride Irrigation, USP (SCI), inoculated onto the surface of the appropriate agar contained in Petri plates (reference Table 1), and incubated at 35 ± 2 °C under the conditions appropriate for each species for approximately 24 hours, or until sufficient growth is observed.

11.2 Approximately 24 hours prior to testing, a suspension of each species will be prepared by rinsing the plates of solid media with sterile SCI. The purity of each suspension will be verified by preparing isolation streaks of each culture on the appropriate agar (reference Table 1). Aliquots of each suspension will then be spread-plated onto the surface of the appropriate agar and incubated at 35 ± 2 °C under the conditions appropriate for each species until sufficient growth is observed. This will produce lawns of the bacteria on the surface of the agar plates, and growth from these will be used to prepare the challenge suspensions.

11.3 Immediately prior to initiating the test procedures, an initial suspension of each challenge microorganism will be prepared in SCI by suspending the microorganisms from the solid media previously prepared to produce suspension concentrations of approximately 10^9 CFU/mL. The suspensions may be centrifuged, if deemed necessary, to achieve the desired concentration.

Bacteroides fragilis (ATCC #25285)

11.4 Four to six days prior to testing, sterile tubes containing Schaedler's Broth (SB) will be inoculated from a lyophilized vial or a cryogenic culture containing this species. The broth cultures will be incubated anaerobically at 35 ± 2 °C for 48 to 72 hours, or until sufficient growth is observed.

11.5 48 to 72 hours prior to testing, the broth cultures prepared will be subcultured into additional tubes of SB and incubated anaerobically at 35 ± 2 °C until sufficient growth is observed. The purity of the broth culture will be verified by preparing duplicate isolation streaks on Chocolate Agar with Enrichment (CAE), and incubating one plate aerobically and one anaerobically at 35 ± 2 °C.

11.6 Immediately prior to initiating the test procedures, an initial suspension of this species containing approximately 10^9 CFU/mL will be prepared by centrifuging the broth cultures tubes, combining the resulting pellets, and re-suspending them SB.

All species not described above

11.7 Two to four days prior to testing, separate sterile tubes containing the appropriate broth (reference Table 1) will be inoculated from lyophilized vials or cryogenic cultures containing the challenge species. The broth cultures will be incubated at the temperature appropriate for each species for 24 to 48 hours, or until sufficient growth is observed (reference Table 1).

11.8 24 to 48 hours prior to testing, the broth cultures will be inoculated onto the surface of the appropriate agar media (reference Table 1) contained in Petri plates and incubated appropriately until sufficient growth is observed. This will produce lawns of the bacteria on the surface of the agar plates, and growth from these will be used to prepare the challenge suspensions. The purity of each broth culture will be verified by preparing an isolation streak on the appropriate agar, and incubating appropriately. Isolation streaks of each drug-resistant ATCC strain will be prepared on Tryptic Soy Agar with 5% Sheep Blood (SBA). These plates will be incubated appropriately for approximately 24 hours and provided to a CLIA[1]-certified laboratory facility for determination/confirmation of antibiotic resistance. The results of the resistance verification of these species will be presented in the Final Report.

11.9 Immediately prior to initiating the test procedures, an initial suspension of each challenge microorganism will be prepared in SCI by suspending the microorganisms from the solid media previously prepared to produce suspension concentrations of approximately 10^9 CFU/mL.

12.0 NEUTRALIZATION STUDIES:

12.1 Neutralization studies of each test material will be performed versus *Bacteroides fragilis* (ATCC #25285), *Escherichia coli* (ATCC #11229), and *Streptococcus pneumoniae* (ATCC #49619) to ensure that the neutralizing solution employed (Butterfield's Phosphate Buffer solution with product neutralizers [BBP++]) effectively neutralizes the antimicrobial properties of each test material and is non-toxic to these representative challenge strains. This neutralization procedure is based on guidelines set forth in ASTM E1054-08 (2013), *Standard Test Methods for Evaluation of Inactivators of Antimicrobial Agents*.

12.2 Neutralization Challenge Suspensions containing approximately 1 x 10^4 CFU/mL will be prepared for *Escherichia coli* (ATCC #11229) and *Streptococcus pneumoniae* (ATCC #49619) by diluting the initial suspensions (Sections 11.3 and 11.9) in additional SCI, as necessary.

12.3 A Neutralization Challenge Suspension containing approximately 1 x 10^4 CFU/mL will be prepared for *Bacteroides fragilis* (ATCC #25285) by diluting the initial suspension (Section 11.6) in additional SB, as necessary.

Neutralization Effectiveness Evaluation (Test A) – *Escherichia coli* (ATCC #11229)

12.4 Three replicates of this procedure will be performed versus *Escherichia coli* (ATCC #11229).

12.5 A 0.1 mL aliquot of a Neutralization Challenge Suspension will be transferred to a test tube containing 8.9 mL of BBP++ (10^0 dilution). A 1.0 mL aliquot of test material will be added to the tube containing inoculum/neutralizing formulation and mixed thoroughly.

[1] = Clinical Laboratory Improvement Amendments.

12.6 The inoculum/neutralizing formulation/product mixture will be exposed for greater than or equal to 15 minutes, timed using a calibrated minute/second timer. Following exposure, a 10-fold dilution (e.g., 10^{-1}) will be prepared in BBP++ and mixed thoroughly.

12.7 1.0 mL and/or 0.1 mL aliquots of each suspension will be pour-plated, in duplicate, using Tryptic Soy Agar with product neutralizers (TSA+) to produce final plated dilutions of e.g., 10^{0}, 10^{-1}, and 10^{-2}. The plates will be incubated at 35 ± 2 °C for 48 to 72 hours, or until sufficient growth is observed (reference Table 1).

Neutralizer Toxicity Evaluation (Test B) – *Escherichia coli* (ATCC #11229)

12.8 Three replicates of this procedure will be performed versus *Escherichia coli* (ATCC #11229).

12.9 A 0.1 mL aliquot of a Neutralization Challenge Suspension will be transferred to a test tube containing 8.9 mL of BBP++ (10^{0} dilution).. A 1.0 mL aliquot of SCI will be added to the tube containing inoculum/neutralizing formulation and mixed thoroughly (10^{0} dilution).

12.10 The inoculum/neutralizing formulation/SCI mixture will be exposed for greater than or equal to 15 minutes, timed using a calibrated minute/second timer. Following exposure, a 10-fold dilution (e.g., 10^{-1}) will be prepared in BBP++ and mixed thoroughly.

12.11 1.0 mL and/or 0.1 mL aliquots of each suspension will be pour-plated, in duplicate, using TSA+ to produce final plated dilutions of e.g., 10^{0}, 10^{-1}, and 10^{-2}. The plates will be incubated at 35 ± 2 °C for 48 to 72 hours, or until sufficient growth is observed (reference Table 1).

Test Organism Viability (Test C) – *Escherichia coli* (ATCC #11229)

12.12 Three replicates of this procedure will be performed versus *Escherichia coli* (ATCC #11229).

12.13 A 0.1 mL aliquot of a Neutralization Challenge Suspension will be transferred to a test tube containing 9.9 mL of SCI and mixed thoroughly (10^{0} dilution).

12.14 The inoculum/SCI mixture will be exposed for greater than or equal to 15 minutes, timed using a calibrated minute/second timer. Following exposure, a 10-fold dilution (e.g., 10^{-1}) will be prepared in SCI and mixed thoroughly.

12.15 1.0 mL and/or 0.1 mL aliquots of each suspension will be pour-plated, in duplicate, using TSA to produce final plated dilutions of e.g., 10^{0}, 10^{-1}, and 10^{-2}. The plates will be incubated at 35 ± 2 °C for 48 to 72 hours, or until sufficient growth is observed (reference Table 1).

Neutralization Effectiveness Evaluation (Test A) – *Bacteroides fragilis* (ATCC #25285) and *Streptococcus pneumoniae* (ATCC #49619)

12.16 Three replicates of this procedure will be performed versus *Bacteroides fragilis* (ATCC #25285) and *Streptococcus pneumoniae* (ATCC #49619).

12.17 A 0.1 mL aliquot of a Neutralization Challenge Suspension will be transferred to a test tube containing 8.9 mL of BBP++ (10^{0} dilution). A 1.0 mL aliquot of test material will be added to the tube containing inoculum/neutralizing formulation and mixed thoroughly.

12.18 The product/neutralizer/inoculum suspension will be diluted and plated immediately (within approximately 1 minute). A 10-fold dilution (10^{-1}) of the suspension of *Bacteroides fragilis* will be prepared in SB; the 10-fold dilutions of the suspension of *Streptococcus pneumoniae* will be prepared in SCI.

12.19 1.0 mL and/or 0.1 mL aliquots of each suspension will be pour- or spread-plated, in duplicate, using the appropriate agar media (reference Table 1) to produce final plated dilutions of e.g., 10^0, 10^{-1}, and 10^{-2}. The plates will be incubated at 35 ± 2 °C under the conditions appropriate for each species for 2 to 5 days, or until sufficient growth is observed (reference Table 1).

Neutralizer Toxicity Evaluation (Test B) – *Bacteroides fragilis* (ATCC #25285) and *Streptococcus pneumoniae* (ATCC #49619)

12.20 Three replicates of this procedure will be performed versus *Bacteroides fragilis* (ATCC #25285) and *Streptococcus pneumoniae* (ATCC #49619).

12.21 A 0.1 mL aliquot of a Neutralization Challenge Suspension will be transferred to a test tube containing 8.9 mL of BBP++ (10^0 dilution).. A 1.0 mL aliquot of SCI will be added to the tube containing inoculum/neutralizing formulation and mixed thoroughly (10^0 dilution).

12.22 The inoculum/neutralizing formulation/SCI mixture will be diluted and plated immediately (within approximately 1 minute). A 10-fold dilution (10^{-1}) of the suspension of *Bacteroides fragilis* will be prepared in SB; the 10-fold dilutions of the suspension of *Streptococcus pneumoniae* will be prepared in SCI.

12.23 1.0 mL and/or 0.1 mL aliquots of each suspension will be pour- or spread-plated, in duplicate, using the appropriate agar media (reference Table 1) to produce final plated dilutions of e.g., 10^0, 10^{-1}, and 10^{-2}. The plates will be incubated at 35 ± 2 °C under the conditions appropriate for each species for 2 to 5 days, or until sufficient growth is observed (reference Table 1).

Test Organism Viability (Test C) – *Bacteroides fragilis* (ATCC #25285) and *Streptococcus pneumoniae* (ATCC #49619)

12.24 Three replicates of this procedure will be performed versus *Bacteroides fragilis* (ATCC #25285) and *Streptococcus pneumoniae* (ATCC #49619).

12.25 A 0.1 mL aliquot of a Neutralization Challenge Suspension will be transferred to a test tube containing 9.9 mL of SCI (*Streptococcus pneumoniae*), or 9.9 mL of SB (*Bacteroides fragilis*), and mixed thoroughly (10^0 dilution).

12.26 The inoculum/SCI or inoculum/SB mixtures will be diluted and plated immediately (within approximately 1 minute). A 10-fold dilution (10^{-1}) of the suspension of *Bacteroides fragilis* will be prepared in SB; the 10-fold dilutions of the suspension of *Streptococcus pneumoniae* will be prepared in SCI.

12.27 1.0 mL and/or 0.1 mL aliquots of each suspension will be pour- or spread-plated, in duplicate, using the appropriate agar media (reference Table 1) to produce final plated dilutions of e.g., 10^0, 10^{-1}, and 10^{-2}. The plates will be incubated at 35 ± 2 °C under the conditions appropriate for each species for 2 to 5 days, or until sufficient growth is observed (reference Table 1).

Data Collection

12.28 Following incubation, the colonies on the plates will be counted manually using a hand-tally counter. Counts in the range of 30 to 300 colony-forming units (CFU) will be used preferentially in the data calculations. If no counts in this range are observed, those plates with colony counts closest to those ranges will be used for the data calculations.

Acceptance Criterion

12.29 The Log_{10} of the number of survivors of each challenge strain from Test A and Test B will be statistically compared to those from Test C using a One-Way Analysis of Variance (ANOVA). If the 95% Confidence Interval of Test A for a product overlaps that of Test C, neutralization will be considered effective for that product. If the 95% Confidence Interval of Test B overlaps that of Test C, the neutralizing formulation (BBP++) will be considered non-toxic to that challenge species.

12.30 Because low variability of the recovery data can affect interpretation of the results from the statistical analysis, an alternative method of assessing the neutralization outcomes may be necessary. As referenced by ASTM E1054-08 (Note 10), the data may be considered equivalent if the microbial recovery populations (the average of the three replicates) are no more than 0.2 $\log_{10}$ lower than those of Test C (the average of the three replicates).

13.0 TIME-KILL METHODOLOGY:

Challenge Suspension Preparation

13.1 Challenge Suspensions of each bacterial and yeast species containing approximately 1 x 10^8 CFU/mL will be prepared by diluting the initial suspensions (reference Sections 11.3, 11.6, and 11.9) in SCI or SB, as appropriate.

Initial and Final Population Methodology

13.2 Prior to use in testing, the initial population of each challenge suspension (except those of *Bacteroides fragilis* [ATCC #25285] and *Streptococcus pneumoniae* [ATCC #49619]) will be determined by preparing 10-fold dilutions (e.g., 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6}) in BBP++. The 10-fold dilutions of *Bacteroides fragilis* will be prepared in SB; the 10-fold dilutions of *Streptococcus pneumoniae* will be prepared in SCI. Using the appropriate agar media (reference Table 1), pour- or spread-plates will be prepared, in duplicate, from the inoculum dilutions by plating 0.1 mL of the final dilutions to achieve plated dilutions of, e.g., 10^{-5}, 10^{-6}, and 10^{-7}. The plates will be incubated at the temperature and under the conditions appropriate for each species for 2 to 5 days, or until sufficient growth is observed (reference Table 1).

13.3 Following the completion of the appropriate testing procedures, the final population of each challenge suspension will be determined, as described above.

13.4 The initial and final population of each challenge suspension must be within 0.5 $\log_{10}$ of each other for the testing to be considered valid.

Numbers Controls (i.e., Blanks)

13.5 A 0.1 mL aliquot of a challenge suspension containing approximately 1 x 10^8 CFU/mL will be transferred to a sterile tube containing 10.0 mL of SCI and mixed thoroughly using a vortex mixer and/or positive displacement pipetter (10^0 dilution).

13.6 Each challenge microorganism will be exposed to the SCI for 60 seconds, timed using a calibrated minute/second timer.

13.7 After each exposure time has elapsed, 1.0 mL will be transferred from each tube containing SCI/challenge suspension to separate sterile test tubes containing 9.0 mL BBP++ (10^{-1} dilution), and mixed thoroughly using a vortex mixer. Ten-fold dilutions (e.g., 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5}) of the suspensions of each challenge species (except those of *Bacteroides fragilis* and *Streptococcus pneumoniae*) will be prepared in BBP++, mixing thoroughly using a vortex mixer between dilutions. The 10-fold dilutions of *Bacteroides fragilis* will be prepared in SB; the 10-fold dilutions of *Streptococcus pneumoniae* will be prepared in SCI.

13.8 From the final dilutions of the SCI/neutralizer/challenge suspension, 0.1 or 1.0 mL aliquots will be pour- or spread-plated, in duplicate, using the appropriate agar (reference Table 1), producing final plated dilutions of, e.g., 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6}. The plates will be incubated at the temperature and under the conditions appropriate for each species (reference Table 1) for 2 to 5 days, or until sufficient growth is observed.

Time-Kill Testing Methodology

13.9 A 0.1 mL aliquot of a challenge suspension containing approximately 1 x 10^8 CFU/mL will be transferred to a sterile tube containing 10.0 mL of a test material and mixed thoroughly using a vortex mixer and/or positive displacement pipetter (10^0 dilution).

13.10 Each challenge microorganism will be exposed to the test materials for 15 seconds only, or for 30 seconds and 60 seconds (reference Section 6.0), timed using a calibrated minute/second timer.

13.11 After each exposure time has elapsed, 1.0 mL will be transferred from each tube containing product/challenge suspension to separate sterile test tubes containing 9.0 mL BBP++ (10^{-1} dilution), and mixed thoroughly using a vortex mixer. Ten-fold dilutions (e.g., 10^{-2}, 10^{-3}, and 10^{-4}) of the suspensions of each challenge species (except those of *Bacteroides fragilis* and *Streptococcus pneumoniae*) will be prepared in BBP++, mixing thoroughly using a vortex mixer between dilutions. The 10-fold dilutions of *Bacteroides fragilis* will be prepared in SB; the 10-fold dilutions of *Streptococcus pneumoniae* will be prepared in SCI.

13.12 From the final dilutions of the product/neutralizer/challenge suspension, 0.1 or 1.0 mL aliquots will be pour- or spread-plated, in duplicate, using the appropriate agar (reference Table 1), producing final plated dilutions of, e.g., 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5}. The plates will be incubated at the temperature and under the conditions appropriate for each species (reference Table 1) for 2 to 5 days, or until sufficient growth is observed.

13.13 The procedures described in Sections 13.9 through 13.12 will be performed in triplicate using each challenge microorganism and each test material.

Data Collection

13.14 Following incubation, the colonies on the plates will be counted manually using a hand-tally counter. Counts in the range of 30 to 300 CFU will be used preferentially in the data calculations. If no counts in this range are observed, those plates with colony counts closest to this range will be used for the data calculations.

TABLE 1: CHALLENGE MICROORGANISMS, MEDIA, AND INCUBATION CONDITIONS

No.	Microorganism Species	ATCC Number	Incubation Time	Incubation Temperature	Media
1	*Acinetobacter baumannii*	19606	24 – 72 Hours	35 ± 2 °C	BHIB/BHIA/BHIA+
2	*Bacteroides fragilis*	25285	24 – 72 Hours	35 ± 2 °C (Anaerobic)	SB/SA-B/CAE
3	*Burkholderia cepacia*	25416	24 – 72 Hours	30 ± 2 °C	TSB/TSA/TSA+
4	*Candida albicans*	10231	24 – 72 Hours	30 ± 2 °C	SDB/SDA/SDA+
5	*Candida tropicalis*	750	24 – 72 Hours	30 ± 2 °C	SDB/SDA/SDA+
6	*Enterobacter aerogenes*	13048	24 – 72 Hours	35 ± 2 °C	TSB/TSA/TSA+
7	*Enterococcus faecalis*	29212	24 – 72 Hours	35 ± 2 °C	TSB/TSA/TSA+
8	*Enterococcus faecalis* MDR, VRE	51575	24 – 72 Hours	35 ± 2 °C	TSB/TSA/TSA+/SBA
9	*Enterococcus faecium* VRE	700221	24 – 72 Hours	35 ± 2 °C	TSB/TSA/TSA+/SBA
10	*Escherichia coli*	11229	24 – 72 Hours	35 ± 2 °C	TSB/TSA/TSA+
11	*Escherichia coli*	25922	24 – 72 Hours	35 ± 2 °C	TSB/TSA/TSA+
12	*Haemophilus influenzae*	19418	24 – 72 Hours	35 ± 2 °C (+ CO_2)	SCI/CAE
13	*Klebsiella pneumoniae*	10031	24 – 72 Hours	35 ± 2 °C	TSB/TSA/TSA+
14	*Micrococcus luteus*	7468	24 – 72 Hours	30 ± 2 °C	TSB/TSA/TSA+
15	*Proteus mirabilis*	7002	24 – 72 Hours	35 ± 2 °C	TSB/TSA/TSA+
16	*Pseudomonas aeruginosa*	15442	24 – 72 Hours	35 ± 2 °C	TSB/TSA/TSA+
17	*Pseudomonas aeruginosa*	27853	24 – 72 Hours	35 ± 2 °C	TSB/TSA/TSA+
18	*Serratia marcescens*	14756	24 – 72 Hours	30 ± 2 °C	TSB/TSA/TSA+
19	*Staphylococcus aureus*	6538	24 – 72 Hours	35 ± 2 °C	TSB/TSA/TSA+
20	*Staphylococcus aureus*	29213	24 – 72 Hours	35 ± 2 °C	TSB/TSA/TSA+
21	*Staphylococcus aureus* MRSA	43300	24 – 72 Hours	35 ± 2 °C	TSB/TSA/TSA+/SBA
22	*Staphylococcus epidermidis*	12228	24 – 72 Hours	35 ± 2 °C	TSB/TSA/TSA+
23	*Staphylococcus epidermidis* MRSE	51625	24 – 72 Hours	35 ± 2 °C	TSB/TSA/TSA+/SBA
24	*Staphylococcus haemolyticus*	29970	24 – 72 Hours	35 ± 2 °C	TSB/TSA/TSA+
25	*Staphylococcus hominis*	27845	24 – 72 Hours	35 ± 2 °C	TSB/TSA/TSA+
26	*Staphylococcus saprophyticus*	15305	24 – 72 Hours	35 ± 2 °C	TSB/TSA/TSA+
27	*Streptococcus pneumoniae*	49619	24 – 72 Hours	35 ± 2 °C (+ CO_2)	SCI/SBA
28	*Streptococcus pyogenes*	12344	24 – 72 Hours	35 ± 2 °C	BHIB/BHIA/BHIA+

Note: Incubation times are nominal, but in practice, incubation will continue until good growth is observed.
VRE = Vancomycin-Resistant *Enterococcus* MRSA = Methicillin-Resistant *Staphylococcus aureus*
MRSE = Methicillin-Resistant *Staphylococcus epidermidis*

14.0 CALCULATIONS:

14.1 The Initial Population (IP) and the Final Population (FP) of each challenge suspension will be calculated as follows:

Log_{10} (IP or FP) = Log_{10} (C_i x 10^{-D})

CFU/mL (IP or FP) = (C_i x 10^{-D})

Where:

C_i = Average of the Two Plates Counted
D = Dilution Factor of the Plates Counted

14.2 The Numbers Control (NC) population recovery (CFU/mL and Log_{10} CFU/mL) will be calculated for each challenge suspension as follows:

Log_{10} (NC) = Log_{10} (C_i x 10^{-D})

CFU/mL (NC) = (C_i x 10^{-D})

Where:

C_i = Average of the Two Plates Counted
D = Dilution Factor of the Plates Counted

14.3 The Post-Exposure Population (P_{EX}) of each challenge suspension following each timed exposure to each test material will be calculated for each replicate of testing as follows:

Log_{10} (P_{EX}) = Log_{10} (C_i x 10^{-D})

CFU/mL (P_{EX}) = (C_i x 10^{-D})

Where:

C_i = Average of the Two Plates Counted
D = Dilution Factor of the Plates Counted

14.4 The Log_{10} Reduction attributable to each test material will be calculated for each replicate of testing as follows:

Log_{10} Reduction = Log_{10} (NC) - Log_{10} (P_{EX})

Where:

NC = Numbers Control Population (CFU/mL)
P_{EX} = Post-Exposure Population (CFU/mL)

14.5 The Percent Reduction attributable to each test material will be calculated for each replicate of testing as follows:

$$\text{Percent Reduction} = \frac{NC - P_{EX}}{NC} \times 100$$

Where:

NC = Numbers Control Population (CFU/mL)
P_{EX} = Post-Exposure Population (CFU/mL)

14.6 The Average Log_{10} Reduction attributable to each test material will be calculated for each time of exposure as follows:

$$\text{Average } Log_{10} \text{ Reduction} = \frac{\sum Log_{10} \text{ Reductions}}{3}$$

Where:

3 = Number of replicates

15.0 STATISTICAL ANALYSIS:

A One-Way Analysis of Variance (ANOVA) will be performed on the data derived from the Neutralization Study. A statistical analysis will not be performed on the data derived from the Time-Kill portion of this evaluation.

16.0 FINAL REPORT:

A Final Report will be issued presenting the results of this evaluation in a clear, concise manner.

17.0 EXCEPTIONAL CONDITIONS:

The Sponsor will be notified by telephone, email, and/or letter of any exceptions encountered in this study. The exceptional conditions or occurrences will be detailed in full and formally recorded. Exceptional conditions that occur and are not addressed in this Protocol will be subject to Out-of-Scope charges (See Proposal/Contract).

19.0 DOCUMENTATION AND RECORD-KEEPING:

All documentation and records will be compiled, analyzed, and retained by BioScience Laboratories, Inc. at its facility in Bozeman, Montana. All raw data for this study, as well as the Final Report, will be retained in safe storage by the Testing Facility for a period of at least 5 years. BioScience Laboratories, Inc. will notify the Study Sponsor before any documents or records are destroyed.

20.0 QUALITY ASSURANCE AUDITS:

The Quality Assurance Unit (QAU) will conduct an in-phase audit of critical processes in testing at least once and advise the Study Director and Management of the outcomes of this. On completion of testing, the QAU will perform an audit of the data and the Final Report in accordance with 21 CFR Part 58.

21.0 LIABILITY AND INDEMNIFICATION:

Test Facility's liability to Sponsor under this Protocol shall be limited to the price of this evaluation. Sponsor shall be responsible to Study Participants (when applicable) and to other third parties for the fitness of the product for use as defined in the Study Protocol.

22.0 REFERENCES:

ASTM E2783-11 *Standard Test Method for Assessment of Antimicrobial Activity for Water Miscible Compounds Using a Time-Kill Procedure*

ASTM E 1054-08 (2013), *Standard Test Methods for Evaluation of Inactivators of Antimicrobial Agents*.

23.0 ACCEPTANCE:

AN IN-VITRO TIME-KILL EVALUATION OF SIX TEST MATERIALS WHEN CHALLENGED WITH TWENTY-EIGHT BACTERIAL AND YEAST SPECIES

ACCEPTED BY: BIOSCIENCE LABORATORIES, INC. (TESTING FACILITY)
1755 South 19th Avenue
Bozeman, Montana 59718

Study Director Name:_____

Title:_____

Signature: _____ _____
Date of Study Initiation

ACCEPTED BY: AMERICAN CLEANING INSTITUTE (SPONSOR)
1331 L Street N.W.
Suite 650
Washington, D.C. 20005

_____ _____
Representative Date

_____
Title

ATTACHMENT 2: DETERMINATION OF THE MINIMUM INHIBITORY CONCENTRATIONS (MIC) AND MINIMUM BACTERICIDAL CONCENTRATIONS (MBC) OF SIX TEST MATERIALS

DRAFT PROTOCOL #150941-202

BIOSCIENCES LABORATORIES, INC.

October 26, 2015

DRAFT PROTOCOL #150941-202

DETERMINATION OF THE MINIMUM INHIBITORY CONCENTRATIONS (MIC) AND MINIMUM BACTERICIDAL CONCENTRATIONS (MBC) OF SIX TEST MATERIALS

Prepared for:

AMERICAN CLEANING INSTITUTE (SPONSOR)
1331 L Street, N.W.
Suite 650
Washington, D.C. 20005

Prepared by:

BIOSCIENCE LABORATORIES, INC. (TESTING FACILITY)
1755 South 19th Avenue
Bozeman, Montana 59718
(406) 587-5735

CONFIDENTIAL

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October 26, 2015

DRAFT PROTOCOL #150941-202

1.0 TITLE: **DETERMINATION OF THE MINIMUM INHIBITORY CONCENTRATIONS (MIC) AND MINIMUM BACTERICIDAL CONCENTRATIONS (MBC) SIX TEST MATERIALS**

2.0 SPONSOR: **AMERICAN CLEANING INSTITUTE**
1331 L Street, N.W.
Suite 650
Washington, D.C. 20005

3.0 TESTING FACILITY: **BIOSCIENCE LABORATORIES, INC.**
1755 South 19th Avenue
Bozeman, Montana 59718

4.0 STUDY DIRECTOR: To Be Determined

5.0 PURPOSE:

This study will evaluate the Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentrations (MBC) of six test materials when challenged with 1,400 different microorganism strains. All testing will be performed in accordance with Good Laboratory Practices, as specified in 21 CFR Part 58, with two exceptions. The characterization of the identity, strength, purity, composition, stability, and solubility of all test materials remains the responsibility of the Study Sponsor and will not be performed by the Testing Facility (GLP 58.105 and GLP 58.113). Verification of the antibiotic resistance of purportedly drug-resistant American Type Culture Collection (ATCC) strains, and determination of antibiotic resistance profiles for each of the clinically-isolated bacterial strains, will be performed by another facility; these procedures are exempt from this GLP statement.

6.0 SCOPE:

6.1 This study, a Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) evaluation of six test materials will be performed based upon the Macrodilution Broth Method outlined in CLSI Document M07-A10, *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically*, Tenth Edition, as well as NCCLS (currently known as CLSI) Document M26-A, *Methods for Determining Bactericidal Activity of Antimicrobial Agents* (September 1999).

6.2 Each test material will be evaluated versus a total of 1400 microorganism strains – 50 strains of each of the 23 microorganism species listed in the Tentative Final Monograph, *Federal Register*, 17 June 1994, vol. 59:116, p. 31444, as well as 50 strains of each of the following organisms: *Burkholderia cepacia*, *Enterococcus faecalis* VRE, *Enterococcus faecium* VRE, *Staphylococcus aureus* MRSA, and *Staphylococcus epidermidis* MRSE. Where possible, 25 American Type Culture Collection (ATCC) strains and 25 Clinical Isolates of each species will be evaluated. Where there are insufficient ATCC strains available, additional Clinical Isolates will be tested to achieve a total of 50 strains for that species. Each of the challenge strains will be exposed to each of eight doubling dilutions of the appropriate test material prepared in sterile nutrient broth (reference Table 1). Following an appropriate incubation period, the Minimum Inhibitory Concentrations (MIC) of each test material will be determined visually and documented.

6.3 Aliquots of the three highest product dilutions that exhibit no visually detectable growth of the challenge strain will be neutralized and subcultured using agar media. Following incubation, the agar subcultures will be examined, and the Minimum Bactericidal Concentration (MBC) of each test material will be reported as the highest dilution (lowest product concentration) resulting in a ≥ 3.0 Log_{10} reduction in the population of the challenge strain.

7.0 TEST MATERIALS:

All test materials will be provided to the Testing Facility by the Study Sponsor, complete with the appropriate documentation. If the product names, lot numbers and expiration dates are not documented below, the information will be presented in the Final Report. Responsibility for determination of the identity, strength, purity, composition, solubility, and stability of the test materials, as well as responsibility for retention of all test materials, rests with the Study Sponsor.

Test Material #1: Benzalkonium Chloride
Lot Number: _____
Manufacture Date: _____
Expiration Date: _____

Test Material #2: Benzethonium Chloride
Lot Number: _____
Manufacture Date: _____
Expiration Date: _____

Test Material #3: Chloroxylenol
Lot Number: _____
Manufacture Date: _____
Expiration Date: _____

Test Material #4: Povidone Iodine
Lot Number: _____
Manufacture Date: _____
Expiration Date: _____

Test Material #5: Ethanol
Lot Number: _____
Manufacture Date: _____
Expiration Date: _____

Test Material #6: Isopropyl Alcohol
Lot Number: _____
Manufacture Date: _____
Expiration Date: _____

8.0 CHALLENGE MICROORGANISM SPECIES:

The list of challenge microorganism species will be determined in close consultation with the U.S. Food and Drug Administration

9.0 EQUIPMENT:

9.1 Steam Autoclaves
9.2 Laminar Biological Flow Hoods
9.3 Water Bath, 47 ± 2 °C
9.4 Water Bath Thermometer
9.5 Continuously Adjustable Pipetters, 100 µL - 1000 µL Capacity
9.6 Continuously Adjustable Pipetters, 20 µL - 200 µL Capacity
9.7 Microman® Positive Displacement Pipetters, 100 µL - 1000 µL Capacity
9.8 Microman® Positive Displacement Pipetters, 10 µL - 100 µL Capacity
9.9 Refrigerators, Temperature Range 2 – 8 °C
9.10 Refrigerator Thermometers
9.11 Incubator, Temperature Range 35 ± 2 °C
9.12 Incubator, Temperature Range 30 ± 2 °C
9.13 Incubator Thermometers
9.14 GasPak™ Anaerobic System (Anaerobic Jars)
9.15 Drummond Pipet-Aid® Portable Pipetters
9.16 Vortex Mixers
9.17 Centrifuges

10.0 SUPPLIES:

10.1 Sterile Disposable Pipettes
10.2 Sterile Disposable Petri Plates
10.3 Glass Test Tubes, Sterilized
10.4 Sterile Disposable Centrifuge Tubes, 15 mL
10.5 Sterile Universal 1.0 mL and 0.1 mL Pipette Tips
10.6 Sterile 1.0 mL and 0.1 mL Positive Displacement Tips
10.7 Sterile Syringes
10.8 Hand-Tally Counters
10.9 Glass Bottles, Sterilized
10.10 Polypropylene Bottles, Sterilized
10.11 Sterile Disposable Containers with Screw-caps
10.12 Inoculating Loops
10.13 GasPak™ EZ CO_2 Container System Sachets, or equivalent
10.14 GasPak™ EZ Anaerobe Container System Sachets, or equivalent
10.15 Dry Anaerobic Indicator Strips, or equivalent
10.16 Sterile Disposable Spreaders

11.0 MEDIA:

11.1 Brain-Heart Infusion Broth (BHIB)
11.2 Brain-Heart Infusion Agar (BHIA)
11.3 RPMI-1640 Medium (buffered with 0.165 mol/L MOPS; with glutamine, without bicarbonate, and with phenol red) (RPMI)
11.4 Cation-Adjusted Mueller-Hinton Broth (CAMHB)
11.5 Cation-Adjusted Mueller-Hinton Broth with 0.5% Lysed Horse Blood (CAMHB-B)
11.6 Cation-Adjusted Mueller-Hinton Broth with 1% Supplement XV (CAMHB-XV)
11.7 Schaedler's Broth (SB)
11.8 Schaedler's Agar with 0.5% Lysed Horse Blood (SA-B)
11.9 Tryptic Soy Broth (TSB)
11.10 Tryptic Soy Agar (TSA)
11.11 Tryptic Soy Agar with 5% Sheep Blood (SBA)
11.12 Chocolate Agar with Enrichment (CAE)
11.13 Sabouraud Dextrose Broth (SDB)
11.14 Sabouraud Dextrose Agar (SDA)
11.15 Sterile 0.9% Sodium Chloride Irrigation, USP (SCI)
11.16 Neutralizing Solution: Butterfield's Phosphate Buffer solution with product neutralizers (BBP++)

12.0 INOCULUM PREPARATION:

All species, except *Bacteroides fragilis* (ATCC strains and Clinical Isolates), *Haemophilus influenzae* (ATCC strains and Clinical Isolates), and *Streptococcus pneumoniae* (ATCC strains and Clinical Isolates)

12.1 Approximately 48 hours prior to testing, separate sterile tubes containing the appropriate broth media (reference Table 1) will be inoculated from lyophilized vials or cryogenic stock cultures containing the challenge microorganisms. The broth cultures will be incubated at the temperatures and under the conditions appropriate for each species for approximately 24 hours, or until sufficient growth is observed (reference Table 1).

12.2 Approximately 24 hours prior to testing, the broth cultures will be used to inoculate the surface of the appropriate agar media (reference Table 1) contained in Petri plates. These plates will be incubated appropriately (reference Table 1) until sufficient growth is observed. This will produce lawns of the bacteria or yeast on the surface of the agar plates, and growth from these will be used to prepare the challenge suspensions. The purity of each suspension will be verified by streaking for isolation on the appropriate agar media and incubating appropriately for approximately 24 hours. Isolation streaks of each clinical isolate strain (bacteria only) and each drug-resistant ATCC strain will be prepared on Tryptic Soy Agar with 5% Sheep Blood (SBA). These plates will be incubated appropriately for approximately 24 hours and provided to a CLIA[1]-certified laboratory facility for determination/confirmation of antibiotic resistance. The results of the resistance verification of these species will be presented in the Final Report.

*Bacter*oides fragilis (ATCC strains and Clinical Isolates)

12.3 48 to 96 hours prior to testing, separate sterile tubes containing Schaedler's Broth (SB) will be inoculated from lyophilized vials or cryogenic stock cultures containing these species. The broth cultures will be incubated anaerobically at 35 ± 2 °C for 24 to 48 hours, or until sufficient growth is observed (reference Table 1).

[1] = Clinical Laboratory Improvement Amendments.

12.4 24 to 48 hours prior to testing, the broth cultures will be subcultured into additional tubes of SB and incubated appropriately (reference Table 1). Following incubation, initial suspensions containing approximately 10^9 CFU/mL will be prepared for each species by centrifuging the broth culture tubes, combining the resulting pellets, and resuspending them in SB.

Haemophilus influenzae (ATCC strains and Clinical Isolates) and *Streptococcus pneumoniae* (ATCC strains and Clinical Isolates)

12.5 Approximately 48 hours prior to testing, inocula from lyophilized vials or cryogenic stock cultures containing these species will be suspended in 0.9% Sodium Chloride Irrigation, USP (SCI), inoculated onto the surface of the appropriate agar media contained in Petri plates, and incubated at 35 ± 2 °C under the appropriate conditions for approximately 24 hours, or until sufficient growth is observed (reference Table 1).

12.6 Approximately 24 hours prior to testing, growth from the agar plates previously prepared will be suspended in SCI. The purity of each suspension will be verified by preparing isolation streaks of each culture on the appropriate agar media (reference Table 1). Aliquots of each suspension will then be spread-plated onto the surface of additional plates of the appropriate agar media and incubated appropriately (reference Table 1) until sufficient growth is observed. This will produce lawns of the bacteria on the surface of the agar plates, and growth from these will be used to prepare the challenge suspensions. Isolation streaks of each clinical isolate strain will be prepared on SBA. These plates will be incubated appropriately for approximately 24 hours and provided to a CLIA-certified laboratory facility for determination of antibiotic resistance. The results of the resistance verification of these species will be presented in the Final Report.

Challenge Suspensions

12.7 Immediately prior to initiating the test procedure, an initial suspension containing approximately 10^9 CFU/mL of each microorganism (except *Bacteroides fragilis* [ATCC Strains and Clinical Isolates]) will be prepared by inoculating a test tube containing SCI with microorganisms taken from the plates of solid media prepared as described in Sections 12.2 and 12.6. The initial suspensions of *Bacteroides fragilis* will be prepared as described in Section 12.4.

12.8 Final challenge suspensions containing approximately 1×10^6 CFU/mL of each microorganism strain will be prepared by transferring aliquots of the 10^9 CFU/mL suspensions into sterile containers containing a sufficient volume of the appropriate broth to complete testing (reference Table 1).

12.9 The final challenge suspensions will be mixed thoroughly prior to use in testing.

Initial Population Determination

12.10 An initial population of each challenge microorganism (except *Bacteroides fragilis* [ATCC Strains and Clinical Isolates]) will be determined from the inoculum container used for testing by preparing ten-fold dilutions (e.g., 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4}) in SCI. The initial populations of *Bacteroides fragilis* will be determined as described above except that SB will be used as the diluent. Using the appropriate solid media (reference Table 1), pour- or spread-plates will be prepared, in duplicate, from the inoculum dilutions by plating 0.1 mL of the final dilutions to achieve plated dilutions of, e.g., 10^{-3}, 10^{-4}, and 10^{-5}. These plates will be incubated for 48 to 72 hours at the temperature and under the conditions appropriate for each challenge microorganism (reference Table 1), until sufficient growth is observed. Following incubation, the colonies on the plates will be counted manually using a hand-tally counter. Counts in the range of 30 to 300 CFU will be used preferentially for the data calculations. If no counts in this range are observed, those plates with colony counts closest to that range will be used for the data calculations.

Calculations (Initial Populations)

12.11 The initial population (CFU/mL) of each challenge suspension will be calculated as follows:

$CFU/mL = (C_i \times 10^{-D})$

Where:

C_i	=	Average of the Two (2) Plates Counted
D	=	Dilution Factor of the Plates Counted

12.12 The population (CFU/mL) per tube of product/broth following inoculation will be calculated as follows:

$$\text{Population per Tube (CFU/mL)} = \frac{\left(C_i \times 10^{-D}\right)}{2}$$

Where:

C_i	=	Average of the Two (2) Plates Counted
D	=	Dilution Factor of the Plates Counted
2	=	Total Volume (milliliters) present in each product/broth tube following inoculation

13.0 TESTING PROCEDURE:

Minimum Inhibitory Concentration (MIC) Evaluation

Product Dilution by Bottle

13.1 A series of 1:2 (v/v) dilutions of each test material will be prepared using the appropriate broth (reference Table 1), resulting in product dilutions of, e.g., 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, and 1:128.

13.2 A 1.0 mL aliquot of undiluted test material and 1.0 mL aliquots of each of the seven dilutions prepared as described in Section 13.1 will be transferred to separate sterile test tubes. A single series of test tubes will be prepared for each test material for challenge with each microorganism strain.

Product Dilution by Tube

13.3 A 1.0 mL aliquot of test material will be dispensed into an empty test tube, and into the first of a series of seven test tubes, each containing 1.0 mL of the appropriate broth (reference Table 1). This tube, containing equal parts of product and broth, constitutes the 1:2 (v/v) dilution of test material. A 1.0 mL aliquot will be removed from the 1:2 dilution tube and used to initiate a 1:2 dilution series. Each dilution will be mixed thoroughly using a vortex mixer before the 1.0 mL aliquot is removed for the next dilution in the series. A 1.0 mL aliquot will be removed from the last tube in the series (1:128 product dilution) and discarded, providing a final volume of 1.0 mL.

13.4 A 1.0 mL aliquot of a challenge suspension containing approximately 1×10^6 CFU/mL will be dispensed into each dilution tube in each series. Following inoculation, final product dilutions of e.g., 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, and 1:256 will be produced. Each dilution tube will contain approximately 5×10^5 CFU/mL of the challenge microorganism. The actual microbial population (CFU/mL) per tube will be calculated as described in Sections 12.10 through 12.12.

13.5 The test procedure outlined in Sections 13.2 and 13.4, or in Sections 13.3 and 13.4, will be performed using each test material versus each microorganism strain.

Controls

13.6 A Positive Control Tube (Growth Control) containing a 1.0 mL aliquot of appropriate broth medium and a 1.0 mL aliquot of challenge suspension will be prepared using each microorganism strain (reference Table 1).

13.7 A Negative Control Tube (Sterility Control; no microbial inoculation) of each broth medium (reference Table 1) will also be prepared.

13.8 Test material turbidity controls will be prepared by transferring 1.0 mL aliquots of each of the product dilutions (e.g., undiluted, 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, and 1:128) into sterile test tubes, adding 1.0 mL per tube of the appropriate sterile broth (reference Table 1), and mixing thoroughly. Alternatively, product turbidity controls will be prepared by creating a product dilution series as described in Section 13.3, then inoculating each tube with 1.0 mL each of the appropriate sterile broth (reference Table 1), and mixing thoroughly. This will result in a final product dilution series identical to that described in Section 13.4.

Incubation

13.9 The challenge suspension/product dilution tubes and all controls will be incubated at 35 ± 2 °C under the appropriate conditions for 16 to 24 hours, or until good growth is apparent in the Positive Control tubes (reference Section 13.6).

Determination of MIC Results

13.10 Following incubation, the tubes will be examined for growth of the challenge microorganism, as determined visually on the basis of turbidity.

13.11 The Minimum Inhibitory Concentration (MIC) of each product versus each microorganism strain will be recorded as the highest dilution of product that completely inhibits growth of the microorganism, as detected by the unaided eye.

Minimum Bactericidal Concentration (MBC) Evaluation

13.12 After the Minimum Inhibitory Concentration (MIC) results have been read and documented, a 0.1 mL aliquot will be removed from each of the three highest product dilution tubes that exhibit no growth, and transferred to separate tubes containing 0.9 mL of Butterfield's Phosphate Buffer Solution with product neutralizers (BBP++; 10^{-1} dilution).

13.13 Each tube containing product/neutralizer will be vortexed thoroughly, and the entire contents of the tube will be plated using the appropriate agar medium (reference Table 1; 10^{-1} dilution).

13.14 These plates will be incubated appropriately for 48 to 72 hours, or until sufficient growth is observed (reference Table 1). Following incubation, the colonies on the plates will be counted manually using a hand-tally counter. Counts in the range of 30 to 300 CFU will be used preferentially for the data calculations. If no counts in this range are observed, those plates with colony counts closest to that range will be used for the data calculations.

13.15 The Minimum Bactericidal Concentration (MBC) of each test material versus a microorganism strain will be recorded as the highest dilution (lowest concentration) of product that produces a ≥ 3.0 Log_{10} reduction in the microbial population, when compared to the population (CFU/mL) per tube of product/broth immediately following inoculation (reference Section 12.12).

13.16 Concurrent with the MBC testing procedure, a neutralization verification will be performed to demonstrate that the neutralizing solution (BBP++) effectively quenches the antimicrobial activity of the test materials. The challenge species used for the neutralization verification procedures will be a single ATCC strain of each of the following species: *Acinetobacter* species, *Bacteroides fragilis*, *Burkholderia cepacia*, *Candida albicans*, *Enterobacter aerogenes*, *Enterococcus faecalis*, *Enterococcus faecium*, *Escherichia coli*, *Haemophilus influenzae*, *Klebsiella pneumoniae*, *Micrococcus luteus*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Staphylococcus hominis*, *Staphylococcus saprophyticus*, *Streptococcus pneumoniae*, and *Streptococcus pyogenes*.

13.16.1 After the three product dilutions to be evaluated for the MBC procedure have been determined, the corresponding product dilutions prepared as turbidity controls (reference Section 13.8) will be used for the neutralization verification.

13.16.2 0.1 mL aliquots will be removed from each tube and transferred to separate sterile tubes containing 0.8 mL of BBP++. A 0.1 mL aliquot of a challenge suspension containing approximately 10^3 CFU/mL of a challenge species will be added to the tube and mixed thoroughly.

13.16.3 The entire contents of each tube containing product/neutralizer/inoculum will be pour- or spread-plated using the appropriate agar medium (reference Table 1).

13.16.4 A control will be prepared by inoculating a tube containing a 0.9 mL aliquot of BBP++ with 0.1 mL of a suspension containing approximately 10^3 CFU/mL of a challenge species and mixing thoroughly. The entire contents of the tube will be pour- or spread-plated using the appropriate agar medium (reference Table 1).

13.16.5 These plates will be incubated appropriately for 48 to 72 hours, or until sufficient growth is observed. Following incubation, the colonies on the plates will be counted manually using a hand-tally counter. Counts in the range of 30 to 300 CFU will be used preferentially for the data calculations. If no counts in this range are observed, those plates with colony counts closest to that range will be used for the data calculations.

13.16.6 If the microbial population recovered from each of the product dilution/neutralizer tubes is not more than 0.5 Log_{10} lower than the control, the neutralizer (BBP++) will be considered effective in neutralizing the antibacterial activity of the test material at the respective concentrations.

TABLE 1: POTENTIAL CHALLENGE MICROORGANISMS, MEDIA, AND INCUBATION CONDITIONS

Microorganism Species	Incubation Time *Inoculum Cultures*	Incubation Time *MIC Tubes*	Incubation Time *Initial Population Determination plates and MBC Evaluation plates*	Incubation Temperature *MIC Tubes*	Incubation Temperature *Inoculum Cultures, Initial Population Determination plates, and MBC Evaluation plates*	Growth Media
Acinetobacter species	22 to 24 hours	18 to 24 hours	48 to 72 hours	35 ± 2 °C	35 ± 2 °C	BHIB/BHIA/CAMHB
Bacteroides fragilis	48 to 72 hours	48 to 72 hours	48 to 72 hours	35 ± 2 °C Anaerobic	35 ± 2 °C Anaerobic	SB/SA-B/CAE
Burkholderia cepacia	22 to 24 hours	18 to 24 hours	48 to 72 hours	35 ± 2 °C	30 ± 2 °C	TSB/TSA/CAMHB
Candida species	22 to 24 hours	24 to 48 hours	48 to 72 hours	35 ± 2 °C	30 ± 2 °C	SDB/SDA/RPMI
Enterobacter species	22 to 24 hours	18 to 24 hours	48 to 72 hours	35 ± 2 °C	35 ± 2 °C	TSB/TSA/CAMHB
Enterococcus species	22 to 24 hours	18 to 24 hours	48 to 72 hours	35 ± 2 °C	35 ± 2 °C	TSB/TSA/CAMHB
Escherichia coli	22 to 24 hours	18 to 24 hours	48 to 72 hours	35 ± 2 °C	35 ± 2 °C	TSB/TSA/CAMHB
Haemophilus influenzae	22 to 24 hours	18 to 24 hours	48 to 72 hours	35 ± 2 °C + 5% CO_2	35 ± 2 °C + 5% CO_2	CAE/CAMHB-XV
Klebsiella species	22 to 24 hours	18 to 24 hours	48 to 72 hours	35 ± 2 °C	35 ± 2 °C	TSB/TSA/CAMHB
Micrococcus luteus	22 to 24 hours	18 to 24 hours	48 to 72 hours	35 ± 2 °C	30 ± 2 °C	TSB/TSA/CAMHB
Pseudomonas aeruginosa	22 to 24 hours	18 to 24 hours	48 to 72 hours	35 ± 2 °C	35 ± 2 °C	TSB/TSA/CAMHB
Proteus mirabilis	22 to 24 hours	18 to 24 hours	48 to 72 hours	35 ± 2 °C	35 ± 2 °C	TSB/TSA/CAMHB
Serratia marcescens	22 to 24 hours	18 to 24 hours	48 to 72 hours	35 ± 2 °C	30 ± 2 °C	TSB/TSA/CAMHB
Staphylococcus species	22 to 24 hours	18 to 24 hours	48 to 72 hours	35 ± 2 °C	35 ± 2 °C	TSB/TSA/CAMHB
Streptococcus pneumoniae	22 to 24 hours	18 to 24 hours	48 to 72 hours	35 ± 2 °C + 5% CO_2	35 ± 2 °C + 5% CO_2	SBA/CAMHB-B
Streptococcus pyogenes	22 to 24 hours	18 to 24 hours	48 to 72 hours	35 ± 2 °C	35 ± 2 °C	BHIB/BHIA/ CAMHB-B

Note: Incubation times are nominal, but in practice, incubation will continue until good growth is observed.

14.0 REFERENCES:

14.1 CSLI Document M7-A10, *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically*, Tenth Edition, January 2015.

14.2 NCCLS (Currently known as CLSI) Document M26-A, *Methods for Determining Bactericidal Activity of Antimicrobial Agents*, September 1999.

14.3 Tentative Final Monograph For Healthcare Antiseptic Drug Products; Proposed Rule, *Federal Register*, 17 June 1994, vol. 59:166, p. 31444.

15.0 STATISTICAL ANALYSIS:

A statistical analysis will not be performed on the data derived from this evaluation.

16.0 FINAL REPORT:

A Final Report will be issued presenting the results of this evaluation in a clear, concise manner.

17.0 EXCEPTIONAL CONDITIONS:

Sponsor will be notified by telephone, email, and/or letter of any exceptions encountered in this study. The exceptional conditions or occurrences will be detailed in full and formally recorded. Exceptional conditions that occur and are not addressed in this Protocol will be subject to Out-of-Scope charges (See Proposal/Contract).

18.0 LIABILITY AND INDEMNIFICATION:

Test Facility's liability to Sponsor under this Protocol shall be limited to the price of this evaluation. Sponsor shall be responsible to Study Participants (when applicable) and to other third parties for the fitness of the product for use as defined in the Study Protocol.

19.0 DOCUMENTATION AND RECORD-KEEPING:

All documentation and records will be compiled, analyzed, and retained by BioScience Laboratories, Inc. at its facility in Bozeman, Montana. All raw data from this study will be retained in safe storage by Company for a period of at least minimum of 5 years. BioScience Laboratories, Inc. will notify the Study Sponsor before any documents or records are destroyed.

20.0 QUALITY ASSURANCE AUDITS:

The Quality Assurance Unit (QAU) will conduct in-phase audits of critical processes in testing at least once and advise the Study Director and Management of the outcomes of these. On completion of testing, the QAU will perform an audit of the data and of the Final Report, in its entirety.

21.0 ACCEPTANCE:

DETERMINATION OF THE MINIMUM INHIBITORY CONCENTRATIONS (MIC) AND MINIMUM BACTERICIDAL CONCENTRATIONS (MBC) OF SIX TEST MATERIALS

ACCEPTED BY: BIOSCIENCE LABORATORIES, INC. (TESTING FACILITY)
1755 South 19th Avenue
Bozeman, Montana 59718

Study
Director: _____ _____
To Be Determined Date of Study Initiation

ACCEPTED BY: AMERICAN CLEANING INSTITUTE (SPONSOR)
1331 L Street N.W.
Suite 650
Washington, D.C. 20005

_____ _____
Representative Date

_____
Title

ATTACHMENT 3: EVALUATION OF THE ANTIMICROBIAL EFFICACY OF FOUR TEST MATERIALS WITH POSITIVE AND NEGATIVE CONTROLS BASED ON THE HEALTH CARE PERSONNEL HANDWASH PROCEDURE

DRAFT PROTOCOL 150942-101

BIOSCIENCES LABORATORIES, INC.

BIOSCIENCE LABORATORIES, INC. DRAFT PROTOCOL 150942-101

EVALUATION OF THE ANTIMICROBIAL EFFICACY OF FOUR TEST MATERIALS WITH POSITIVE AND NEGATIVE CONTROLS BASED ON THE HEALTH CARE PERSONNEL HANDWASH PROCEDURE

1.0 TITLE PAGE

Active Ingredients:	Benzalkonium Chloride Benzethonium Chloride Chloroxylenol Povidone Iodine
Sponsor:	The American Cleaning Institute 1331 L Street N.W. Suite 650 Washington D.C. 20005
Study Number:	150942-101
Sponsor Representative:	Francis H. Kruszewski
Principal Investigator:	To Be Determined
Sub-Investigator:	To Be Determined
Clinical Site:	BioScience Laboratories, Inc. 1765 South 19th Avenue Bozeman, Montana 59718 Telephone: (406) 587-5735
Date:	October 23, 2015

Confidentiality Statement

This document contains the confidential information of The American Cleaning Institute and BioScience Laboratories, Inc. It is intended solely for the guidance of the clinical investigation. This protocol may not be disclosed to parties not associated with the clinical investigation or used for any purpose without the prior written consent of The American Cleaning Institute and BioScience Laboratories, Inc. Permission to release the Protocol and Study Results to the United States Food and Drug Administration (FDA) or other regulatory agency to which this study will be submitted is explicitly granted.

This Protocol has been approved by the GIRB on _____

2.0 PROTOCOL SYNOPSIS

Name of Sponsor: The American Cleaning Institute	
Name and Concentration of Active Ingredients: Test Material #1: Benzalkonium Chloride Test Material #2: Benzethonium Chloride Test Material #3: Chloroxylenol Test Material #4: Povidone Iodine Positive Control: Hibiclens®, Chlorhexidine gluconate solution, 4% (w/v) Negative Control: Normal Physiological Saline	**Protocol Number** **150942-101**

Title of Study:	**EVALUATION OF THE ANTIMICROBIAL EFFICACY OF FOUR TEST MATERIALS WITH POSITIVE AND NEGATIVE CONTROLS BASED ON THE HEALTH CARE PERSONNEL HANDWASH PROCEDURE**
Principal Investigator:	To Be Determined
Sub-Investigator:	To Be Determined
Study Center:	BioScience Laboratories, Inc.
Publications (References)	ASTM E1174-13, *Standard Test Method for Evaluation of the Effectiveness of Health Care Personnel Handwash Formulations* 1994 FDA TFM, 21 CFR Parts 333 and 369, *Health-Care Antiseptic Drug Products*; *Effectiveness testing of an antiseptic handwash or health-care personnel handwash;* Proposed Rule. (FR59: No. 116, 17 June 94. pp 31448 to 31450) 2015 FDA TFM, 21 CFR Part 310, *Safety and Effectiveness of Health Care Antiseptics; Topical Antimicrobial Drug Products for Over-the-Counter Human Use; Proposed Amendment of the Tentative Final Monograph; Reopening of Administrative Record;* Proposed Rule. (FR80: No. 84, 1 May 2015. pp 25166 to 25205)

This Protocol has been approved by the GIRB on _____

Name of Sponsor: The American Cleaning Institute	**Protocol Number** **150942-101**
Name and Concentration of Active Ingredients: Test Material #1: Benzalkonium Chloride Test Material #2: Benzethonium Chloride Test Material #3: Chloroxylenol Test Material #4: Povidone Iodine Positive Control: Hibiclens®, Chlorhexidine gluconate solution, 4% (w/v) Negative Control: Normal Physiological Saline	

Study Duration:	A pre-test conditioning period of 7 days and a test period of 1 day.
Objectives:	The purpose of this study is to evaluate the antimicrobial efficacy of four test materials with positive and negative controls, for use as Health Care Personnel Handwashes following single test material applications.
Methodology:	The testing methods are based on the standardized test method ASTM E1174-13, *Standard Test Method for Evaluation of the Effectiveness of Health Care Personnel Handwash Formulations,* for use as a Health Care Personnel Handwash following a single test material application. Evaluations of the test materials will be made following subjects being inoculated with the test organism in a broth media, and after a single test material application. Samples for baseline and following test material application will be processed for bacterial enumeration. The indicator microorganism will be *Serratia marcescens* (ATCC #14756).
Number of Subjects:	The number of subjects will be evaluated per test material based on pilot study data. Subjects will be tested in a randomized evaluation.
Main Criteria for Inclusion:	A sufficient number of overtly healthy subjects at least 18 years of age will be admitted into the study. Insofar as possible, the groups of subjects selected will be of mixed sex, age, and race. All subjects' hands will be free from clinically evident dermatoses, injuries to the hands or forearms, hangnails, or any other disorders that may compromise the subject or the study.

This Protocol has been approved by the GIRB on _____

Name of Sponsor: The American Cleaning Institute	**Protocol Number** **150942-101**
Name and Concentration of Active Ingredients: Test Material #1: Benzalkonium Chloride Test Material #2: Benzethonium Chloride Test Material #3: Chloroxylenol Test Material #4: Povidone Iodine Positive Control: Hibiclens®, Chlorhexidine gluconate solution, 4% (w/v) Negative Control: Normal Physiological Saline	
Duration of treatment:	One of the test materials will be applied by subjects one time.
Criteria for Evaluation:	**Efficacy:** Mean log_{10} reductions from baseline of the indicator microorganism will be used to determine the antimicrobial effectiveness of the test materials. The critical index is mean log_{10} reductions in microorganisms of ≥ 2.0 log_{10} within 5 minutes after one application with a 70% responder rate. A non-antimicrobial negative control is included to show the contribution of the active ingredients to effectiveness. The test materials should be statistically superior to the negative control for the clinical simulation to be considered successful at showing that the test materials are effective for use in health care antiseptic products. A positive control is included to validate the study conduct to assure that the expected results are produced. For the results to be valid, the positive control should meet the appropriate log_{10} reduction criteria.
	Safety: Evaluation for safety of use of the test materials will consist of Adverse Event-reporting and assessment for skin reactions following testing.
Statistical Methods:	Log_{10} reductions from baseline population recovered from each of a subject's hands will be calculated by subtracting the log_{10} number of viable *Serratia marcescens* recovered following test material application from the log_{10} baseline population recovered from that hand. Log_{10} microbial data

<table>
<tr><td colspan="2">Name of Sponsor:
The American Cleaning Institute</td><td rowspan="2">Protocol Number
150942-101</td></tr>
<tr><td colspan="2">Name and Concentration of Active Ingredients:
Test Material #1: Benzalkonium Chloride
Test Material #2: Benzethonium Chloride
Test Material #3: Chloroxylenol
Test Material #4: Povidone Iodine
Positive Control: Hibiclens®, Chlorhexidine gluconate solution, 4% (w/v)
Negative Control: Normal Physiological Saline</td></tr>
<tr><td></td><td colspan="2">and population reductions from each of a subject’s hands will be presented in tabular form.

Statistical calculations of mean and standard deviation will be generated on the $\log_{10}$ data from baseline samples, post-test material application samples, and the reductions from baseline.

The statistical analysis will determine the portion of subjects who meet the $\log_{10}$ reduction criteria based on a two-sided statistical test for superiority to the negative control and a 95 percent confidence interval approach.</td></tr>
</table>

This Protocol has been approved by the GIRB on _____

3.0 TABLE OF CONTENTS

SECTION **PAGE**

This Protocol has been approved by the GIRB on _____

This Protocol has been approved by the GIRB on _____

4.0 LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

AIDS	Acquired Immune Deficiency Syndrome
ASTM	American Society for Testing and Materials
ATCC	American Type Culture Collection
BBP++	Butterfield's Phosphate Buffer Solution with product neutralizers
BSLI	BioScience Laboratories, Inc.
CFR	Code of Federal Regulations
CFU	Colony Forming Units
DHHS	Department of Health and Human Services
FR	Federal Register
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GIRB	Gallatin Institutional Review Board
HIPAA	Health Insurance Portability and Accountability Act
HIV	Human Immunodeficiency Virus
ICH	International Conference on Harmonisation
PBS	Phosphate Buffered Saline
SAE	Serious Adverse Event
SSF	Stripping Suspending Fluid
SFF++	Stripping Suspending Fluid with neutralizers / 10% Tween
TFM	Tentative Final Monograph
TSA	Tryptic Soy Agar
TSA+	Tryptic Soy Agar with neutralizers
TSB	Tryptic Soy Broth
UV	Ultraviolet
Glove Juice Sampling Procedure	A procedure used to sample bacteria from the hands using a sterile glove and a specified volume of sampling fluid.

Mean log_{10} reductions	Average of the differences between the baseline microbial populations expressed as log_{10} CFU/hand and the populations in log_{10} CFU/hand recovered from the post-application samples.
Source Documents	Recorded results of original observations and activities of a clinical investigation.
Subject	Healthy human paid participant that has consented to test in the study.
Test Material	The test material, negative control or positive control that are to be tested according to procedures in this protocol.
Negative Control	A material that is to be tested according to procedures in this protocol with no active ingredient.
Positive Control	A product that is to be tested according to procedures in this protocol in order to validate the testing procedures and to be used as a control.

5.0 ETHICS

5.1 Institutional Review Board

Informed Consent Forms and any other supportive material relevant to the safety of the subjects will be supplied to the Gallatin Institutional Review Board (GIRB) for their review and approval. The primary purpose of the GIRB is the protection of the rights and welfare of the subjects involved (reference CFR 21, Parts 50, 56, 312, and 314). This study will begin only after GIRB approval has been obtained.

5.2 Ethical Conduct of Study

The study will be conducted in compliance with the Good Laboratory Practice standards (21 CFR Part 58) and Good Clinical Practice standards (21 CFR Parts 50, 56, 312, and 314, and ICH E6), the United States Food and Drug Administration regulations, Standard Operating Procedures of BioScience Laboratories, Inc., the study protocol, and any protocol amendments.

5.3 Subject Information and Consent

The Informed Consent Form, Authorization to Use and Disclose Protected Health Information Form, and List of Restricted Products will be provided to each subject prior to beginning the study. Trained personnel will explain the study to each subject and will be available to answer any questions that may arise.

6.0 INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

BioScience Laboratories, Inc.
1765 S 19th Ave.
Bozeman, Montana 59715

Contact: To Be Determined
Phone: (406) 587-5735 Ext. XXX
Fax: (406) 586-7930

Principal Investigator: To Be Determined

Sub-Investigator: To Be Determined

Quality Assurance Monitor: Amy L. Juhnke, RQAP-GLP

Statistical Consultant: Daryl S. Paulson, Ph.D.

Subject Recruitment and Consenting: Chelsey Allison

Consulting Medical Experts: Gabor Benda, M.D. and David McLaughlin, M.D.

Gallatin Institutional Review Board (GIRB)
3006 Secor Avenue
Bozeman, Montana 59715
Phone: (406) 581-8559
DHHS Number: IRB00005939

6.1 Monitoring

The American Cleaning Institute, as Sponsor of this study, is responsible for ensuring the proper conduct of the study with regard to protocol adherence and validity of the data recorded on the study documents. The American Cleaning Institute has therefore assigned a study monitor to this study. The progress of the study may be monitored by:

- Periodic on-site review
- Telephone communications
- E-mail communications
- Review of sample data sheets and source documents

The Investigator will give The American Cleaning Institute study monitor direct access to source documents that support data on the study documents and make available such records to authorized The American Cleaning Institute personnel, quality assurance, IRB, and regulatory personnel for inspection and/or copying.

Note: The Federal Privacy rule (HIPAA) specifically permits the use and disclosure of protected health information "to a person subject to the jurisdiction of the Food and Drug Administration (FDA) [e.g. study sponsor] with respect to an FDA-related product or activity for which that person has responsibility, for the purpose of activities related to the quality, safety, or effectiveness of such FDA-regulated product or activity" [45 CFR 164.512(b)(1)(iii)].

This Protocol has been approved by the GIRB on _____

7.0 INTRODUCTION

Healthcare Personnel, as a standard of care, wash their hands with an antiseptic handwash and/or apply a leave-on hand antiseptic prior to and following performing care of a patient. The proposed Tentative Final Monograph (TFM) for *Health-Care Antiseptic Drug Products* (Vol. 59, No. 116, June 17, 1994) describes an *in-vivo* procedure for evaluating these types of test materials, as well as expected performance criteria. The 2015 FDA TFM, 21 CFR Part 310, *Safety and Effectiveness of Health Care Antiseptics; Topical Antimicrobial Drug Products for Over-the-Counter Human Use; Proposed Amendment of the Tentative Final Monograph; Reopening of Administrative Record;* Proposed Rule now proposes that additional safety data are necessary to support the safety of antiseptic active ingredients for these uses. The new effectiveness criteria described in the 2015 proposed rule includes mean $\log_{10}$ reductions in microorganisms of ≥ 2.5 $\log_{10}$ within 5 minutes after a single application. This guideline efficacy hurdle is not achievable. As this guideline is unachievable by the FDA recommended active control at a 70% responder rate, this procedure has been modified to meet a ≥ 2.0 $\log_{10}$ reduction in microorganisms within 5 minutes after a single application. This hurdle is representative of the cidal performance of the NDA active control.

8.0 STUDY OBJECTIVES

The purpose of this study is to evaluate the antimicrobial efficacy of four test materials with positive and negative controls, for use as Health Care Personnel Handwashes following single test material applications.

9.0 INVESTIGATIONAL PLAN

9.1 Overall Study Design and Plan

This study is designed to determine the antimicrobial effectiveness of four test materials (Benzalkonium Chloride, Benzethonium Chloride, Chloroxylenol, and Povidone Iodine), and positive and negative controls intended for use as Healthcare Personnel Handwashes. At least 47 subjects will be evaluated per test material for a total of at least 282 subjects tested in a randomized evaluation. Evaluations of the test materials will be made following subjects being inoculated with the test organism in a broth media, and after a single test material application. Samples for baseline and following test material application will be processed for bacterial enumeration. The indicator microorganism will be *Serratia marcescens* (ATCC #14756). The testing methods are based on the standardized test method ASTM E1174-13, *Standard Test Method for Evaluation of the Effectiveness of Health Care Personnel Handwash Formulations*. Mean $\log_{10}$ reductions of the indicator microorganism will be used to determine the antimicrobial effectiveness of the test materials. The critical index is mean $\log_{10}$ reductions in microorganisms of ≥ 2.0 $\log_{10}$ after one application with a 70% responder rate at the 95% confidence level.

9.2 Discussion of Study Design, Including Choice of Sample Size

The sample size for this study will be determined using the following formula:

$$\mathbf{N} \geq \frac{S^2 \left(Z_{\alpha/2} + Z_{\mathrm{B}}\right)^2}{D^2}$$

Where:

N= Sample size per test material configuration arm

S= Estimate of variance (Based on pilot study for each active)

$Z_{\alpha/2}$= 0.05 level of significance (two-tail) = 1.96, Type I error (probability of stating a significant effect exists when one does not)

Z_{β}= 0.842 level of significance for Type II (beta) error (probability of stating no significant effect exists when one does)

D= Detectable difference (sensitivity) = 0.5

$$\mathbf{N} \geq \left(\frac{(S)^2 \ (1.96 + 0.842)^2}{0.5^2} \right) = X$$

9.3 Selection of Study Population

A sufficient number of overtly healthy subjects at least 18 years of age will be admitted into the study to ensure that the required subjects complete the study. Insofar as possible, the groups of subjects selected will be of mixed sex, age, and race. All subjects' hands will be free from clinically evident dermatoses, injuries to the hands or forearms, open wounds, hangnails, and/or any other disorders that may compromise the subject and the study. All subjects will sign the Informed Consent Form, Authorization to Use and Disclose Protected Health Information Form, and List of Restricted Products prior to participating in the study. The above forms are provided as separate Informed Consent documents.

An Informed Consent Form, Authorization to Use and Disclose Protected Health Information Form, and List of Restricted Products will be provided to each subject prior to beginning the study. Trained personnel will explain the study to each participant and will be available to answer any questions that may arise.

9.3.1 Inclusion Criteria

- Subjects may be of either sex, at least 18 years of age, and of any race.
- Subjects must possess both hands and all ten digits.
- Subjects must be in good general health.
- Subjects must have read and signed an Informed Consent Form, Authorization to Use and Disclose Protected Health Information Form, and List of Restricted Products prior to participating in the study, all located in the separate Informed Consent documents.

9.3.2 Exclusion Criteria

- Known allergies to sunscreens, deodorants, laundry detergents, fragrances, latex (rubber), alcohols, to common antibacterial agents found in soaps or lotions, particularly Benzalkonium Chloride, Benzethonium Chloride, Chloroxylenol, Povidone Iodine or chlorhexidine gluconate, or to topical antibiotic ointments (e.g., Neosporin® or Polysporin® [neomycin/bacitracin/polymyxin B]).
- Exposure of ungloved hands or forearms to antimicrobial agents, medicated soaps, medicated shampoos (e.g., anti-dandruff), hair mousses, or medicated lotions, during the 7-day pre-test conditioning period or on the single test day.
- Use of biocide-treated pools or hot tubs, or use of UV tanning beds or sunbathing during the 7-day pre-test conditioning period or on the single test day.
- Exposure of ungloved hands or forearms to strong detergents, solvents, or other irritants during the 7-day pre-test conditioning period or on the single test day.
- Use of systemic or topical antibiotic medications, during the 7-day pre-test conditioning period or on the single test day.
- Use of systemic or topical steroids other than for contraception or post-menopausal indications during the 7-day pre-test conditioning period or on the single test day. This includes steroid medications used to treat asthma.
- Application or presence of nail polish, artificial nails, or nail polish remover, or having undergone nail treatments during the 7-day pre-test conditioning period or on the single test day.
- A medical diagnosis of a physical condition, such as a current or recent severe illness, medicated or uncontrolled diabetes, hepatitis B, hepatitis C, an organ transplant, a heart murmur, mitral valve prolapse with heart murmur, congenital heart disease, an immunocompromised condition such as AIDS (or HIV positive), lupus, or medicated multiple sclerosis.
- Any prosthetic device in the neck or spine including pins, screws, plates, or rods.

- Any prosthetic joints (movable parts of the body).
- Any pins, screws, plates, or rods installed within the last 6 months.
- Any type of port (or portacath).
- Pregnancy, plans to become pregnant or impregnate a sexual partner within the pre-test or on the single test day, or nursing a child. Females must continue to take birth control precautions one month following the last day of testing. Males must continue to take birth control precautions through one week following the last day of testing.
- Any active skin rashes, dermatoses, hangnails, or breaks in the skin of the hands or forearms; skin blemishes such as dry scabs or warts may be permissible, with the specific approval of the Principal Investigator or consulting physician.
- An inflammatory skin condition, such as dermatitis, eczema, or psoriasis, anywhere on the body, that in the opinion of the Principal Investigator or consulting physician should preclude participation.
- Participation in a clinical study in the past 7 days or current participation in another clinical study.
- Any medical condition or use of any medications that, in the opinion of the Principal Investigator or consulting physician, should preclude participation.
- Unwillingness to fulfill the performance requirements of the study.

9.3.3 Subject Withdrawal

After admission to the study, the subject may withdraw at any time for any reason. If possible, the reason for withdrawal will be recorded. Any subject not adhering to Protocol requirements will be disqualified.

9.4 Test Methods

9.4.1 Equipment, Supplies, Test Solutions and Media

The equipment used during this study will be detailed on Clinical Trials Equipment Tracking Forms (Form No. 01-L-009), and the forms will be included in the Final Report.

The supplies used during this study will be detailed on Clinical Trials Supplies Tracking Forms (Form No. 01-L-008), and the forms will be included in the Final Report.

The Test Solutions and Media will be prepared in accordance with BioScience Laboratories, Inc. Standard Operating Procedures. The Test Solutions and Media used for this study are listed below:

<u>Sampling Solution</u>

Stripping Suspending Fluid (SSF)

Stripping Suspending Fluid with neutralizers /10% Tween (SSF++)

Neutralizing/Diluting Fluid

Butterfield's Phosphate Buffer Solution with Product Neutralizers (BBP++)

Media

Tryptic Soy Agar (TSA) for Inoculum Preparation

Tryptic Soy Agar with product neutralizers (TSA+)

Tryptic Soy Broth (TSB) for Inoculum Preparation and Neutralization Study

Phosphate Buffered Saline Solution (PBS) for Neutralization Study

Johnson & Johnson Head to Toe

Soft Soap (per ASTM E1174-13):

Ingredients:

Linseed oil (50 parts by weight)

Potassium hydroxide (9.5 parts)

Ethanol (7 parts)

Distilled or high purity water (as needed)

Linseed oil will be added to a solution of potassium hydroxide in 15 parts water and heated to approximately 70 °C while constantly stirring.

Ethanol will be added and heating continued while stirring until the saponification process is completed and a sample dissolves clearly in water and almost clearly in alcohol. The weight of the Soft Soap will then be brought up to 100 parts by addition of hot water. 200 g of the soft soap will be diluted in 1 L of water. The diluted Soft Soap will then be dispensed into appropriate containers and sterilized in an autoclave.

9.4.2 Identity of Test Materials

The test materials will be provided to the Testing Facility by the Study Sponsor, complete with appropriate documentation. The test materials will be received and stored by BioScience Laboratories, Inc. (BSLI) in accordance with instructions from the Sponsor and retained in secure quarantine when not being used in testing. BSLI will maintain an inventory of the test materials and a log of use. Responsibility for determination of the identity, strength, purity, composition, stability, and solubility of the test materials, as well as responsibility for retention of the test materials rests with the Sponsor. Unused, sealed test and control materials will be stored by BSLI until the Sponsor specifies their disposition. In the absence of a disposition request from the Sponsor within 1 year of their planned usage, the test materials will be returned to the Sponsor. No test or control material will be destroyed unless so requested by the Sponsor. If the test material names

and lot numbers are not stated below, they will be provided by the Sponsor prior to the start of testing.

Test Material #1: _____
Active Ingredient: Benzalkonium Chloride
Lot Number: _____
Expiration Date: _____
Manufacture Date: _____

Test Material #2: _____
Active Ingredient: Benzethonium Chloride
Lot Number: _____
Expiration Date: _____
Manufacture Date: _____

Test Material #3: _____
Active Ingredient: Chloroxylenol
Lot Number: _____
Expiration Date: _____
Manufacture Date: _____

Test Material #4: _____
Active Ingredient: Povidone Iodine
Lot Number: _____
Expiration Date: _____
Manufacture Date: _____

Positive Control: Hibiclens®
Active Ingredient: Chlorhexidine gluconate solution 4% (w/v)
Lot Number: _____
Expiration Date: _____
Manufacture Date: _____

Negative Control: _Normal Physiological Saline__
Active Ingredient: N/A
Lot Number: _____
Expiration Date: _____
Manufacture Date: _____

9.4.3 Method of Assigning Subjects to Treatment Groups

Subjects will be assigned randomly to test with one, and only one, of the six test materials.

9.4.4 Inoculum Preparation

Serratia marcescens (ATCC #14756) will be used to challenge the efficacy of the test materials.

Stock cultures containing approximately 10^9 of *Serratia marcescens* (ATCC #14756) will be prepared by aseptically transferring contents of a lyophilized vial to approximately 5 mL of sterile Tryptic Soy Broth (TSB), which will then be incubated at 25 °C ± 2 °C for 24 hours ± 4 hours. One or more appropriately sized flasks containing TSB (for example, 2-L flasks containing approximately 1,000 mL TSB) will be inoculated with approximately 1 mL of a 24-hour broth culture, and incubated for 24 hours ± 4 hours at 25 °C ± 2 °C. Prior to any withdrawal of culture, whether for hand contamination or for numbers assay, the suspension will be stirred or swirled. The suspension will be assayed for number of organisms at the beginning and end of the use period. A suspension will not be used for more than 8 hours.

9.4.5 Pre-Test Conditioning Period

The 7 days prior to the test portion of the study will constitute the pre-test conditioning period. During this time, subjects will avoid the use of medicated soaps, lotions, deodorants and shampoos, as well as skin contact with solvents, detergents, acids and bases, or any other products known to affect the normal microbial populations of the skin. Subjects will be supplied a personal hygiene kit containing nonmedicated soap, shampoo, lotion, and rubber gloves to be worn when contact with antimicrobials, solvents, detergents, acids, or bases cannot be avoided. Subjects will be instructed to use the contents of this kit exclusively during their participation in the study. Subjects must also avoid using UV tanning beds or sunbathing, and swimming or bathing in biocide-treated pools or hot tubs.

9.4.6 Test Period

Each subject will be in testing for 1 to 2 hours on a single day. Prior to being admitted into testing, subjects will be questioned regarding their adherence to the Protocol requirements. Subjects will clip their fingernails to a free edge of ≤ 1 mm, if they have not already done so. All jewelry will be removed from the hands and arms prior to washing.

Subjects will perform a practice hand-contamination, but using tap water instead of the challenge suspension and not performing an air-dry. A 4.5-mL aliquot of water will be transferred into each subject's cupped hands in three aliquots of 1.5 mL. Each aliquot of water will be distributed over the entire surface of the hands (front and back), not reaching above the wrists, for 20 seconds ± 5 seconds.

Following the practice contamination, a 30-seconds ± 5 seconds handwash using 5 mL of Soft Soap and a 30-seconds ± 5 seconds rinse of the hands will be performed to remove dirt and oil from the hands. The temperature of the water used for this and subsequent

wash procedures will be controlled at 40 °C ± 2 °C.

A 5.0-mL aliquot of the assigned challenge suspension will be transferred into each subject's cupped hands in three aliquots, as follows:

- Approximately 1.5 mL of the challenge suspension will be dispensed into the subject's cupped hands. The suspension will be distributed over the entire surface of the hands (front and back), not reaching above the wrists, for 20 seconds ± 5 seconds. Following distribution of the inoculum, the hands will be held motionless, away from the body, and allowed to air-dry for 30 seconds ± 5 seconds.
- A second aliquot of approximately 1.5 mL of the challenge suspension will be dispensed into the subject's cupped hands, and the distribution and drying procedure described above will be repeated.
- The third and final remaining volume of the suspension will be dispensed into the subject's cupped hands and distributed over the entire surface of the hands (front and back), not reaching above the wrists, for 20 seconds ± 5 seconds. The hands will be allowed to air-dry for 90 seconds ± 5 seconds.

After the timed 90-second air-dry, the Glove Juice Sampling Procedure (Section 9.4.6.2) will be performed. This first contamination cycle will provide the baseline population titer. It will be followed by a 30-seconds ± 5 seconds handwash using 5 mL of Soft Soap and a 30-seconds ± 5 seconds rinse. Subjects will then use disposable paper towels to pat-dry their hands.

A 4.5-mL aliquot of the challenge suspension will again be evenly distributed over both hands, as described above. Subjects will then apply their randomly assigned test material according to the directions below.

9.4.6.1 Application Procedures

Subject will sparingly wet hands and forearms in running tap water by rapidly passing them one time through the tap water controlled at a temperature of 40 °C ± 2 °C.

A volume of test material indicated in the table below will be dispensed into subject's cupped hands.

Test Material	**Test Material Identity**	**ApplicationVolume**
Test Material #1	Benzalkonium Chloride	___ mL
Test Material #2	Benzethonium Chloride	___ mL
Test Material #3	Chloroxylenol	___ mL
Test Material #4	Povidone Iodine	___ mL
Positive Control	Hibiclens®	___ mL
Negative Control	Normal Physiological Saline	___ mL

Subject will perform a handwash with the dispensed test material for 30 seconds ± 5 seconds. Subject will then rinse thoroughly from elbows to fingertips under running tap water controlled at a temperature of 40 °C ± 2 °C for 30 seconds.

The hands will be gloved while hands are still wet and the Glove Juice Sampling Procedure (Section 9.4.6.2) will be performed.

9.4.6.2 Glove Juice Sampling Procedures

Within 1 minute after contamination for baseline, the subject's dry hands will be placed into powder-free, sterile synthetic gloves, and 75.0 mL of sterile SSF will be instilled into each of the gloves.

Within 1 minute after the test or control material application procedure, the subject's hands will be placed into powder-free, sterile synthetic gloves while still wet, and 75.0 mL of sterile SSF++ will be instilled into each of the gloves.

Within 1 minute of donning the gloves, the wrists will be secured, and technicians will massage the hands through the gloves in a standardized manner for 1 minute ± 5 seconds, paying particular attention to the fingers and flipping the hand after 30 seconds to ensure both the palm and back of the hand are thoroughly massaged. Within 1 minute of completing the massage following sampling for baseline and after test material application a 5 mL aliquot of the solution will be removed from each of the gloves and diluted separately in 5.0 mL BBP++ (dilution 10^0). The 10^0 dilutions will then be serially diluted in BBP++.

9.4.7 Blinding

In order to ensure blinded microbiologists, technicians who participate in test material application or the collection of samples from subjects during test material testing will not participate in plating samples and/or counting plates from samples collected from subjects after test material testing.

9.4.8 Subject Safety

Subjects will not be allowed to leave the laboratory for any reason once testing begins, except in an emergency. Additionally, subjects will be required to wear protective garments and not touch their clothing, faces, or any other body parts with their hands during the test period.

On completion of testing, subjects will be required to perform a 1-minute rinse with 70% ethanol and air-dry, followed by a supervised 4-minute wash with a 4% chlorhexidine gluconate solution. A topical antibiotic ointment will be applied to the hands following the decontamination procedure.

An antibiotic sensitivity profile for the *Serratia marcescens* (ATCC #14756) used in this study will be retained on file at BSLI.

9.4.9 Plating

Duplicate spiral and/or spread plates on TSA+ will be prepared from appropriate dilutions. The plates will be incubated at 25 °C ± 2 °C for approximately 48 hours, or until sufficient growth is observed. *Serratia marcescens* (ATCC #14756) will produce red colonies, and only those colonies will be counted. If colonies on one of the plates are uncountable, the count from the remaining plate will be used.

9.5 Efficacy and Safety Variables

9.5.1 Efficacy and Safety Measurements and Flow Chart

Mean $\log_{10}$ reductions of the indicator microorganism will be used to determine the antimicrobial effectiveness of the test materials.

Subject safety will be monitored by adverse event reporting (Section 11).

Flow Chart

Procedure	Day							
	-7 or more	-6	-5	-4	-3	-2	-1	0
Informed Consent Provided	X							
Pre-Test Conditioning Period	X	X	X	X	X	X	X	
Inclusion/Exclusion Criteria Reviewed								X
Begin Testing								X
Baseline Sample								X
Test Material Application								X
Post-Application Sample								X
Decontamination Procedure								X

9.5.2 Appropriateness of Measurements

The testing methods are based on the standardized test method ASTM E1174-13, *Standard Test Method for Evaluation of the Effectiveness of Health Care Personnel Handwash Formulations,* for use as a Health Care Personnel Handwash following a single test material application. The critical index is mean $\log_{10}$ reductions in microorganisms of ≥ 2.0 $\log_{10}$ within 5 minutes after one application. The hurdle of 2.5 $\log_{10}$ within 5 minutes after one application is not achievable at a 70% responder rate , as recommended by the 2015 FDA TFM, 21 CFR Part 310, *Safety and Effectiveness of Health Care Antiseptics; Topical Antimicrobial Drug Products for Over-the-Counter Human Use; Proposed Amendment of the Tentative Final Monograph; Reopening of Administrative Record;* Proposed Rule.

9.5.2.1 Neutralization

A neutralization study will be performed to assure that the neutralizers used in the recovery medium quench the antimicrobial activity of each test material and are not toxic to the challenge species. Study procedures are based on ASTM E1054-08(2013), *Standard Test Methods for Evaluation of Inactivators of Antimicrobial Agents*. *Serratia marcescens* (ATCC #14756) will be used as the challenge species in the neutralization study.

9.5.3 Primary Efficacy Variables

Mean $\log_{10}$ reductions of the indicator microorganism will be used to determine the antimicrobial effectiveness of the test materials. The critical index is mean $\log_{10}$ reductions in microorganisms of ≥ 2.0 $\log_{10}$ within 5 minutes after one application with a 70% responder rate.

A normal physiological saline negative control is included to show the contribution of the active ingredients to effectiveness. The test materials should be statistically superior to the negative control for the clinical simulation to be considered successful at showing that the test material is effective for use in health care antiseptic products.

A positive control is included to validate the study conduct to assure that the expected results are produced. For the results to be valid, the positive control should meet the appropriate log reduction criteria.

An analysis will be performed of the proportion of subjects who meet the log reduction criteria based on a two-sided statistical test for superiority to the negative control and a 95 percent confidence interval approach.

9.5.4 Data Collection and Microbial Recoveries

Colonies will be counted and data recorded using a computerized plate-counting system. If 10^0 spiral plates give an average count of zero, the average plate count will be expressed as 1.00×10^1.

The plate count data collected from this study will be evaluated using MiniTab® statistical computer software.

The estimated $\log_{10}$ number of viable microorganisms recovered from each hand will be designated the "*R*-value." It is the adjusted average $\log_{10}$ colony count measurement at each sampling time. Each *R*-value will be determined using the following formula:

$$R = \log_{10} [75 \times C_i \times 10^{-D} \times 2]$$

where: 75 = the amount (mL) of stripping solution instilled into each glove
C_i = the arithmetic average colony count of the two plate counts for each subject at a particular dilution level
D = the dilution factor
2 = the neutralization dilution

The $\log_{10}$ transformation is performed on these data to convert them to a linear scale. A linear scale, more appropriately a $\log_{10}$ linear scale, is a requirement of the statistical models to be used.

9.6 Data Quality Assurance

The Sponsor's designated Quality Assurance Representative may conduct audits at the study site. Audits will include, but are not limited to test materials, presence of required documents, the informed consent process, and review of source documents. The Investigator agrees to participate with audits conducted at a reasonable time in a reasonable manner.

The study will be inspected by the Quality Assurance Unit at BioScience Laboratories, Inc., and reports will be submitted to the Principal Investigator and Management in accordance with BSLI Standard Operating Procedures.

9.7 Statistical Methods and Determination of Sample Size

9.7.1 Statistical and Analytical Plans

The MiniTab® 17 Statistical Computer Package will be used for all statistical calculations.

A one-factor Analysis of Variance (ANOVA) model will be used to determine differences. It will have the form:

$$\hat{y} = A + e$$

where:

$\hat{y}$ = Log_{10} reductions = Log_{10} baseline – Log_{10} recoveries

A = Product

1, if Benzalkonium Chloride
2, if Benzethonium Chloride
3, if Chloroxylenol
4, if Povidone Iodine
5, if Positive Control (Hibiclens)
6, if Negative Control

e = Error Term

The variances will be evaluated using a Dunnett's Test. The alpha (α) value will be set at 0.05.

The 95% confidence intervals will be calculated according to the Bonferroni method.

Descriptive statistics will be generated on the $\log_{10}$ microbial populations recovered in the samples and the reductions from the controls, including sample size, means, standard deviations, and ranges.

Mean $\log_{10}$ reductions of the indicator microorganism will be used to determine the antimicrobial effectiveness of the test materials. The critical index is mean $\log_{10}$ reductions in microorganisms of 2.0 $\log_{10}$ within five minutes after one application with a 70% responder rate.

A normal physiological saline negative control is included to show the contribution of the active ingredients to effectiveness. The test products should be statistically superior to the negative control for the clinical simulation to be considered successful at showing that the test product is effective for use in healthcare antiseptic products.

A positive control is included to validate the study conduct to assure that the expected results are produced. For the results to be valid, the positive control should meet the appropriate log reduction criteria.

9.7.2 Determination of Sample Size

The sample size for this study will be determined using the following formula:

$$\mathbf{N} \geq \frac{S^2 \left(Z_{\alpha/2} + Z_{\mathrm{B}}\right)^2}{D^2}$$

Where:

- N= Sample size per test material configuration arm
- S= Estimate of variance (Based on pilot data per active)
- $Z_{\alpha/2}$= 0.05 level of significance (two-tail) = 1.96, Type I error (probability of stating a significant effect exists when one does not)
- Z_{β}= 0.842 level of significance for Type II (beta) error (probability of stating no significant effect exists when one does)
- D= Detectable difference (sensitivity) = 0.5

$$\mathbf{N} \geq \left(\frac{(S)^2 \left(1.96 + 0.842\right)^2}{0.5^2} \right) = X$$

9.8 Changes in the Conduct of the Study or Planned Analyses

Neither the Investigator nor the Sponsor will modify or alter this protocol without first obtaining agreement from the other parties. All protocol modifications including, but not limited to, changes in the Principal Investigator, inclusion/exclusion criteria, number of subjects to be enrolled, study sites, or procedures must be submitted to the GIRB as a written amendment for review and approval prior to implementation.

10.0 STUDY SUBJECTS

10.1 Disposition of Subjects

A written Informed Consent Form will be obtained from each subject and filed by the Investigator with the subject's records, in accordance with 21 CFR Parts 50 and 56.

The Investigator may discontinue individual subjects from the study at any time. Subjects may voluntarily withdraw from the study at any time. The Investigator (or designee) will provide a written report on the appropriate study document describing the reason for discontinuance and the date of discontinuance. A subject who discontinues will be replaced with another qualified subject who will follow the same treatment (randomization) scheme as the discontinued subject. The discontinued or withdrawn subject who participated in testing may not re-enter the study.

In order to implement a valid revocation of authorization to use and disclose private health information, the subject or their representative must make the request in writing to BioScience Laboratories, Inc., 1765 South 19th Avenue, Bozeman, Montana 59718. The revocation cannot stop the use or disclosure of information that has been collected prior to the revocation, is needed to ensure complete and accurate study results, or is required by law or government regulation (e.g. reporting adverse events, etc.). Revocation of an authorization may not be used to withhold normal medical care from the subject, but will make the subject ineligible to receive the study treatment or care.

10.2 Protocol Deviations

This study will be conducted as described in this protocol, except for an emergency situation in which the protection, safety, and well-being of the subject requires immediate interventions, based on the judgment of the Principal Investigator. In the event of a significant deviation from the protocol due to an emergency, accident, or mistake, the Investigator or designee will document the details of the situation and any subsequent decisions. All deviations from the Protocol or approved amendments shall be documented by BSLI. Any deviation to the Protocol that may have an effect on the safety or rights of the subjects or the integrity of the study must be reported to the GIRB within two business days from the time the deviation is identified.

The Sponsor or Investigator have the right to discontinue the study at any time for medical and/or administrative reasons. As far as possible, this should occur after mutual consultation.

11.0 SAFETY EVALUATION

11.1 Safety Assessments

The subject's safety will be monitored by evaluations of reactions observed on the skin of the test sites and any adverse reactions. Adverse reactions will be fully documented, reported as an Adverse Event, and followed to resolution.

11.2 Evaluation of Test Sites

Prior to performing any test procedures, the test sites will be examined to ensure no evidence of injury, dermatosis, or dermatitis is present. On completion of testing, the subject's hands will be examined and scored for skin irritation using the Skin Irritation Scoring System (Draize).

SKIN IRRITATION SCORING SYSTEM (Draize)

Erythema	0	No reaction
	1	Mild and/or transient redness limited to sensitive area
	2	Moderate redness persisting over much of the test material-exposed area
	3 *	Severe redness extending over most or all of the test material-exposed area
Edema	0	No reaction
	1	Mild and/or transient swelling limited to sensitive area
	2	Moderate swelling persisting over much of the test material -exposed area
	3 *	Severe swelling extending over most or all of the test material -exposed area
Rash	0	No reaction
	1	Mild and/or transient rash limited to sensitive area
	2	Moderate rash persisting over much of the test material-exposed area
	3 *	Severe rash extending over most or all of the test material-exposed area
Dryness	0	No reaction
	1	Mild and/or transient dryness limited to sensitive area
	2	Moderate dryness persisting over much of the test material-exposed area
	3 *	Severe dryness extending over most or all of the test material-exposed area

* = A score of 3 in one or more of the conditions evaluated represents significant irritation and qualifies as an Adverse Event.

11.3 Adverse Events

Adverse events will be captured for all subjects from the time baseline samples are taken to the time of subject discharge from the study. Adverse events will be categorized in relationship to the test materials that were applied. Trained personnel and emergency treatment (e.g., for anaphylaxis) are available onsite in the laboratory facility, and medical facilities/personnel are in close proximity.

In the event that either the Principal Investigator or the Sponsor determines that continuation of the study poses a hazardous risk of serious injury or death to the subject, the study will be stopped.

11.3.1 Adverse Event/Experience

An Adverse Event/Experience is any unexpected or undesirable experience occurring to a subject during a study, which may or may not be related to the test materials. All adverse event/experiences will be recorded and reported using an Adverse Event Report Form according to the Standard Operating Procedures of the laboratory.

All adverse events, regardless of severity or the cause/effect relationship, are to be recorded. The severity of the effect will be noted as *"Mild," "Moderate,"* or *"Severe"* according to the following definitions:

Mild	Awareness of signs or symptom, but easily tolerated.
Moderate	Discomfort to a degree as to cause interference with normal daily life activities and /or requiring medication.
Severe	Incapacity with inability to work or do usual daily life activities and requiring medical attention/intervention.

11.3.2 Causal Relations of Adverse Event/Experience

When determining the causal/effect relationship to a test material, the relationship will be described as *"None,*" *"Possible,*" *"Probable,*" or *"Definite."* The following definitions will be utilized:

None	No association to the test materials. Related to other etiologies such as concomitant medications or conditions or subject's known clinical state.
Possible	Uncertain association. Other etiologies are also possible.
Probable	Clear-cut association with improvement upon withdrawal of the test materials. Not reasonably explained by the subject's known clinical state but not an anticipated event.
Definite	An adverse event with a clear-cut temporal association and laboratory confirmation if possible.

11.3.3 Serious Adverse Event/Experience – During this Study

A Serious Adverse Event/Experience is any adverse experience occurring that results in any of the following outcomes:

Death;
A life-threatening adverse drug experience;
Inpatient hospitalization or prolongation of existing hospitalization;
A persistent or significant disability/incapacity;
Congenital anomaly/birth defect;
An important medical event that may require medical or surgical intervention to prevent one of the previously listed outcomes.

11.3.4 Unexpected Adverse Event/Experience

An Unexpected Adverse Event/Experience is any adverse event/experience not listed in the current labeling for the test materials, the current investigator's brochure, or the Anticipated Reactions Section 11.3.7 of this Protocol. Where test material labeling or investigator's brochure is not available, anticipated experiences will be based on the known pharmacological/toxicological properties of the test materials or ingredients.

11.3.5 Follow-up

If an adverse event/experience occurs, the Sponsor will be monetarily responsible for all costs associated with the follow-up for said event including, but not limited to, medical visits and medication prescribed by a medical professional directly related to the adverse event along with an administration fee that covers the Principal Investigator's time resolving the Adverse Event. If it is determined by Test Facility Management that the adverse event is due to negligence on the part of the Test Facility, no cost will be passed through to the Sponsor. The subject under the direction of the Principal Investigator (or designee) may be referred to the nearest acute care facility for treatment. Serious or Unexpected Event/Experiences will be followed to resolution. Any adverse event will be documented on an Adverse Event Report Form.

11.3.6 Notification

The Sponsor and the reviewing IRB will be notified of all adverse events/experiences within 2 business days. Any Serious or Unexpected Adverse Event/Experience that occurs during the study must be reported immediately by the Principal Investigator to the Sponsor and the reviewing IRB, followed by written notification within three business days of the information being reported to the investigative study team.

The Principal Investigator is required to review all unanticipated problems involving risk to volunteers or others, serious adverse events, and all subject deaths associated with the protocol and provide an unbiased written report of the event. At a minimum, the Principal Investigator must comment on the outcomes of the event or problem, and in the case of a serious adverse event or death, comment on the relationship to participation in the study.

11.3.7 Anticipated Reactions

It is possible but not likely, that a rash, allergic reaction or infection will develop. The risks associated with this test are primarily related to application of the test materials and the indicator microorganism. Mild skin irritation is anticipated and in some cases mild to heavy erythema, swelling, itching, cracking, peeling, or in rare cases, blistering and/or an allergic reaction might occur.

12.0 EXCEPTIONAL CONDITIONS

The Sponsor will be notified within 24 hours by telephone, email, and/or letter of any exceptions encountered in this study. The exceptional conditions or occurrences will be detailed in full and formally recorded. Exceptional conditions that occur and are not addressed in this Protocol will be subject to Out-of-Scope charges (see Proposal/Contract).

13.0 REFERENCES

1994 FDA Tentative Final Monograph 21 CFR Parts 333 and 369, *Health-Care Antiseptic Drug Products*; *Proposed Rule* in *Effectiveness testing of an antiseptic handwash or health care personnel handwash products*. (FR59: No. 116, 17 June 94. pp 31448 to 31450)

2015 FDA TFM, 21 CFR Part 310, *Safety and Effectiveness of Health Care Antiseptics; Topical Antimicrobial Drug Products for Over-the-Counter Human Use; Proposed Amendment of the Tentative Final Monograph; Reopening of Administrative Record;* Proposed Rule. (FR80: No. 84, 1 May 2015. pp 25166 to 25205)

ASTM E1174-13, *Standard Test Method for Evaluation of the Effectiveness of Health Care Personnel Handwash Formulations.*

ASTM E1054-08(13), *Standard Test Methods for Evaluation of Inactivators of Antimicrobial Agents*.

Code of Federal Regulations Title 21 Parts 50, 54, 56, 58 and 312.

ICH E6 Good Clinical Practice Guidelines.

14.0 FINAL REPORT

A Final Report will be prepared describing the methodology and results of the study in a clear and concise manner.

15.0 DOCUMENTATION AND RECORD KEEPING

All documentation and records will be compiled, analyzed, and retained by BioScience Laboratories, Incorporated, at its facility in Bozeman, Montana. All raw data for this study, as well as the Final Report, will be retained in safe storage by the Testing Facility for a period of at least 5 years. BioScience Laboratories, Inc. will notify the Study Sponsor before any documents or records are destroyed.

This Protocol has been approved by the GIRB on _____

15.1 Study Center File Management

It will be the responsibility of the Investigator to ensure that the Study File is maintained. The Study File for this protocol may contain, but will not be limited to, the information listed below:

- Investigational Brochure (if applicable) or other appropriate test material safety information
- GIRB Approved Signed Protocol
- Revised Protocol (if applicable)
- GIRB-Approved Informed Consent Form (blank)
- Copy of Signed Form(s) FDA-1572 (if applicable)
- Financial Disclosure for the Principal Investigator and Sub-investigators (if applicable)
- Curriculum Vitae of Principal Investigator and Sub-investigators
- DHHS Number for GIRB, or other documentation of IRB compliance with FDA regulation (Included in this Protocol)
- Documentation of GIRB approval of protocol, consent form, any protocol amendments and any consent form revisions
- All correspondence between the Principal Investigator, IRB, and Sponsor relating to study conduct
- Copies of information related to SAE and the information on Immediately Reported Adverse Events
- Copy of the Approval Letter from the GIRB
- Copy of Notification of Initiation of Clinical Testing (Initiation Letter to the GIRB)
- Copy of Notification of Completion of Clinical Testing (Completion Letter to the GIRB)
- Research Site Signature Log/Delegation of Duties
- FDA's Clinical Investigator Information Sheets (if applicable)

To protect privacy and maintain the confidentiality of data, each subject will be assigned a unique study number, all study samples and research records will be identified using the subject's study number, research records will be kept in a locked room with access limited to study personnel, and electronic databases will be maintained on password-protected computers.

16.0 LIABILITY AND INDEMNIFICATION

Test Facility's liability to Sponsor under this Protocol shall be limited to the price of this evaluation. Sponsor shall be responsible to Study Participants (when applicable) and to other third parties for the fitness of the test material for use as defined in the Study Protocol.

17.0 ACCEPTANCE

EVALUATION OF THE ANTIMICROBIAL EFFICACY OF FOUR TEST MATERIALS WITH POSITIVE AND NEGATIVE CONTROLS BASED ON THE HEALTH CARE PERSONNEL HANDWASH PROCEDURE

BIOSCIENCE LABORATORIES, INC. (TESTING FACILITY)
1765 South 19th Avenue
Bozeman, Montana 59718

President
& CEO: _____ _____
Daryl S. Paulson, Ph.D. Date

Principal
Investigator: _____ _____
To Be Determined Date of Study Initiation

Sub-Investigator: _____ _____
To Be Determined Date

REVIEWED BY:

Manager of
Quality Assurance: _____ _____
Amy L. Juhnke, RQAP-GLP Date

ACCEPTED BY: **THE AMERICAN CLEANING INSTITUTE** (SPONSOR)
1331 L Street N.W. Suite 650
Washington D.C. 20005

_____ _____
Representative Date

_____
Title

This Protocol has been approved by the GIRB on _____

ATTACHMENT 4: META-ANALYSIS OF HEALTH CARE PERSONNEL HAND WASH STUDIES CONDUCTED AT BIOSCIENCES LABORATORIES, INC.

META-ANALYSIS OF THE HEALTHCARE PERSONNEL HANDWASH STUDIES CONDUCTED AT BIOSCIENCE LABORATORIES, INC.

Meta-analysis is a way of taking the results of many different studies and running them in one analysis in order to see what the various studies provide.

This meta-analysis is conducted on data acquired from healthcare personnel handwash studies conducted at BioScience Laboratories, Inc. on behalf of GOJO Industries, Inc., using 4% chlorhexidine gluconate (Hibiclens) as a control product. Table 1 presents the data. Appendix A presents the raw data, including the study numbers.

Table 1. Data

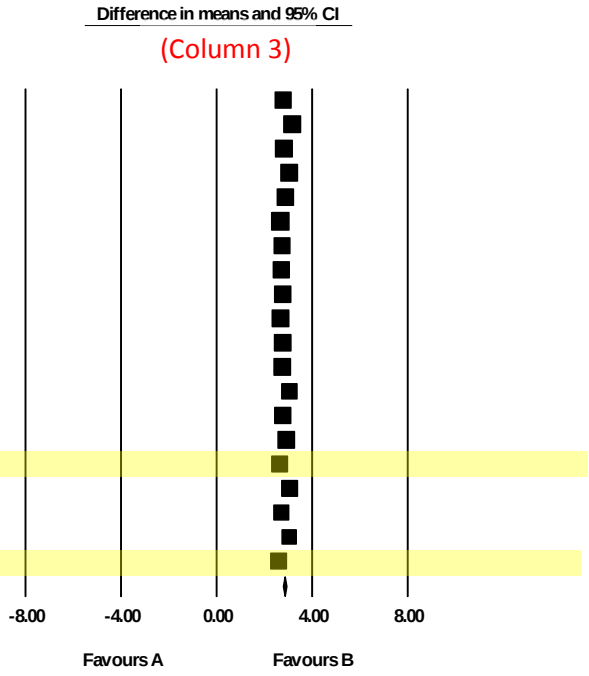
	A = Baseline Samples			B = Wash 1 Samples		
GOJO Study Number	Group A Mean	Group A Standard Deviation	Group A Sample Size	Group B Mean	Group B Standard Deviation	Group B Sample Size
1	9.15	0.13	16	6.36	0.28	16
2	9.18	0.13	48	6.01	0.39	48
3	9.15	0.27	46	6.33	0.31	46
4	9.18	0.24	47	6.14	0.36	47
5	9.24	0.08	24	6.36	0.34	24
6	9.16	0.09	48	6.49	0.27	48
7	9.16	0.14	48	6.42	0.53	48
8	9.14	0.22	16	6.43	0.20	16
9	9.10	0.21	48	6.33	0.44	48
10	9.20	0.18	48	6.52	0.38	48
11	9.02	0.22	48	6.25	0.40	48
12	8.87	0.23	48	6.12	0.37	48
13	9.07	0.47	54	6.02	0.47	54
14	9.11	0.11	36	6.34	0.43	36
15	9.21	0.12	36	6.29	0.37	36
16	9.23	0.11	36	6.59	0.47	36
17	9.09	0.15	36	6.03	0.45	36
18	9.02	0.25	36	6.31	0.52	36
19	9.03	0.19	36	5.99	0.68	36
20	9.08	0.14	33	6.48	0.44	33

Table 2 presents the results of the meta-analysis.

Table 2. Meta-Analysis

Meta Analysis

Studyname	Difference in means (Column 1)	Standard error	Variance	95% CI (Column 2) Lower limit	Upper limit	Z-Value	p-Value
1.000	2.790	0.077	0.006	2.639	2.941	36.151	0.000
2.000	3.170	0.059	0.004	3.054	3.286	53.424	0.000
3.000	2.820	0.061	0.004	2.701	2.939	46.525	0.000
4.000	3.040	0.063	0.004	2.916	3.164	48.169	0.000
5.000	2.880	0.071	0.005	2.740	3.020	40.394	0.000
6.000	2.670	0.041	0.002	2.589	2.751	64.996	0.000
7.000	2.740	0.079	0.006	2.585	2.895	34.630	0.000
8.000	2.710	0.074	0.006	2.564	2.856	36.459	0.000
9.000	2.770	0.070	0.005	2.632	2.908	39.363	0.000
10.000	2.680	0.061	0.004	2.561	2.799	44.158	0.000
11.000	2.770	0.066	0.004	2.641	2.899	42.039	0.000
12.000	2.750	0.063	0.004	2.627	2.873	43.733	0.000
13.000	3.050	0.090	0.008	2.873	3.227	33.720	0.000
14.000	2.770	0.074	0.005	2.625	2.915	37.445	0.000
15.000	2.920	0.065	0.004	2.793	3.047	45.042	0.000
16.000	2.640	0.080	0.006	2.482	2.798	32.815	0.000
17.000	3.060	0.079	0.006	2.905	3.215	38.706	0.000
18.000	2.710	0.096	0.009	2.522	2.898	28.181	0.000
19.000	3.040	0.118	0.014	2.809	3.271	25.834	0.000
20.000	2.600	0.080	0.006	2.442	2.758	32.347	0.000
Grand Average	2.827	0.037	0.001	2.754	2.900	75.990	0.000



Meta Analysis

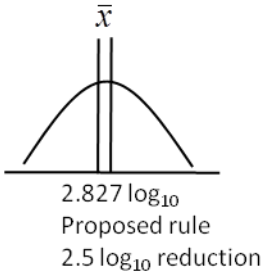
2.5 Log_{10} Reductions

There were 20 studies evaluated in this meta-analysis, all done by GOJO Industries. To perform this analysis, the Wash 1 log_{10} reductions were subtracted from Baseline log_{10} counts to get the reduction or difference in the means, as shown in Column 1. The 95% confidence intervals are shown in Column 2. Note the lower bounds, which have to be greater than 2.5 log_{10} reductions to pass the new FDA's Tentative Final Monograph (TFM). The right side of column 3 presents these three columns into one in graph form. The 95% confidence intervals are the width of the boxes (■).

Two studies with Hibiclens failed the 2.5 $\log_{10}$ reduction requirement (Studies 16 and 20). The lower bounds of the 95% confidence interval was lower than 2.5 $\log_{10}$ reduction.

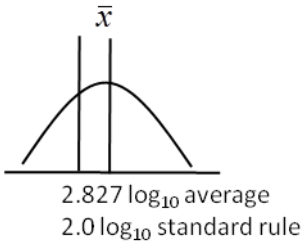
However, the 70% responder rate would be impossible to pass for any of these products at the 2.5 $\log_{10}$ reduction requirement. For example, to get the product to pass the 70% responder rate when the $\log_{10}$ reduction is 2.5 $\log_{10}$ will be impossible to accomplish with a $\log_{10}$ reduction average of 2.827. This would be very difficult to pass unless the products had the lower bound of the 95% confidence level at 3.10 or greater. In this analysis, none of the studies achieved it.

Figure 2. Proposed 2.5 $\log_{10}$ Reduction Rule



Looking at this, there is no doubt the 70% responder rate will be impossible to pass. The grand average reduction was 2.827 $\log_{10}$ and the proposed $\log_{10}$ reduction was 2.5 reductions. There will never be 70% of the data above the 2.5 $\log_{10}$ reduction at the 95% confidence interval.

Figure 3. Standard 2.0 Log_{10} Reduction Rule



The grand average (bottom line in Table 2) was 2.827.

70% Responder Rate

The analysis was performed on data from the 13 studies that could be recovered, as older studies had been destroyed, per Sponsor request.

The 70% responder rate for the 2.5 $\log_{10}$ reduction is presented in Table 3.

Table 3. 70% Responder Rates for 2.50 Log_{10} Reductions

Study Code	Study #	Pass/Fail	Actual responder rate (2.5 LR)	95% CI for Responder rate (2.5 LR)
1	140827-101	P	0.875	0.708 – 1.167
2	140547-101	P	0.917	0.829 – 1.048
3	140409-101	F	0.783	0.636 – 0.973
4	121014-101	F	0.833	0.667 – 1.088
5	121008-101	F	0.75	0.598 – 0.944
6	120604-101	F	0.667	0.499 – 0.876
7	111230-101	P	0.875	0.708 – 1.167
8	111016-101	F	0.667	0499 – 0.876
9	111016-101.2	F	0.708	0.547 – 0.911
10	110238-101	F	0.667	0.499 – 0.876
11	110102-101	F	0.792	0.650 – 0.975
12	100635-101	P	0.885	0.781 – 1.027
13	060227-101	F	0.833	0.689 – 1.033
	The yellow highlight indicates that it barely passed the 70% responder rate.			

For the 70% responder rate, the results for 2.5 $\log_{10}$ reductions are as follows:

4 studies passed

9 studies failed

31% chance of passing all the time with the control Hibiclens on the first wash.

The 70% responder rate for the 2.0 log_{10} reduction is presented in Table 4.

Table 4. 70% Responder Rates for 2.0 Log_{10} Reductions

Study Code	Study #	Pass/Fail	Actual responder rate (2.0 LR)	95% CI for Responder rate (2.0 LR)
1	140827-101	P	1.0	1.0625 – 1.0625
2	140547-101	P	1.0	1.021 – 1.021
3	140409-101	P	0.913	0.820 – 1.050
4	121014-101	P	1.0	1.042 – 1.042
5	121008-101	P	1.0	1.021 – 1.021
6	120604-101	P	0.958	0.899 – 1.059
7	111230-101	P	0.875	0.708 – 1.167
8	111016-101	P	0.917	0.827 – 1.078
9	111016-101.2	P	1.0	1.021 – 1.021
10	110238-101	P	0.917	0.827 – 1.048
11	110102-101	P	0.958	0.899 – 1.059
12	100635-101	P	0.961	0.907 – 1.055
13	060227-101	P	0.944	0.866 – 1.078
	The yellow highlight indicates that it barely passed the 70% responder rate.			

For the 70% responder rate, the results for 2.0 log_{10} reductions are as follows:

13 studies passed

0 studies failed

100% chance of passing all the time with the control Hibiclens at the first wash.

Table 5. Cumulative Results for 13 Studies Conducted at BioScience Laboratories, Inc.

Study Code	Study #	Date	Mean log reduction	Actual responder rate (2.5 LR)	95% CI for Responder rate (2.5 LR)	Meets FDA req at 2.5 log reduction (Y or N)	Actual responder rate (2.0 LR)	95% CI for Responder rate (2.0 LR)	Meets FDA req at 2.0 log reduction (Y or N)
1	140827-101	08/2014	2.7906	0.875	0.708 – 1.167	Y	1.0	1.0625 – 1.0625	Y
2	140547-101	05/2014	3.1627	0.917	0.829 – 1.048	Y	1.0	1.021 – 1.021	Y
3	140409-101	04/2014	2.8196	0.783	0.636 – 0.973	N	0.913	0.820 – 1.050	Y
4	121014-101	10/2012	2.8754	0.833	0.667 – 1.088	N	1.0	1.042 – 1.042	Y
5	121008-101	10/2012	2.6742	0.75	0.598 – 0.944	N	1.0	1.021 – 1.021	Y
6	120604-101	06/2012	2.7448	0.667	0.499 – 0.876	N	0.958	0.899 – 1.059	Y
7	111230-101	12/2011	2.7081	0.875	0.708 – 1.167	Y	0.875	0.708 – 1.167	Y
8	111016-101	10/2011	2.7633	0.667	0499 – 0.876	N	0.917	0.827 – 1.078	Y
9	111016-101.2	10/2011	2.6790	0.708	0.547 – 0.911	N	1.0	1.021 – 1.021	Y
10	110238-101	02/2011	2.7677	0.667	0.499 – 0.876	N	0.917	0.827 – 1.048	Y
11	110102-101	02/2011	2.7531	0.792	0.650 – 0.975	N	0.958	0.899 – 1.059	Y
12	100635-101	06/2010	3.0471	0.885	0.781 – 1.027	Y	0.961	0.907 – 1.055	Y
13	060227-101	02/2006	2.859	0.833	0.689 – 1.033	N	0.944	0.866 – 1.078	Y

META-ANALYSIS performed this 25th day of August, 2015.

Daryl S. Paulson, PhD
President/CEO/Chief Statistician
BioScience Laboratories, Inc.

APPENDIX A

Raw Data Table

STUDY	STUDYCODE	PRODUCT	BL_N	BL_Mean	BL_SD	W1_N	W1_Mean	W1_SD	Reduction N	Reduction Mean	Reduction SD
140827-101	1	CHG	16	9.15	0.13	16	6.36	0.28	16	2.79	0.29
140547-101	2	CHG	48	9.18	0.13	48	6.01	0.39	48	3.16	0.36
140409-101	3	CHG	46	9.15	0.27	46	6.33	0.31	46	2.82	0.47
130330-101	4	CHG	47	9.18	0.24	48	6.14	0.36	47	3.04	0.48
121014-101	5	CHG	24	9.24	0.08	24	6.36	0.34	24	2.88	0.34
121008-101	6	CHG	48	9.16	0.09	48	6.49	0.27	48	2.67	0.29
120604-101	7	CHG	48	9.16	0.14	48	6.42	0.53	48	2.74	0.49
111230-101	8	CHG	16	9.14	0.22	16	6.43	0.20	16	2.71	0.23
111016-101	9	CHG	48	9.10	0.21	48	6.33	0.44	48	2.76	0.43
111016-101	10	CHG	48	9.20	0.18	48	6.52	0.38	48	2.68	0.44
110238-101	11	CHG	48	9.02	0.22	48	6.25	0.40	48	2.77	0.43
110102-101	12	CHG	48	8.87	0.23	48	6.12	0.37	48	2.75	0.42
100635-101	13	CHG	54	9.07	0.47	52	6.02	0.47	52	3.05	0.49
060227-101	14	CHG	36	9.11	0.11	36	6.34	0.43	*	*	*
050328-101	15	CHG	36	9.21	0.12	36	6.29	0.37	*	*	*
050201-101	16	CHG	36	9.23	0.11	36	6.59	0.47	*	*	*
040518-101	17	CHG	36	9.09	0.15	36	6.03	0.45	*	*	*
040414-101	18	CHG	36	9.02	0.25	36	6.31	0.52	*	*	*
021004-101	19	CHG	36	9.03	0.19	36	5.99	0.68	*	*	*
020917-101	20	CHG	33	9.08	0.14	34	6.48	0.44	*	*	*

ATTACHMENT 5: META-ANALYSIS OF HEALTH CARE PERSONNEL HAND WASH STUDIES CONDUCTED AT HILL TOP RESEARCH AND HENKEL

REPORT FOR

Henkel for a Review of CHG Study Data

September 11, 2015

FOR
HENKEL
7201 E. Henkel Way
Scottsdale, AZ 85255

BY
James P Bowman & Associates
6409 Roth Ridge Dr.
Loveland, OH 45140

IX. Method of statistical analysis

CHG Data Review: Health Care Personnel Handwash E1174

Thirteen studies from Hill Top Research that included CHG as the control were reviewed in 2004.

Thirteen studies (2009 – 2014) from Henkel that included CHG as the control were analyzed in a similar fashion to the data from 2004 from Hill Top Research.

For both sets of data descriptive statistics were calculated on the log10 reductions (i.e. mean, standard deviation, etc.). Additionally, an independent t-test was conducted comparing the mean log10 reductions from the Hill Top Research and the Henkel data.

The frequency of log10 reductions were presented in graphical format – histogram, for each set of data.

Calculations were done using the FDA criteria of a mean log10 reduction of 2.5 and 70% of the test subjects having to achieve that reduction to determine whether or not each individual study would pass the monograph.

Additionally, calculations were done using a modified criteria of a mean log10 reduction of 2.0 and 70% of the test subjects having to achieve that reduction to determine whether or not each individual study would pass this modified criteria.

CHG Data Review - Simulations

Simulations were conducted using the mean log10 reduction of 2.51 and the variability variable around 0.3318 (values from the data review).

1000 studies were simulated utilizing sample sizes of 6, 12, 18, 24, 30 and 36.

Objective:

Calculations were done using the FDA criteria of a mean log10 reduction of 2.5 as well as the modified criteria of 2.0 and 70% of the test subjects having to achieve those reductions to determine how many studies out of the 1000 simulations would pass the criteria.

X. Results

Dataset #1 – HTR

- ***13 Studies***
- ***Sample Sizes Ranged from N=4 to N=30***
- ***Mean Log10 reduction = 2.5065 (0.3318)***

Dataset #2 – Henkel

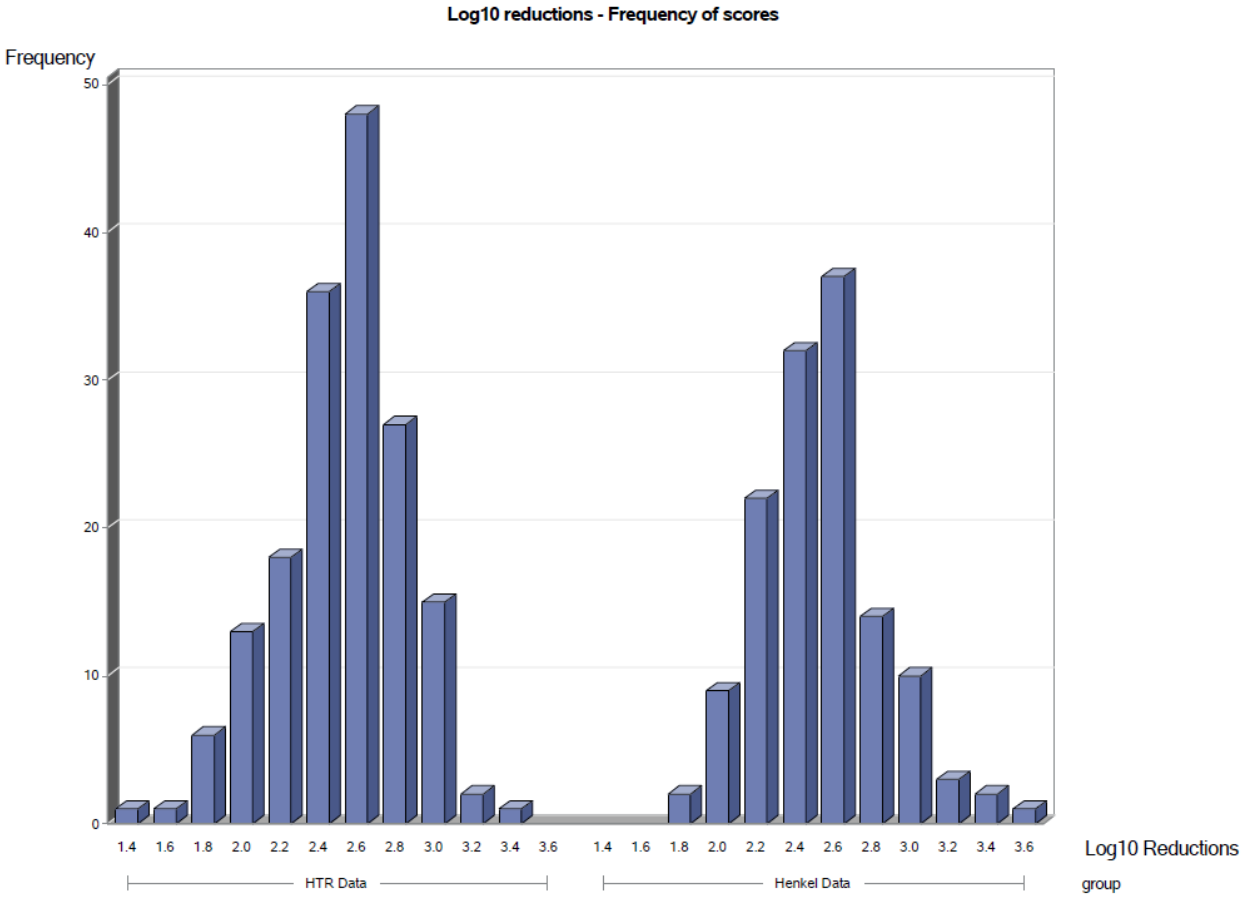
- ***13 Studies***
- ***Sample Sizes Ranged from N=8 to N=12***
- ***Mean Log10 reduction = 2.5165 (0.3284)***

Note similar means and standard deviations

T-test data analysis indicated no significant difference between HTR and Henkel Data (p>0.5000)

Results (continued)

The following graph indicates the distribution of log10 reductions from the two datasets.



Results (continued)

A summary of the results of the two datasets versus the FDA criteria are shown below.

Dataset #1 – HTR

- FDA Criteria:
  - Mean Log Reduction 2.5
  - 70% of Subjects Reduction >2.5
- 15% of the studies pass FDA Criteria
- FDA Criteria
  - Mean Log Reduction 2.0
  - 70% of Subjects Reduction >2.0
- 100% of the studies pass FDA Criteria

Dataset #2 – Henkel

- FDA Criteria:
  - Mean Log Reduction 2.5
  - 70% of Subjects Reduction >2.5
- 13% of the studies pass FDA Criteria
- FDA Criteria
  - Mean Log Reduction 2.0
  - 70% of Subjects Reduction >2.0
- 92% of the studies pass FDA Criteria

XI. RESULTS of the Simulations

Simulations were done:

1000 studies were simulated with the assumption of the mean value being 2.51 and the variability variable around 0.3318.

1000 studies were simulated each utilizing sample sizes of 6, 12, 18, 24, 30 and 36.

What is the percent of the time that we would have passed the FDA Criteria?

Sample Size	***2.5 Log Reduction***	***2.0 Log Reduction***
N=6	***55.6%***	***91.2%***
N=12	***53.8%***	***95.2%***
N=18	***53.9%***	***96.0%***
N=24	***55.2%***	***98.1%***
N=30	***58.1%***	***98.5%***
N=36	***55.1%***	***98.9%***

XII. Conclusions

So, if in fact the mean value for this product is around 2.5 log10 reduction, even using sample sizes as small as 6 test subjects, you will achieve a mean value of greater than 2.0 log10 with greater than 70% of the subjects seeing a 2.0 log10 reduction over 90% of the time (larger percent with larger sample sizes).

However, when using the criteria of 2.5 log10 reduction, for all of the sample sizes, passing the FDA criteria occurs less than 60% of the time.

Based on this data, it would seem appropriate to have the criteria for the already approved product control (i.e. CHG) set to be 2.0 log10 reduction with 70% of the individual test subjects achieving a 2.0 log10 reduction.

It would seem that sample sizes of 12 would be appropriate.

Attachments:

5.1 HTR and Henkel Tables_CHG_081815

5.2 HTR and Henkel_CHG data review August 2015

5.3 HTR and Henkel Graphs_CHG_081815

ATTACHMENT 5.1:

HTR AND HENKEL TABLES_CHG_081815

HTR AND HENKEL - CHG

CHG DATASET

TABLE 1. TABULATION OF THE CHG DATA - LOG10 REDUCTIONS FROM BASELINE

	2	3	4	5	6	7	8	9	10	11	12	13	14
Subject													
1	2.6577	2.2212	2.5843	2.5753	2.4650	2.1005	2.9417	2.9300	3.0213	1.8499	2.7891	2.0185	2.6800
2	2.8164	3.1414	2.4811	2.8432	2.4116	2.3171	2.8462	2.0915	2.4383	2.4049	2.9255	2.4950	1.7125
3	2.9815	2.6409	2.0501	2.9085	2.5641	2.0278	2.3026	2.7832	2.6851	2.4288	2.9731	2.4454	2.9389
4	2.7983	2.5551	2.1854	2.2701	2.6322	2.8751	2.4303	2.6354	2.6161	2.8873	2.7496	2.7715	2.6814
5	2.6805	2.4485	2.0436	2.7482	ND	2.4200	2.5752	2.5442	2.4487	2.0839	2.6559	2.3946	2.6475
6	2.7297	2.4541	2.1645	2.3289	ND	2.8480	2.6314	2.7997	2.0414	2.6961	2.7127	2.8308	1.9365
7	2.9682	2.9339	2.1146	2.7757	ND	2.1840	2.6176	2.3574	2.9973	2.1869	ND	2.6694	2.2676
8	2.6625	ND	2.3210	2.7832	ND	2.6693	2.4168	2.5703	2.9963	2.7061	ND	2.3415	2.3425
9	ND	ND	1.6579	2.5176	ND	ND	2.5817	2.2156	2.3610	2.5421	ND	2.2943	3.0969
10	ND	ND	2.4167	2.0415	ND	ND	2.8047	2.6528	2.1739	2.5654	ND	2.7176	2.7230
11	ND	ND	2.7559	2.6259	ND	ND	2.4296	2.3083	2.3978	3.3208	ND	2.5718	1.7505
12	ND	ND	2.6282	2.3623	ND	ND	2.2215	2.8140	1.8236	2.6858	ND	2.4371	2.5073
13	ND	ND	2.1096	2.5777	ND	ND	2.8407	2.5736	2.6166	2.3368	ND	2.4025	3.1667
14	ND	ND	2.3454	2.6491	ND	ND	2.8495	2.4511	2.5270	1.9456	ND	2.2664	1.3846
15	ND	ND	1.9876	2.2513	ND	ND	ND	1.9995	2.5826	2.9689	ND	2.4566	2.4914
16	ND	ND	ND	ND	ND	ND	ND	2.6233	ND	ND	ND	ND	1.7710
17	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	2.6760
18	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	2.3519
19	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	1.9720
20	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	1.7466
21	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	2.7704
22	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	2.5681
23	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	2.6217
24	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	2.5003
25	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	2.6767
26	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	3.0924
27	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	2.2966
28	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	2.1164
29	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	2.5296
30	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	2.6643
Mean	2.7868	2.6279	2.2564	2.5506	2.5182	2.4302	2.6064	2.5219	2.5151	2.5073	2.8010	2.4742	2.4227
Std	0.1301	0.3137	0.2875	0.2518	0.0989	0.3325	0.2260	0.2674	0.3424	0.3970	0.1239	0.2134	0.4509
N	8	7	15	15	4	8	14	16	15	15	6	15	30

HTR and HENKEL - CHG
CHG Dataset
Table 1. Tabulation of the CHG Data - log10 reductions from baseline

	1	2	3	4	5	6	7	8	9	10	11	12	13
Subject													
1	2.5242	2.1556	2.6123	2.3839	2.5579	2.7708	2.7518	2.6157	2.3091	2.0675	1.9318	2.5972	2.6633
2	2.6796	2.3263	2.6630	2.4947	2.3991	2.2861	2.2283	2.6313	2.5656	2.0558	2.3905	2.3803	2.7742
3	2.3012	2.2585	1.9886	2.1235	2.6766	2.0222	3.0612	2.5236	2.5503	1.7923	1.9912	2.3659	2.7369
4	2.9952	2.4838	2.2488	2.3663	2.5533	2.5492	2.2333	2.9713	2.6718	2.4369	2.4106	3.0467	2.9512
5	2.8319	2.2952	2.3682	2.3317	3.6113	2.6678	2.6494	3.1725	2.1987	2.3418	2.5535	2.0045	3.0360
6	2.2500	2.9375	2.5498	2.7437	3.0349	2.5782	2.2936	2.2912	2.4053	2.1622	2.6078	2.0677	2.2977
7	2.1452	2.3762	2.4072	2.3917	2.2755	2.8193	2.6735	2.2610	2.3994	1.7173	2.5823	2.3543	2.9581
8	2.4974	2.8181	2.6853	2.7517	2.6132	2.5262	2.9802	2.5463	2.2104	1.9283	3.1825	2.5560	2.6312
9	2.5118	2.8243	2.5581	2.6912	ND	2.4128	ND	3.4703	2.4473	2.3056	2.7390	2.8459	3.2803
10	ND	2.4054	2.1251	2.2652	ND	2.2163	ND	2.7723	2.3811	2.3045	2.3725	2.5126	2.5457
11	ND	ND	ND	ND	ND	ND	ND	2.7723	2.6191	ND	2.4415	2.6075	3.4159
12	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	2.1573	2.4821
Mean	2.5263	2.4881	2.4206	2.4544	2.7152	2.4849	2.6089	2.7298	2.4326	2.1112	2.4730	2.4580	2.8144
Std	0.2762	0.2728	0.2375	0.2126	0.4245	0.2522	0.3282	0.3630	0.1574	0.2432	0.3402	0.3053	0.3277
N	9	10	10	10	8	10	8	11	11	10	11	12	12

HTR and HENKEL - CHG
CHG Dataset
Table 2A. Overall Mean CHG data

The MEANS Procedure

Analysis Variable : score		
Mean	Std Dev	N
2.5108758	0.3297906	300

HTR and HENKEL - CHG
CHG Dataset
Table 2B. Mean CHG data - by group
The MEANS Procedure

Analysis Variable : score				
group	N Obs	Mean	Std Dev	N
HTR Data	390	2.5064521	0.3318289	168
Henkel Data	156	2.5165060	0.3283532	132

HTR and HENKEL - CHG
CHG Dataset
Table 2C. T-test for mean CHG data - by group

The TTEST Procedure

Variable: score

group	N	Mean	Std Dev	Std Err	Minimum	Maximum
HTR Data	168	2.5065	0.3318	0.0256	1.3846	3.3208
Henkel Data	132	2.5165	0.3284	0.0286	1.7173	3.6113
Diff (1-2)		-0.0101	0.3303	0.0384		

group	Method	Mean	95% CL Mean		Std Dev	95% CL Std Dev	
HTR Data		2.5065	2.4559	2.5570	0.3318	0.2997	0.3717
Henkel Data		2.5165	2.4600	2.5730	0.3284	0.2930	0.3736
Diff (1-2)	Pooled	-0.0101	-0.0857	0.0656	0.3303	0.3058	0.3591
Diff (1-2)	Satterthwaite	-0.0101	-0.0856	0.0655			

Method	Variances	DF	t Value	Pr > |t|
Pooled	Equal	298	-0.26	0.7937
Satterthwaite	Unequal	282.77	-0.26	0.7935

Equality of Variances				
Method	Num DF	Den DF	F Value	Pr > F
Folded F	167	131	1.02	0.9040

HTR and HENKEL - CHG
CHG Dataset
Table 3A. Normality test of the CHG data

The UNIVARIATE Procedure
Variable: score

Moments			
N	300	Sum Weights	300
Mean	2.51087582	Sum Observations	753.262745
Std Deviation	0.32979055	Variance	0.10876181
Skewness	-0.0488199	Kurtosis	0.6072769
Uncorrected SS	1923.86899	Corrected SS	32.5197804
Coeff Variation	13.1344827	Std Error Mean	0.01904047

Basic Statistical Measures			
Location		Variability	
Mean	2.510876	Std Deviation	0.32979
Median	2.535828	Variance	0.10876
Mode	2.772284	Range	2.22668
		Interquartile Range	0.39609

Note: The mode displayed is the smallest of 2 modes with a count of 2.

Tests for Location: Mu0=0				
Test	Statistic		p Value	
Student's t	t	131.8705	Pr > |t|	<.0001
Sign	M	150	Pr >= |M|	<.0001
Signed Rank	S	22575	Pr >= |S|	<.0001

Tests for Normality				
Test	Statistic		p Value	
Shapiro-Wilk	W	0.994496	Pr < W	0.3543
Kolmogorov-Smirnov	D	0.041241	Pr > D	>0.1500
Cramer-von Mises	W-Sq	0.082621	Pr > W-Sq	0.1985
Anderson-Darling	A-Sq	0.500255	Pr > A-Sq	0.2154

HTR and HENKEL - CHG
CHG Dataset
Table 3A. Normality test of the CHG data

The UNIVARIATE Procedure
Variable: score

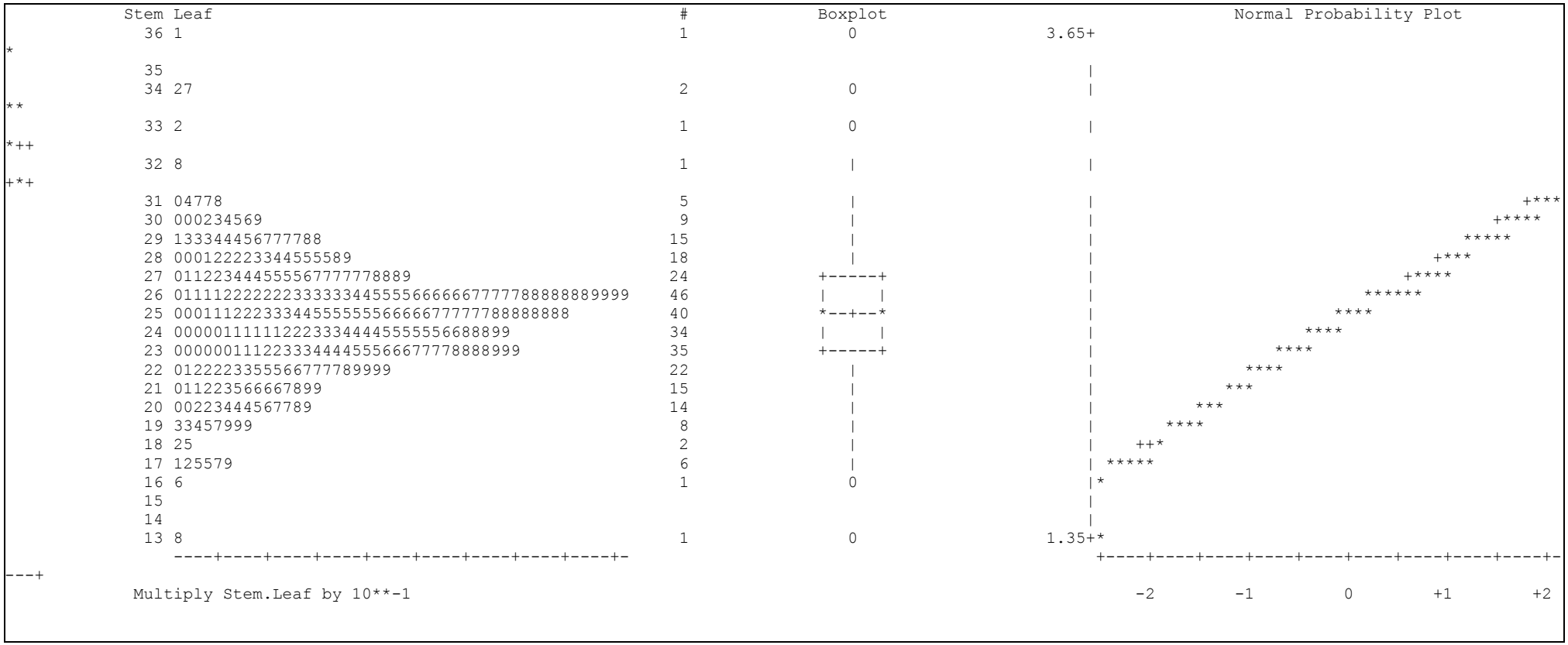
Quantiles (Definition 5)	
Quantile	Estimate
100% Max	3.61128
99%	3.36832
95%	3.02808
90%	2.93571
75% Q3	2.70114
50% Median	2.53583
25% Q1	2.30505
10%	2.07580
5%	1.97978
1%	1.71491
0% Min	1.38461

Extreme Observations			
Lowest		Highest	
Value	Obs	Value	Obs
1.38461	182	3.28033	507
1.65788	107	3.32078	140
1.71255	26	3.41586	533
1.71727	478	3.47029	502
1.74657	260	3.61128	447

Missing Values			
		Percent Of	
Missing Value	Count	All Obs	Missing Obs
.	246	45.05	100.00

HTR and HENKEL - CHG
CHG Dataset
Table 3A. Normality test of the CHG data

The UNIVARIATE Procedure
Variable: score



HTR and HENKEL - CHG
CHG Dataset
Table 3B. Normality test of the CHG data - by group

The UNIVARIATE Procedure
Variable: score

group=HTR Data

Moments			
N	168	Sum Weights	168
Mean	2.50645213	Sum Observations	421.083957
Std Deviation	0.3318289	Variance	0.11011042
Skewness	-0.5121015	Kurtosis	0.35919855
Uncorrected SS	1073.81522	Corrected SS	18.3884397
Coeff Variation	13.2389881	Std Error Mean	0.02560116

Basic Statistical Measures			
Location		Variability	
Mean	2.506452	Std Deviation	0.33183
Median	2.564749	Variance	0.11011
Mode	2.783172	Range	1.93618
		Interquartile Range	0.40122

Tests for Location: Mu0=0				
Test	Statistic		p Value	
Student's t	t	97.90387	Pr > |t|	<.0001
Sign	M	84	Pr >= |M|	<.0001
Signed Rank	S	7098	Pr >= |S|	<.0001

Tests for Normality				
Test	Statistic		p Value	
Shapiro-Wilk	W	0.982634	Pr < W	0.0339
Kolmogorov-Smirnov	D	0.074858	Pr > D	0.0214
Cramer-von Mises	W-Sq	0.160938	Pr > W-Sq	0.0183
Anderson-Darling	A-Sq	0.914292	Pr > A-Sq	0.0208

Quantiles (Definition 5)	
Quantile	Estimate
100% Max	3.32078
99%	3.16668
95%	2.98151

Quantiles (Definition 5)	
Quantile	Estimate
90%	2.92550
75% Q3	2.72028
50% Median	2.56475
25% Q1	2.31907
10%	2.04149
5%	1.93649
1%	1.65788
0% Min	1.38461

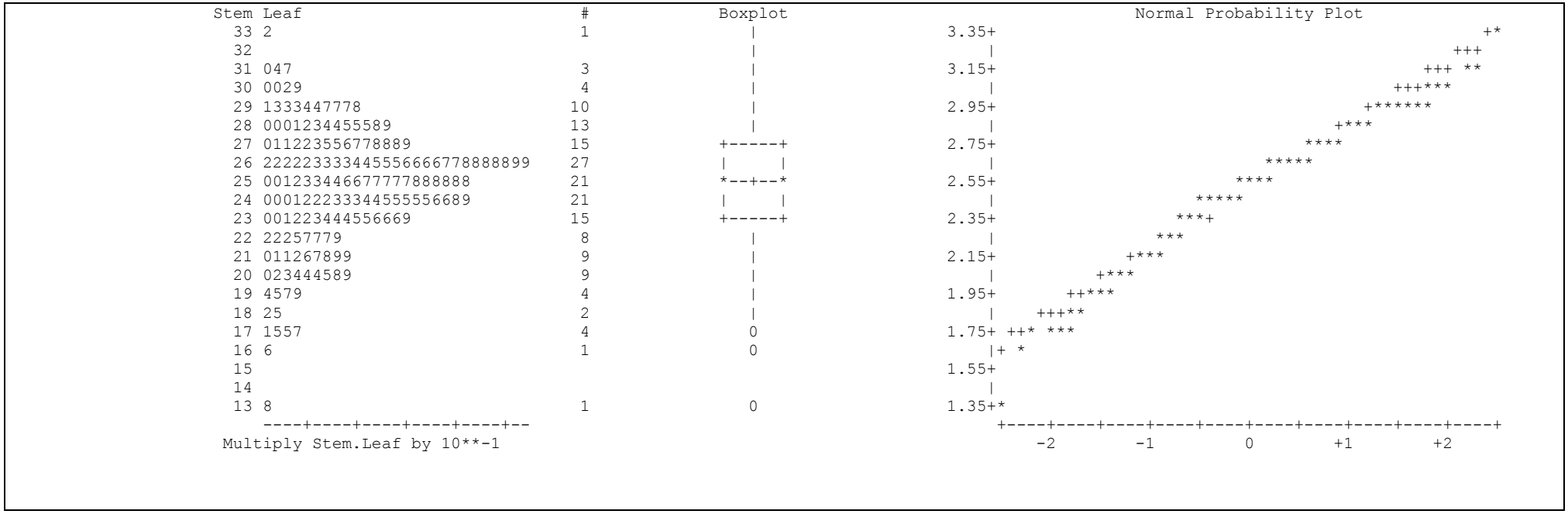
Extreme Observations			
Lowest		Highest	
Value	Obs	Value	Obs
1.38461	182	3.09237	338
1.65788	107	3.09685	117
1.71255	26	3.14141	15
1.74657	260	3.16668	169
1.75045	143	3.32078	140

Missing Values			
		Percent Of	
Missing Value	Count	All Obs	Missing Obs
.	222	56.92	100.00

HTR and HENKEL - CHG
CHG Dataset
Table 3B. Normality test of the CHG data - by group

The UNIVARIATE Procedure
Variable: score

group=HTR Data



HTR and HENKEL - CHG
CHG Dataset
Table 3B. Normality test of the CHG data - by group

The UNIVARIATE Procedure
Variable: score

group=Henkel Data

Moments			
N	132	Sum Weights	132
Mean	2.51650597	Sum Observations	332.178788
Std Deviation	0.32835315	Variance	0.10781579
Skewness	0.56333046	Kurtosis	0.93431333
Uncorrected SS	850.053774	Corrected SS	14.1238689
Coeff Variation	13.0479783	Std Error Mean	0.02857947

Basic Statistical Measures			
Location		Variability	
Mean	2.516506	Std Deviation	0.32835
Median	2.512225	Variance	0.10782
Mode	2.772284	Range	1.89401
		Interquartile Range	0.37871

Tests for Location: Mu0=0				
Test	Statistic		p Value	
Student's t	t	88.05292	Pr > |t|	<.0001
Sign	M	66	Pr >= |M|	<.0001
Signed Rank	S	4389	Pr >= |S|	<.0001

Tests for Normality				
Test	Statistic		p Value	
Shapiro-Wilk	W	0.9776	Pr < W	0.0280
Kolmogorov-Smirnov	D	0.070062	Pr > D	0.1109
Cramer-von Mises	W-Sq	0.126536	Pr > W-Sq	0.0488
Anderson-Darling	A-Sq	0.792064	Pr > A-Sq	0.0408

Quantiles (Definition 5)	
Quantile	Estimate
100% Max	3.61128
99%	3.47029
95%	3.06118

Quantiles (Definition 5)	
Quantile	Estimate
90%	2.95808
75% Q3	2.67813
50% Median	2.51222
25% Q1	2.29942
10%	2.14516
5%	2.00449
1%	1.79235
0% Min	1.71727

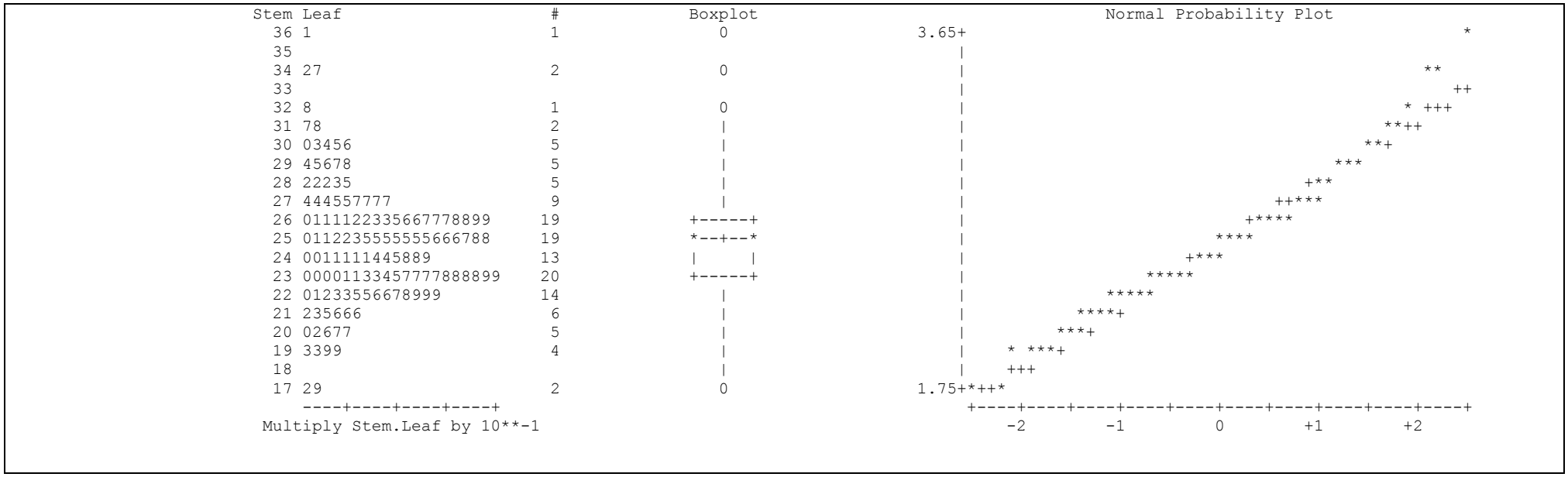
Extreme Observations			
Lowest		Highest	
Value	Obs	Value	Obs
1.71727	478	3.18253	492
1.79235	426	3.28033	507
1.92826	491	3.41586	533
1.93183	401	3.47029	502
1.98856	419	3.61128	447

Missing Values			
		Percent Of	
Missing Value	Count	All Obs	Missing Obs
.	24	15.38	100.00

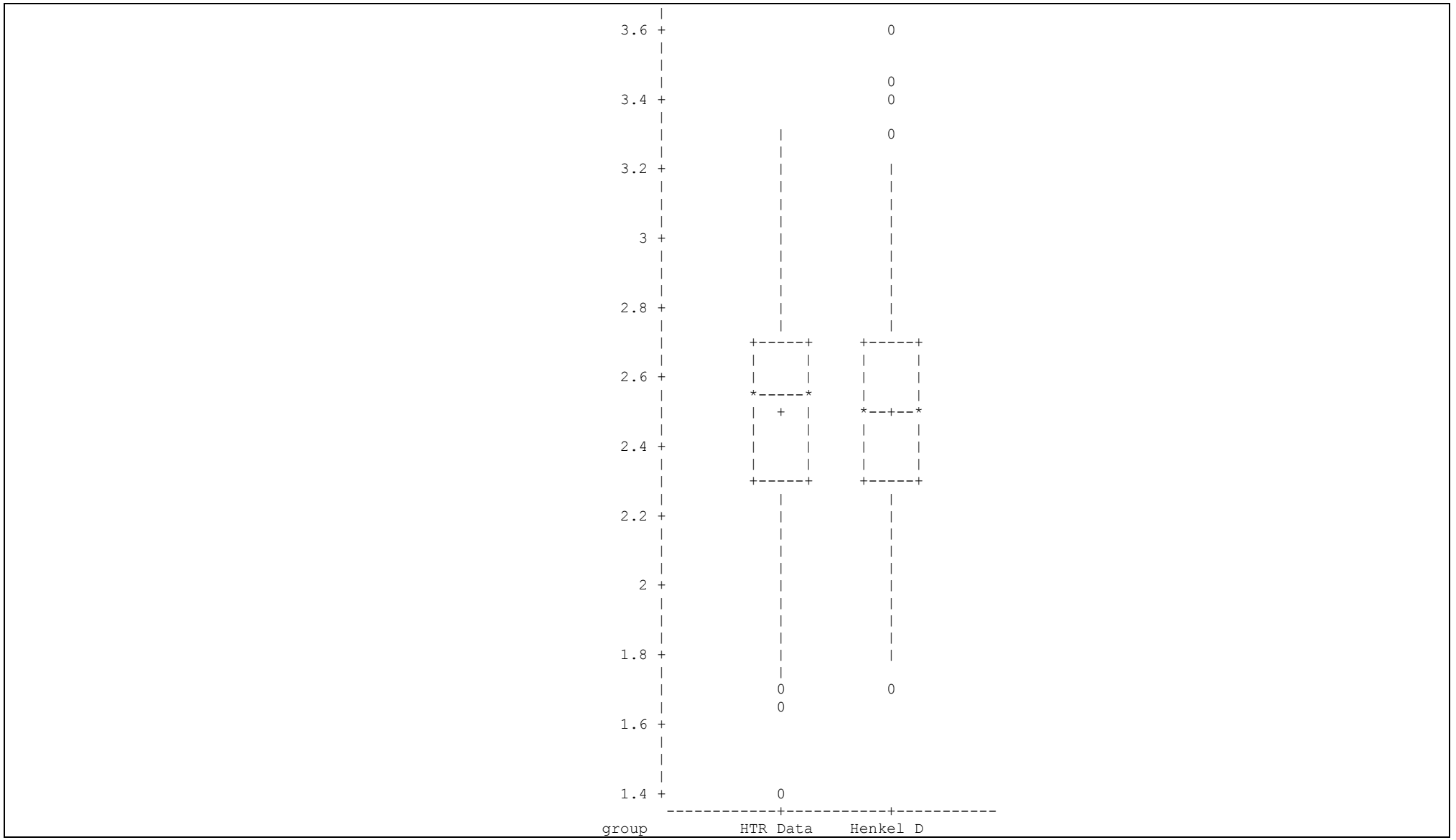
HTR and HENKEL - CHG
CHG Dataset
Table 3B. Normality test of the CHG data - by group

The UNIVARIATE Procedure
Variable: score

group=Henkel Data



HTR and HENKEL - CHG
CHG Dataset
Table 3B. Normality test of the CHG data - by group
The UNIVARIATE Procedure
Variable: score
Schematic Plots



ATTACHMENT 5.2:

HTR AND HENKEL CHG DATA REVIEW AUGUST 2015

CHG Study Data Review

Presented to FDA

By ACI

CHG Data Review
Studies from 2004 (HTR)
Studies from 2015 (Henkel)

CHG Data Review

Dataset #1 – HTR

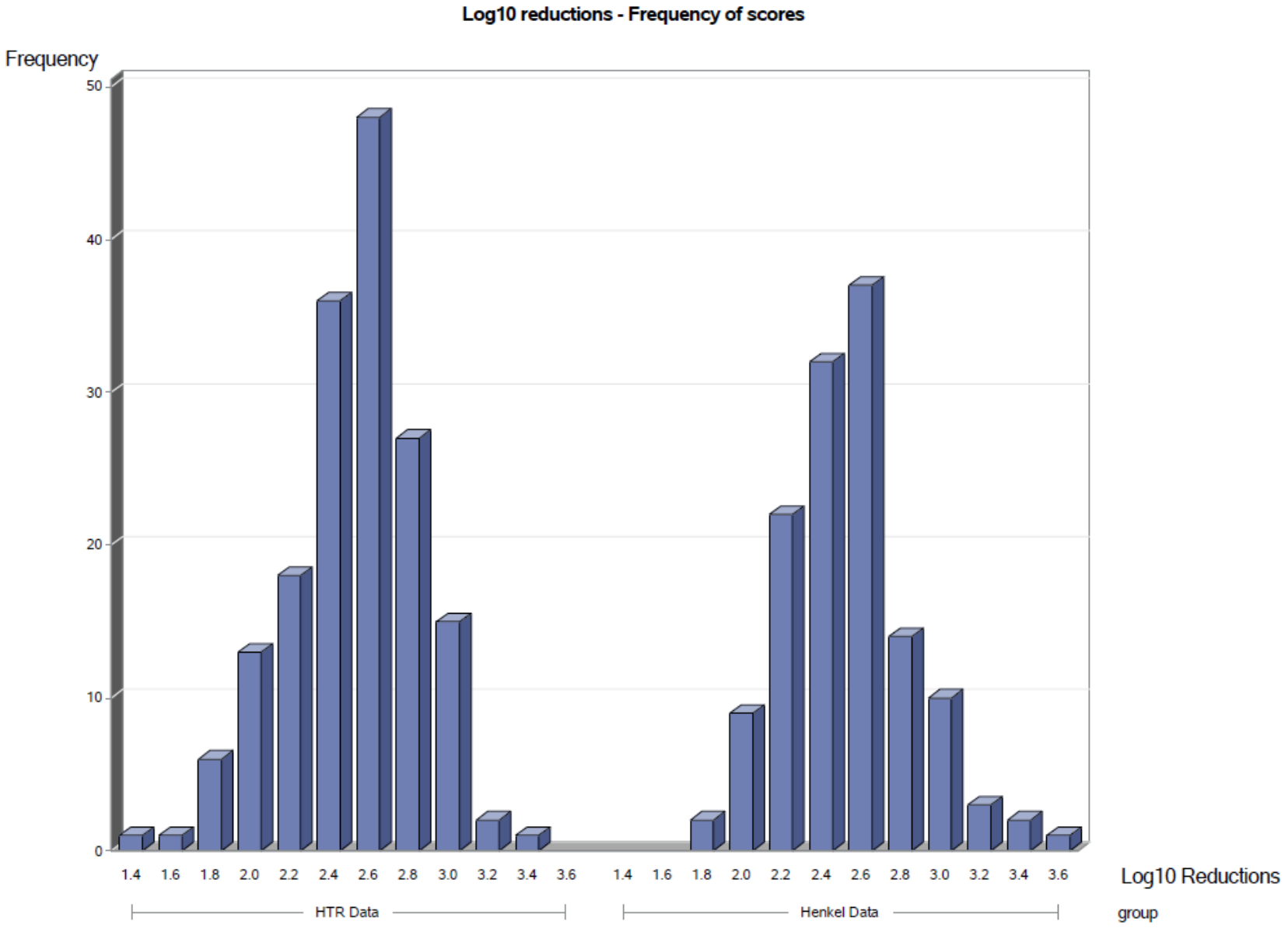
- 13 Studies
- n=4 to 30
- Mean Log10 reduction = 2.5065 (0.3318)

Dataset #2 – Henkel

- 13 Studies
- n=8 to 12
- Mean Log10 reduction = 2.5165 (0.3284)

Note similar means and standard deviations

T-test data analysis indicated no significant difference between HTR and Henkel Data (p>0.5000)


Log10 reductions - Frequency of scores
Frequency
50
40
30
20
10
0
1.4
1.6
1.8
2.0
2.2
2.4
2.6
2.8
3.0
3.2
3.4
3.6
HTR Data
Henkel Data
Log10 Reductions
group

CHG Data Review

Dataset #1 – HTR

- FDA Criteria:
  - Mean Log Reduction 2.5
  - 70% of Subjects Reduction >2.5
- 15% of the studies pass FDA Criteria

- FDA Criteria
  - Mean Log Reduction 2.0
  - 70% of Subjects Reduction >2.0
- 100% of the studies pass FDA Criteria

Dataset #2 – Henkel

- FDA Criteria:
  - Mean Log Reduction 2.5
  - 70% of Subjects Reduction >2.5
- 13% of the studies pass FDA Criteria

- FDA Criteria
  - Mean Log Reduction 2.0
  - 70% of Subjects Reduction >2.0
- 92% of the studies pass FDA Criteria

CHG Data Review

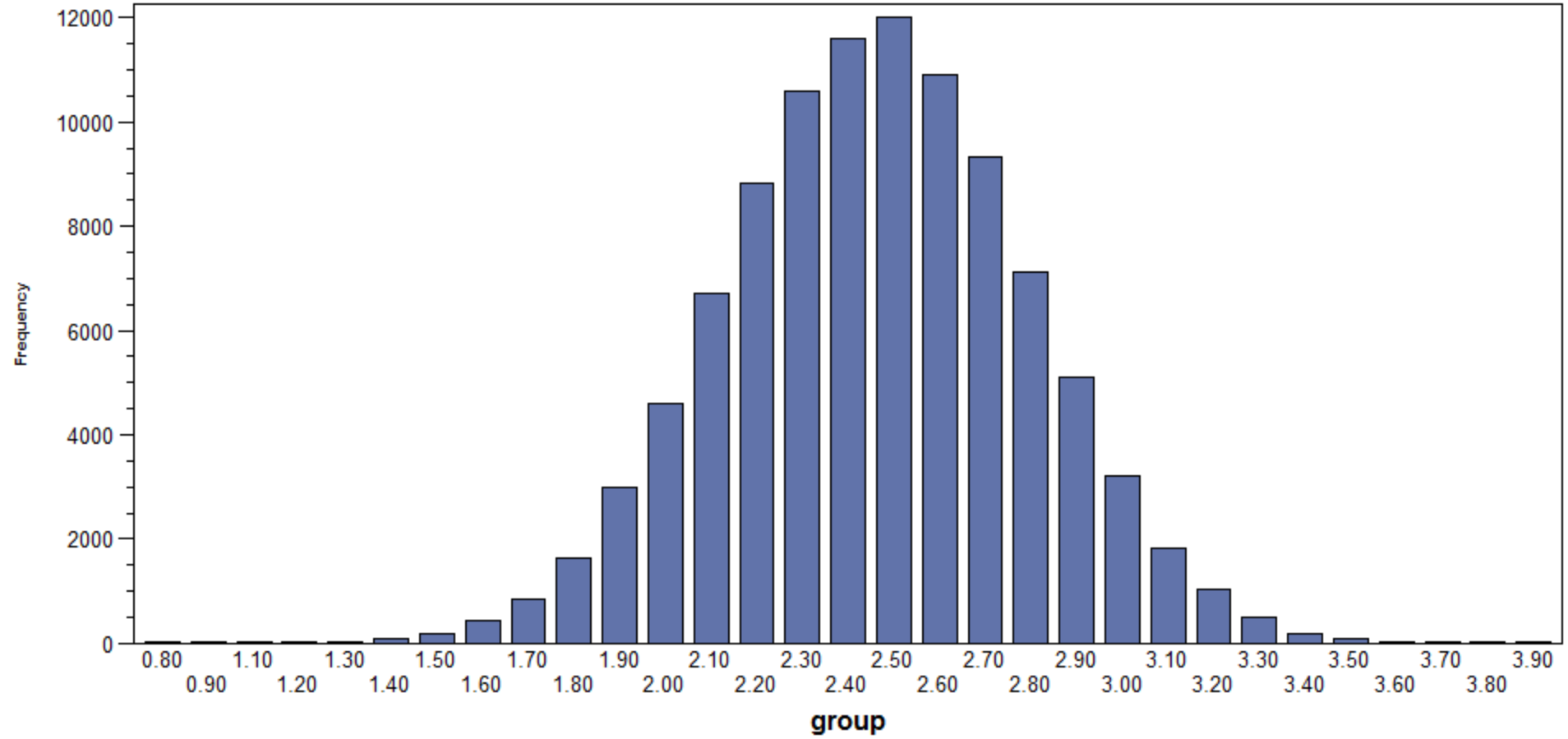
Exercise:

"Variability in CHG Testing"

Simulated Data

100,000 Simulated Scores –

Baseline Mean = 2.51, Std = 0.3318



CHG Data Review - Simulations

Objective: FDA Criteria Evaluation

Question 1: If we use the FDA Criteria of 2.5 Log Reduction and 70% of subjects achieving a 2.5 Log Reduction. How often will studies pass that criteria if we use simulated sample sizes of 6, 12, 18, 24, 30 and 36?

Similarly:
Question 2: If we use the FDA Criteria of 2.0 Log Reduction and 70% of subjects achieving a 2.0 Log Reduction. How often will studies pass that criteria if we use simulated sample sizes of 6, 12, 18, 24, 30 and 36?

CHG Data Review - Simulations

Simulations were done

1000 studies simulated with assumptions of mean value being 2.51 and the variability variable around 0.3318.

1000 studies utilizing sample sizes of 6, 12, 18, 24, etc.

The following graphs display the results of the simulations.

Figure 3–6 1000 Simulated CHG Studies – Tests of panel size 6
True mean 2.51 Std. variable about 0.3318

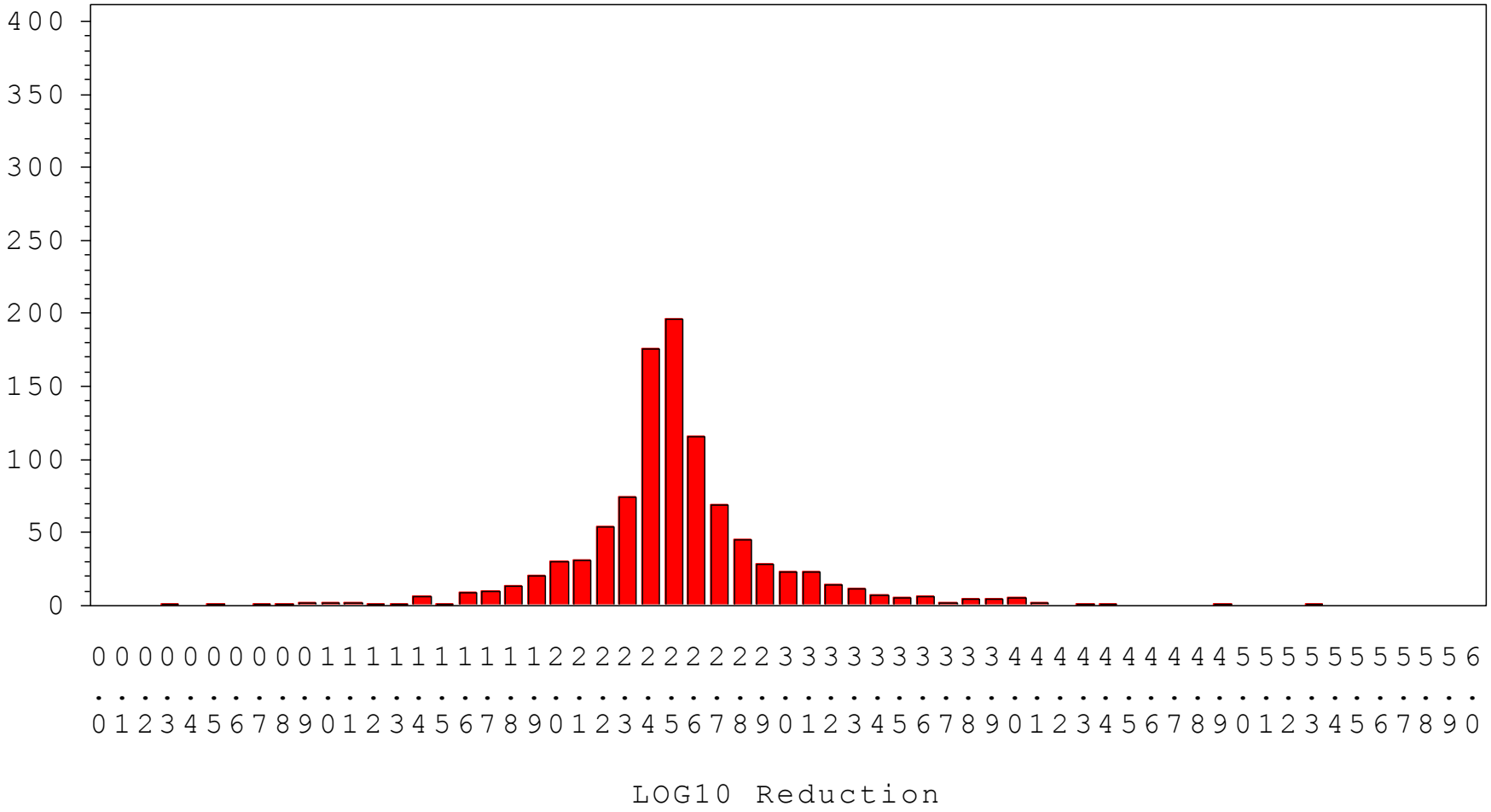


Figure 3–12 1000 Simulated CHG Studies – Tests of panel size 12
True mean 2.51 Std. variable about 0.3318

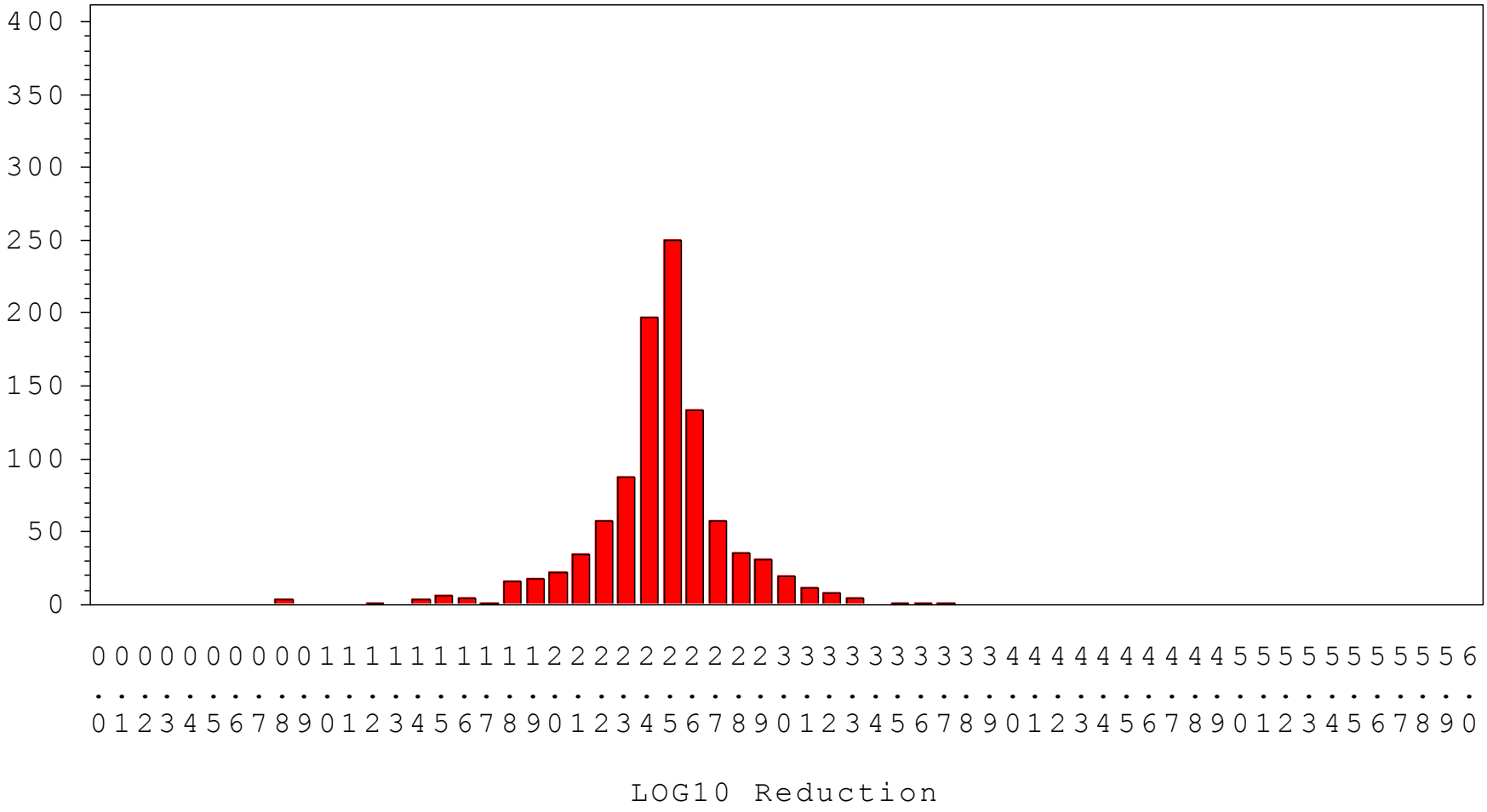


Figure 3–18 1000 Simulated CHG Studies – Tests of panel size 18
True mean 2.51 Std. variable about 0.3318

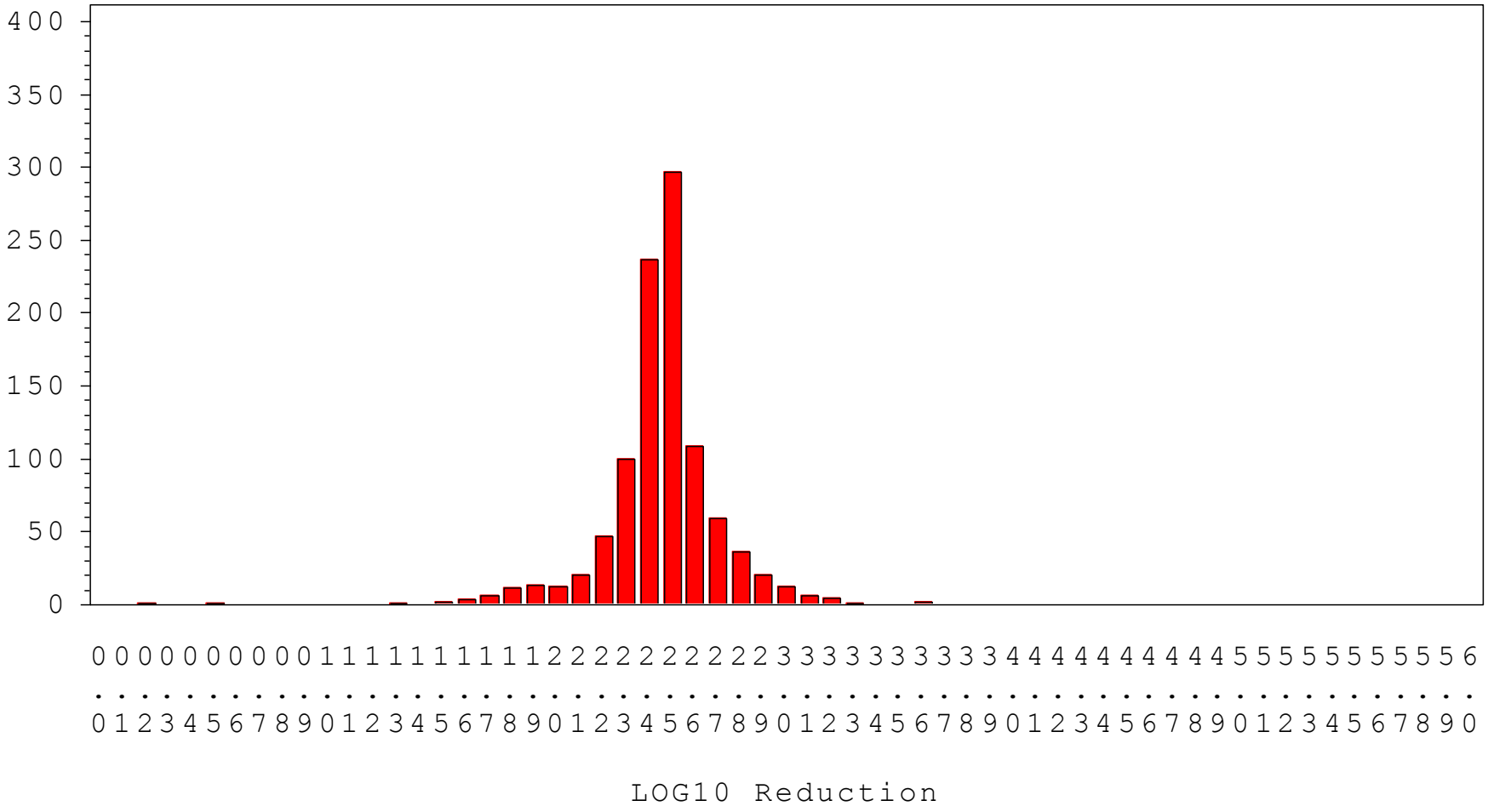


Figure 3–24 1000 Simulated CHG Studies – Tests of panel size 24
True mean 2.51 Std. variable about 0.3318

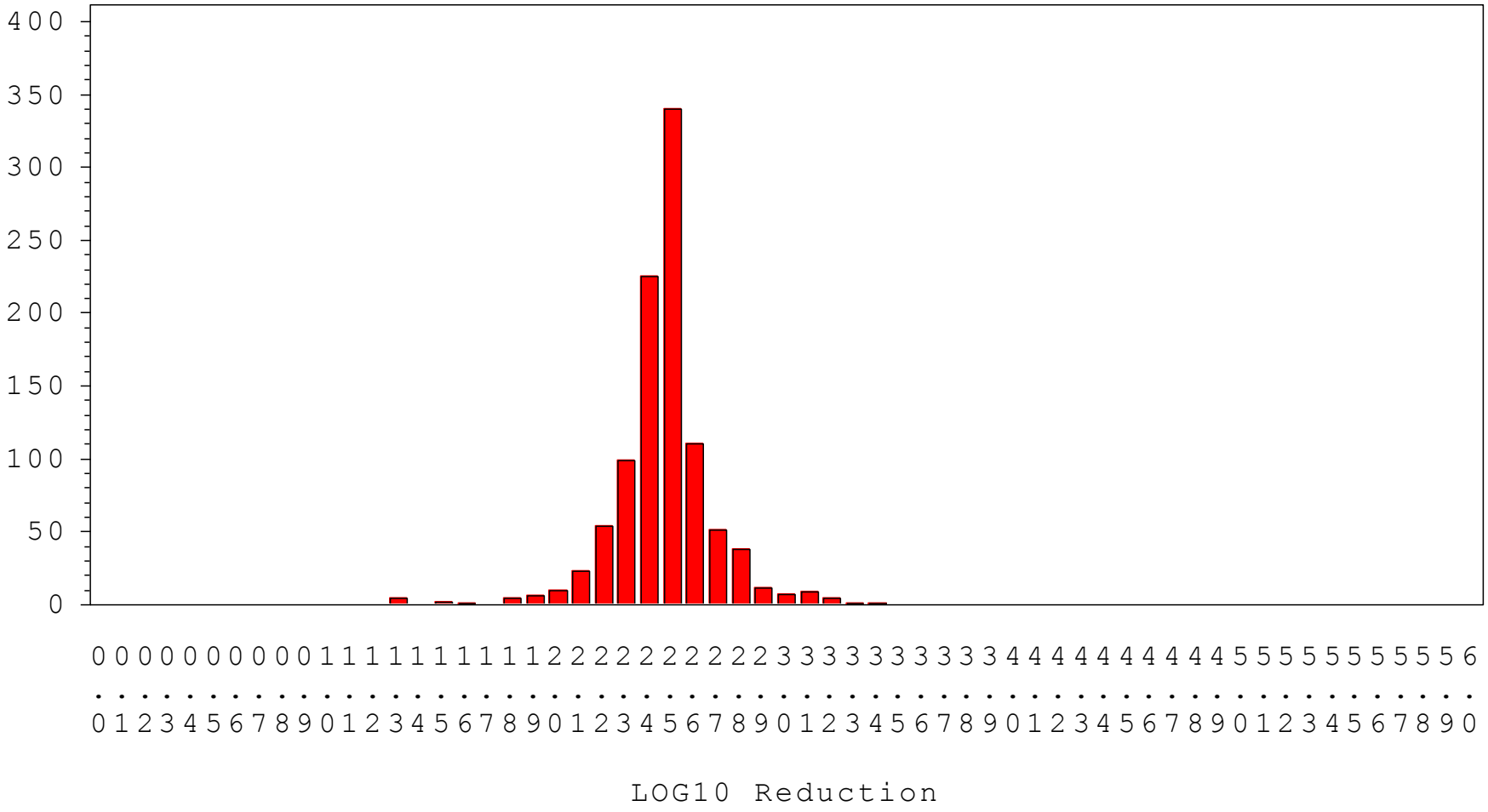


Figure 3 – 30 1000 Simulated CHG Studies – Tests of panel size 30
True mean 2.51 Std. variable about 0.3318

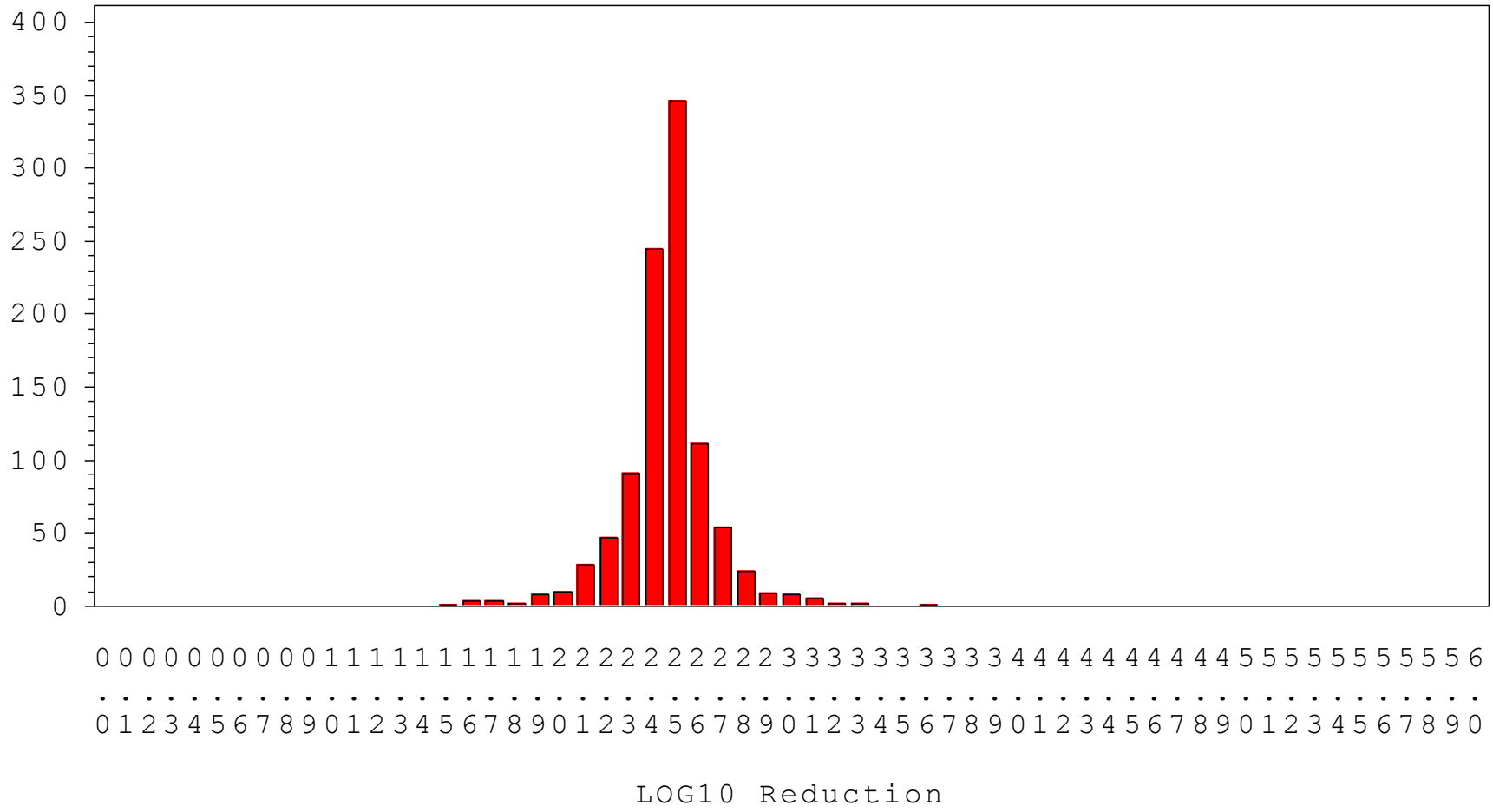
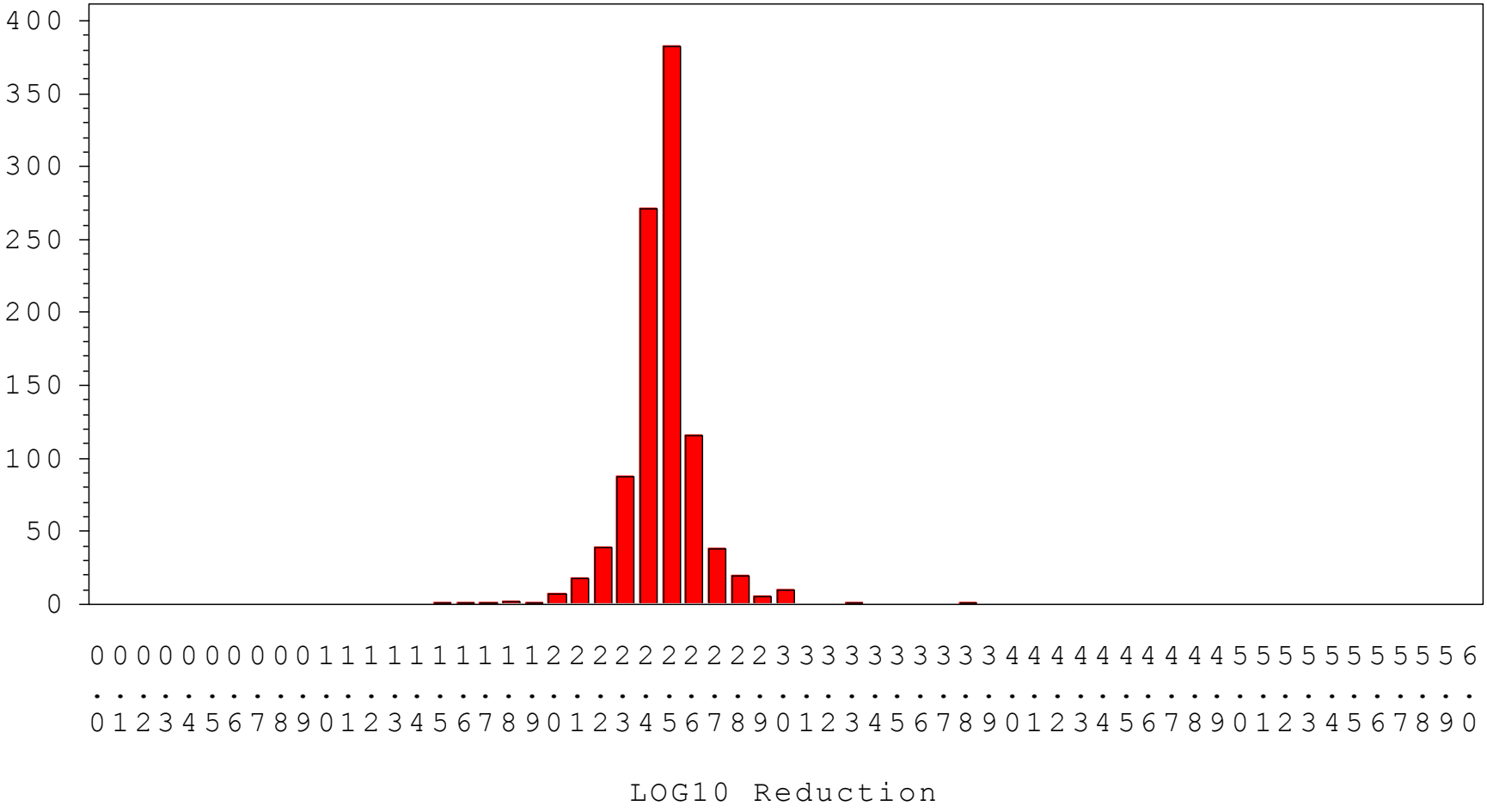


Figure 3 – 48 1000 Simulated CHG Studies – Tests of panel size 48
True mean 2.51 Std. variable about 0.3318



1,000 Simulated Studies

What is the percent of the time that we would have passed the FDA Criteria?

	2.5 Log Reduction	2.0 Log Reduction
N=6	55.6%	91.2%
N=12	53.8%	95.2%
N=18	53.9%	96.0%
N=24	55.2%	98.1%
N=30	58.1%	98.5%
N=36	55.1%	98.9%

CHG Data Review

So, if in fact the mean value for this product is around 2.5 log10 reduction, even using sample sizes as small as 6 test subjects, you will achieve a mean value of greater than 2.0 log10 with greater than 70% of the subjects seeing a 2.0 log10 reduction reduction over 90% of the time (larger percent with larger sample sizes).

However, when using the criteria of 2.5%, for all of the sample sizes, passing the FDA criteria occurs less than 60% of the time.

CHG Data Review

Based on this data, it would seem appropriate to have the criteria for the already approved product control (i.e. CHG) set to be 2.0 log10 reduction with 70% of the individual test subjects achieving a 2.0 log10 reduction.

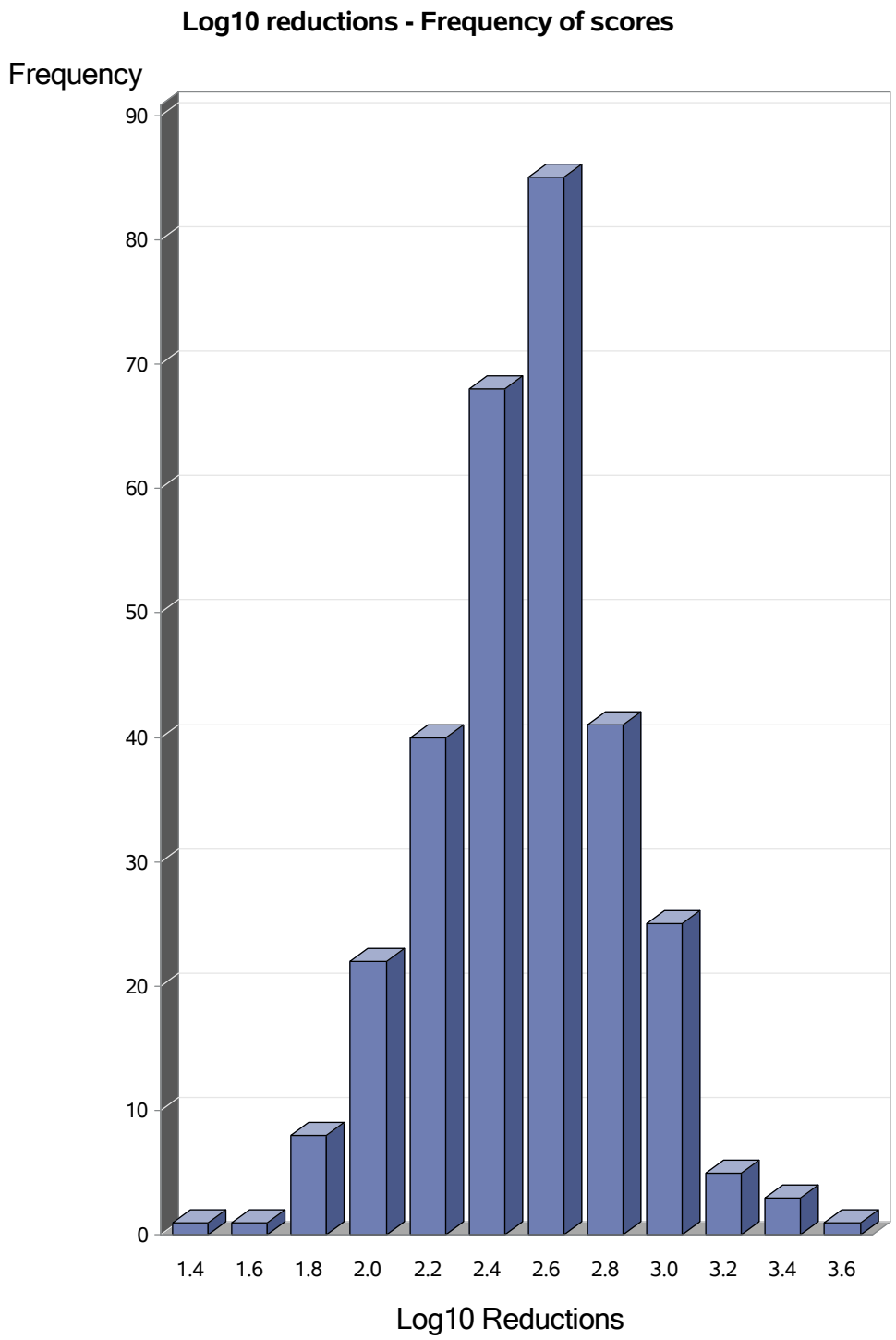
It would seem that sample sizes of 12 would be appropriate.

THANK YOU

Questions?

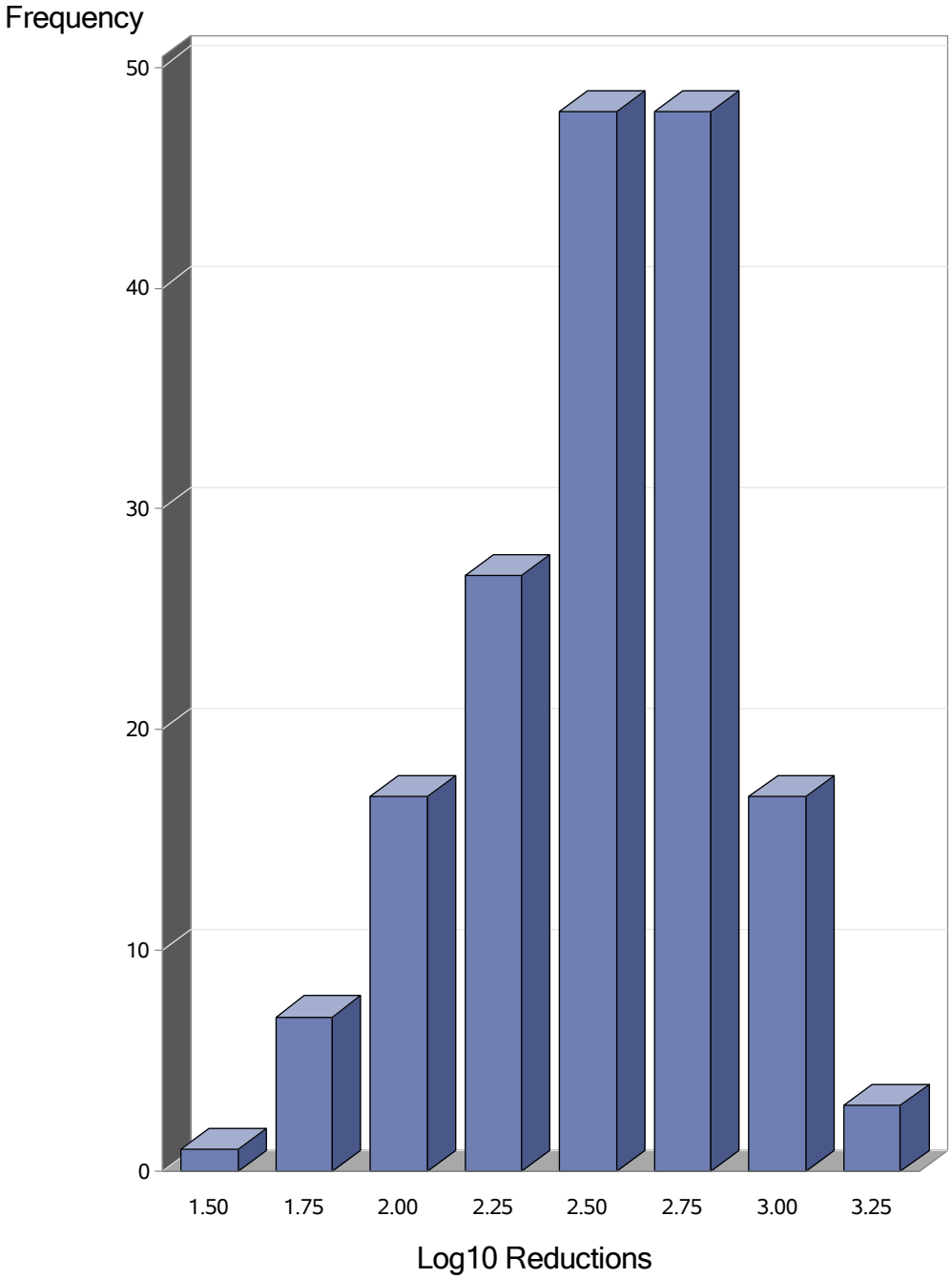
ATTACHMENT 5.3:

HTR AND HENKEL GRAPHS CHG 081815


Log10 reductions - Frequency of scores
Frequency
90
80
70
60
50
40
30
20
10
0
1.4
1.6
1.8
2.0
2.2
2.4
2.6
2.8
3.0
3.2
3.4
3.6
Log10 Reductions

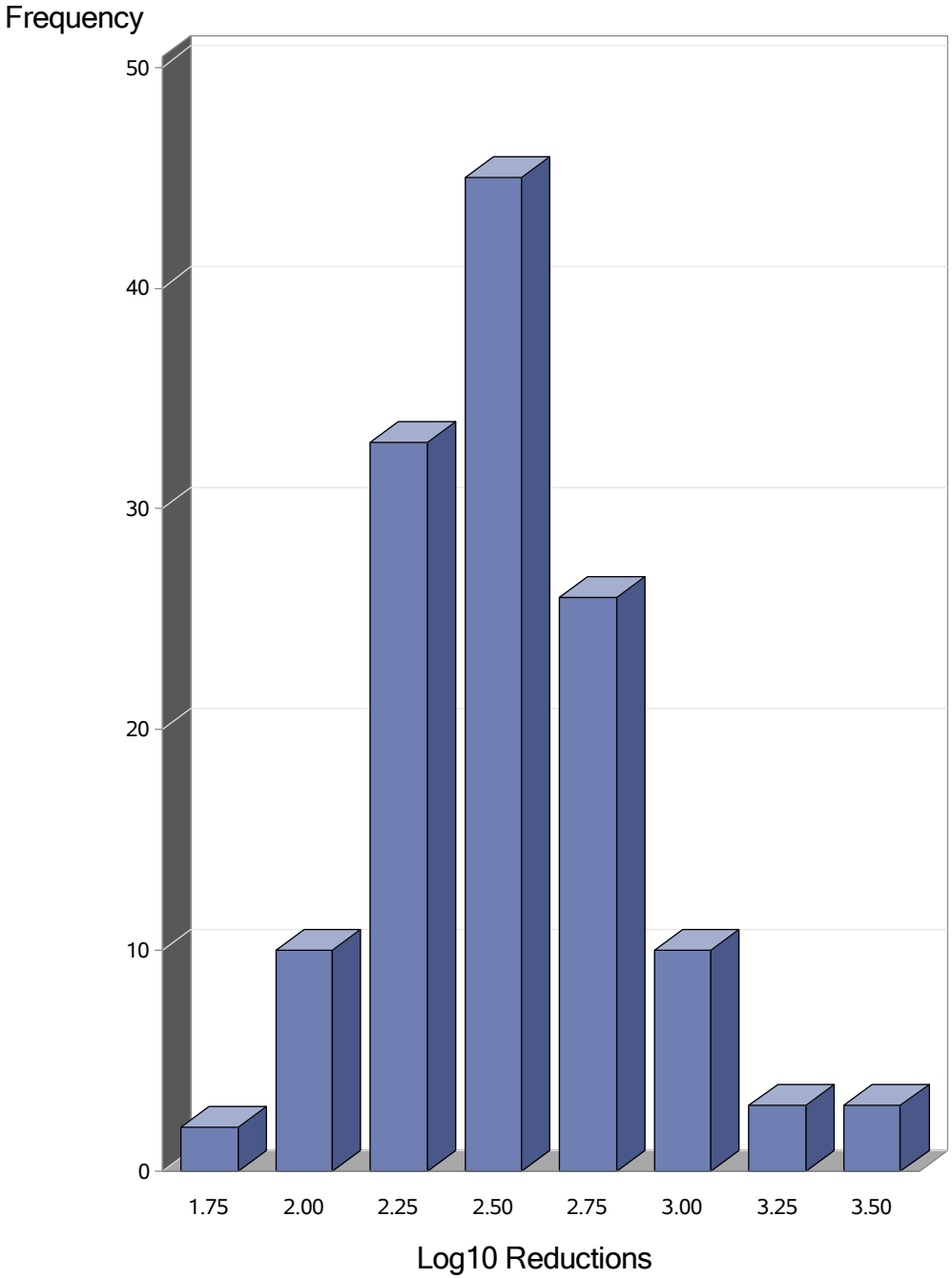
Log10 reductions - Frequency of scores

group=HTR Data

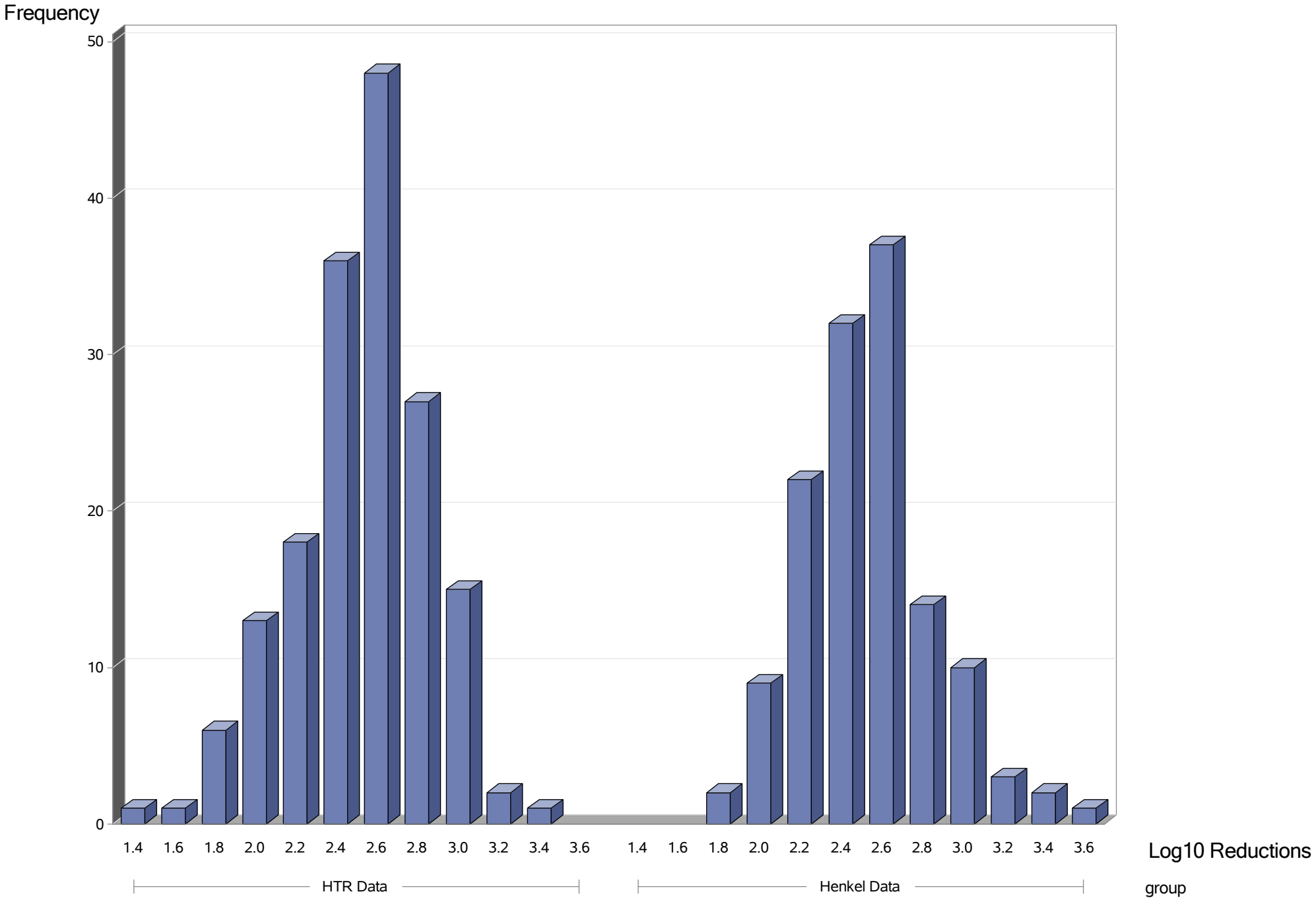


Log10 reductions - Frequency of scores

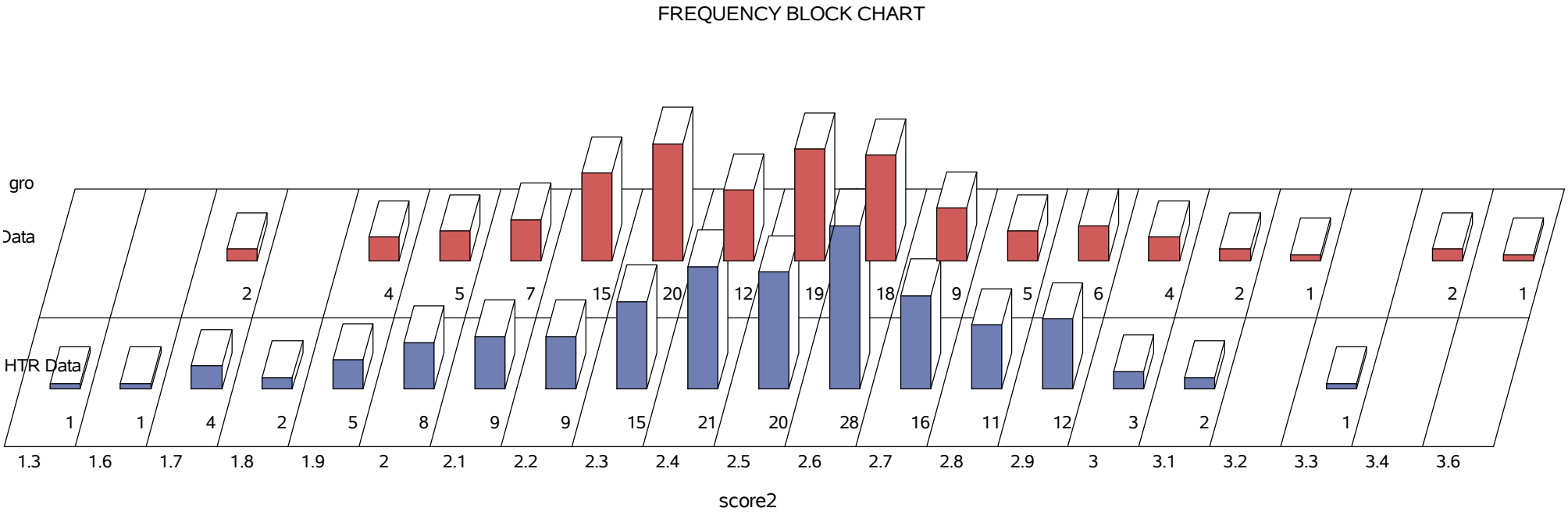
group=Henkel Data



Log10 reductions - Frequency of scores



Log10 reductions - Frequency of scores



ATTACHMENT 6: EVALUATION OF THE ANTIMICROBIAL EFFICACY OF THREE TEST MATERIALS WITH POSITIVE AND NEGATIVE CONTROLS BASED ON THE HEALTH CARE PERSONNEL HAND RUB PROCEDURE

DRAFT PROTOCOL 150943-101

BIOSCIENCES LABORATORIES, INC.

BIOSCIENCE LABORATORIES, INC. DRAFT PROTOCOL 150943-101

EVALUATION OF THE ANTIMICROBIAL EFFICACY OF THREE TEST MATERIALS WITH POSITIVE AND NEGATIVE CONTROLS BASED ON THE HEALTH CARE PERSONNEL HAND RUB PROCEDURE

1.0 TITLE PAGE

Active Ingredients:	Test Material #1 Test Material #2 Test Material #3
Sponsor:	The American Cleaning Institute 1331 L Street N.W. Suite 650 Washington D.C. 20005
Study Number:	150943-101
Sponsor Representative:	Francis H. Kruszewski
Principal Investigator:	To Be Determined
Sub-Investigator:	To Be Determined
Clinical Site:	BioScience Laboratories, Inc. 1765 South 19th Avenue Bozeman, Montana 59718 Telephone: (406) 587-5735
Date:	October 23, 2015

Confidentiality Statement

This document contains the confidential information of The American Cleaning Institute and BioScience Laboratories, Inc. It is intended solely for the guidance of the clinical investigation. This protocol may not be disclosed to parties not associated with the clinical investigation or used for any purpose without the prior written consent of The American Cleaning Institute and BioScience Laboratories, Inc. Permission to release the Protocol and Study Results to the United States Food and Drug Administration (FDA) or other regulatory agency to which this study will be submitted is explicitly granted.

This Protocol has been approved by the GIRB on _____

2.0 PROTOCOL SYNOPSIS

Name of Sponsor: The American Cleaning Institute	**Protocol Number** **150943-101**
Name and Concentration of Active Ingredients: Test Material #1: Test Material #2: Test Material #3: Positive Control: Avagard®, 61% w/w Ethanol Negative Control: Normal Physiological Saline	
Title of Study:	**EVALUATION OF THE ANTIMICROBIAL EFFICACY OF THREE TEST MATERIALS WITH POSITIVE AND NEGATIVE CONTROLS BASED ON THE HEALTH CARE PERSONNEL HANDWASH PROCEDURE**
Principal Investigator:	To Be Determined
Sub-Investigator:	To Be Determined
Study Center:	BioScience Laboratories, Inc.
Publications (References)	ASTM E2755-15, *Standard Test Method for Determining the Bacteria-Eliminating Effectiveness of Healthcare Personnel Hand Rub Formulations Using Hands of Adults* 1994 FDA TFM, 21 CFR Parts 333 and 369, *Health-Care Antiseptic Drug Products*; *Effectiveness testing of an antiseptic handwash or health-care personnel handwash;* Proposed Rule. (FR59: No. 116, 17 June 94. pp 31448 to 31450) 2015 FDA TFM, 21 CFR Part 310, *Safety and Effectiveness of Health Care Antiseptics; Topical Antimicrobial Drug Products for Over-the-Counter Human Use; Proposed Amendment of the Tentative Final Monograph; Reopening of Administrative Record;* Proposed Rule. (FR80: No. 84, 1 May 2015. pp 25166 to 25205)
Study Duration:	A pre-test period of 7 days and a test period of 1 day.
Objectives:	The purpose of this study is to evaluate the antimicrobial efficacy of three test materials with positive and negative controls, for use as Health Care Personnel Handwashes following single test material applications.

This Protocol has been approved by the GIRB on _____

Name of Sponsor: The American Cleaning Institute	
Name and Concentration of Active Ingredients: Test Material #1: Test Material #2: Test Material #3: Positive Control: Avagard®, 61% w/w Ethanol Negative Control: Normal Physiological Saline	**Protocol Number** **150943-101**
Methodology:	The testing methods are based on the standardized test method ASTM E2755-15, *Standard Test Method for Determining the Bacteria-Eliminating Effectiveness of Healthcare Personnel Hand Rub Formulations Using Hands of Adults*, for use as a Health Care Personnel Handwash following a single test material application. Evaluations of the test materials will be made following subjects being inoculated with the test organism in a broth media, and after a single test material application. Samples for baseline and following test material application will be processed for bacterial enumeration. The indicator microorganism will be *Serratia marcescens* (ATCC #14756).
Number of Subjects:	At least 40 subjects will be evaluated per test material for a total of at least 200 subjects tested in a randomized evaluation.
Main Criteria for Inclusion:	A sufficient number of overtly healthy subjects at least 18 years of age will be admitted into the study. Insofar as possible, the groups of subjects selected will be of mixed sex, age, and race. All subjects' hands will be free from clinically evident dermatoses, injuries to the hands or forearms, hangnails, or any other disorders that may compromise the subject or the study.
Duration of treatment:	One of the test materials will be applied by subjects one time.
Criteria for Evaluation:	**Efficacy:** Mean $\log_{10}$ reductions of the indicator microorganism will be used to determine the antimicrobial effectiveness of the test materials. The critical index is mean $\log_{10}$ reductions in microorganisms of ≥ 2.0 $\log_{10}$ within 5 minutes after one application.

This Protocol has been approved by the GIRB on _____

Name of Sponsor: The American Cleaning Institute	
Name and Concentration of Active Ingredients: Test Material #1: Test Material #2: Test Material #3: Positive Control: Avagard®, 61% w/w Ethanol Negative Control: Normal Physiological Saline	**Protocol Number** **150943-101**
	A non-antimicrobial negative control is included to show the contribution of the active ingredients to effectiveness. The test materials should be statistically superior to the negative control for the clinical simulation to be considered successful at showing that the test materials are effective for use in health care antiseptic products. A positive control is included to validate the study conduct to assure that the expected results are produced. For the results to be valid, the positive control should meet the appropriate log reduction criteria.
	Safety: Evaluation for safety of use of the test materials will consist of Adverse Event-reporting and assessment for skin reactions following testing.
Statistical Methods:	Log_{10} reductions from baseline population recovered from each of a subject's hands will be calculated by subtracting the log_{10} number of viable *Serratia marcescens* recovered following test material application from the log_{10} baseline population recovered from that hand. Log_{10} microbial data and population reductions from each of a subject's hands will be presented in tabular form. Statistical calculations of mean and standard deviation will be generated on the log_{10} data from baseline samples, post- test material application samples, and the reductions from baseline. The statistical analysis will determine the portion of subjects who meet the log_{10} reduction criteria based on a two-sided statistical test for superiority to the negative control and a 95 percent confidence interval approach.

This Protocol has been approved by the GIRB on _____

3.0 TABLE OF CONTENTS

SECTION **PAGE**

This Protocol has been approved by the GIRB on _____

This Protocol has been approved by the GIRB on _____

4.0 LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

AIDS	Acquired Immune Deficiency Syndrome
ASTM	American Society for Testing and Materials
ATCC	American Type Culture Collection
BBP++	Butterfield's Phosphate Buffer Solution with product neutralizers
BSLI	BioScience Laboratories, Inc.
CFR	Code of Federal Regulations
CFU	Colony Forming Units
DHHS	Department of Health and Human Services
FR	Federal Register
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GIRB	Gallatin Institutional Review Board
HIPAA	Health Insurance Portability and Accountability Act
HIV	Human Immunodeficiency Virus
ICH	International Conference on Harmonisation
PBS	Phosphate Buffered Saline
SAE	Serious Adverse Event
SSF	Stripping Suspending Fluid
SFF++	Stripping Suspending Fluid with neutralizers / 10% Tween
TFM	Tentative Final Monograph
TSA	Tryptic Soy Agar
TSA+	Tryptic Soy Agar with neutralizers
TSB	Tryptic Soy Broth
UV	Ultraviolet
Glove Juice Sampling Procedure	A procedure used to sample bacteria from the hands using a sterile glove and a specified volume of sampling fluid.

Mean log_{10} reductions	Average of the differences between the baseline microbial populations expressed as log_{10} CFU/hand and the populations in log_{10} CFU/hand recovered from the post-application samples.
Source Documents	Recorded results of original observations and activities of a clinical investigation.
Subject	Healthy human paid participant that has consented to test in the study.
Test Material	The test material, negative control or positive control that are to be tested according to procedures in this protocol.
Negative Control	A material that is to be tested according to procedures in this protocol with no active ingredient.
Positive Control	A product that is to be tested according to procedures in this protocol in order to validate the testing procedures and to be used as a control.

5.0 ETHICS

5.1 Institutional Review Board

Informed Consent Forms and any other supportive material relevant to the safety of the subjects will be supplied to the Gallatin Institutional Review Board (GIRB) for their review and approval. The primary purpose of the GIRB is the protection of the rights and welfare of the subjects involved (reference CFR 21, Parts 50, 56, 312, and 314). This study will begin only after GIRB approval has been obtained.

5.2 Ethical Conduct of Study

The study will be conducted in compliance with the Good Laboratory Practice standards (21 CFR Part 58) and Good Clinical Practice standards (21 CFR Parts 50, 56, 312, and 314, and ICH E6), the United States Food and Drug Administration regulations, Standard Operating Procedures of BioScience Laboratories, Inc., the study protocol, and any protocol amendments.

5.3 Subject Information and Consent

The Informed Consent Form, Authorization to Use and Disclose Protected Health Information Form, and List of Restricted Products will be provided to each subject prior to beginning the study. Trained personnel will explain the study to each subject and will be available to answer any questions that may arise.

6.0 INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

BioScience Laboratories, Inc.
1765 South 19th Avenue.
Bozeman, Montana 59718

Contact: To Be Determined
Phone: (406) 587-5735 Ext. XXX
Fax: (406) 586-7930

Principal Investigator: To Be Determined

Sub-Investigator: To Be Determined

Quality Assurance Monitor: Amy L. Juhnke, RQAP-GLP

Statistical Consultant: Daryl S. Paulson, Ph.D.

Subject Recruitment and Consenting: Chelsey Allison

Consulting Medical Experts: Gabor Benda, M.D. and David McLaughlin, M.D.

Gallatin Institutional Review Board (GIRB)
3006 Secor Avenue
Bozeman, Montana 59715
Phone: (406) 581-8559
DHHS Number: IRB00005939

6.1 Monitoring

The American Cleaning Institute, as Sponsor of this study, is responsible for ensuring the proper conduct of the study with regard to protocol adherence and validity of the data recorded on the study documents. The American Cleaning Institute has therefore assigned a study monitor to this study. The progress of the study may be monitored by:

- Periodic on-site review
- Telephone communications
- E-mail communications
- Review of sample data sheets and source documents

The Investigator will give The American Cleaning Institute, study monitor direct access to source documents that support data on the study documents and make available such records to authorized The American Cleaning Institute personnel, quality assurance, IRB, and regulatory personnel for inspection and/or copying.

Note: The Federal Privacy rule (HIPAA) specifically permits the use and disclosure of protected health information "to a person subject to the jurisdiction of the Food and Drug Administration (FDA) [e.g. study sponsor] with respect to an FDA-related product or activity for which that person has responsibility, for the purpose of activities related to the quality, safety, or effectiveness of such FDA-regulated product or activity" [45 CFR 164.512(b)(1)(iii)].

This Protocol has been approved by the GIRB on _____

7.0 INTRODUCTION

Healthcare Personnel, as a standard of care, wash their hands with an antiseptic handwash and/or apply a leave-on hand antiseptic prior to and following performing care of a patient. The proposed Tentative Final Monograph (TFM) for *Health-Care Antiseptic Drug Products* (Vol. 59, No. 116, June 17, 1994) describes an *in-vivo* procedure for evaluating these types of test materials, as well as expected performance criteria. The 2015 FDA TFM, 21 CFR Part 310, *Safety and Effectiveness of Health Care Antiseptics; Topical Antimicrobial Drug Products for Over-the-Counter Human Use; Proposed Amendment of the Tentative Final Monograph; Reopening of Administrative Record;* Proposed Rule now proposes that additional safety data are necessary to support the safety of antiseptic active ingredients for these uses. The new effectiveness criteria described in the 2015 proposed rule includes mean $\log_{10}$ reductions in microorganisms of ≥ 2.5 $\log_{10}$ within 5 minutes after a single application.

8.0 STUDY OBJECTIVES

The purpose of this study is to evaluate the antimicrobial efficacy of three test materials with positive and negative controls, for use as Health Care Personnel Handwashes following single test material applications.

9.0 INVESTIGATIONAL PLAN

9.1 Overall Study Design and Plan

This study is designed to determine the antimicrobial effectiveness of three test materials (Test Materials #1, #2, and #3), and positive and negative controls intended for use as Healthcare Personnel Handwashes. At least 40 subjects will be evaluated per test material for a total of at least 195 subjects tested in a randomized evaluation. Evaluations of the test materials will be made following subjects being inoculated with the test organism in a broth media, and after a single test material application. Samples for baseline and following test material application will be processed for bacterial enumeration. The indicator microorganism will be *Serratia marcescens* (ATCC #14756). The testing methods are based on the standardized test method ASTM E2755-15, *Standard Test Method for Determining the Bacteria-Eliminating Effectiveness of Healthcare Personnel Hand Rub Formulations Using Hands of Adults*. Mean $\log_{10}$ reductions of the indicator microorganism will be used to determine the antimicrobial effectiveness of the test materials. The critical index is mean $\log_{10}$ reductions in microorganisms of ≥ 2.0 $\log_{10}$ after one application.

9.2 Discussion of Study Design, Including Choice of Sample Size

The sample size for this study was determined using the following formula:

$$\mathbf{N} \geq \frac{5S^2\left(Z_{\alpha/2} + Z_{\mathrm{B}}\right)^2}{D^2}$$

Where:

- N= Sample size per test material configuration arm
- S= Estimate of variance =0.50
- $Z_{\alpha/2}$= 0.05 level of significance (two-tail) = 1.96, Type I error (probability of stating a significant effect exists when one does not)
- Z_{β}= 0.842 level of significance for Type II (beta) error (probability of stating no significant effect exists when one does)
- D= Detectable difference (sensitivity) = 0.5

$$\mathbf{N} \geq \mathbf{5}\left(\frac{(0.50)^2\,(1.96 + 0.842)^2}{0.5^2}\right) = 39.25$$

At least 40 subjects will be evaluated per test material for a total of at least 200 subjects tested in a randomized evaluation.

9.3 Selection of Study Population

A sufficient number of overtly healthy subjects at least 18 years of age will be admitted into the study to ensure that at least 200 subjects complete the study. Insofar as possible, the groups of subjects selected will be of mixed sex, age, and race. All subjects' hands will be free from clinically evident dermatoses, injuries to the hands or forearms, open wounds, hangnails, and/or any other disorders that may compromise the subject and the study. All subjects will sign the Informed Consent Form, Authorization to Use and Disclose Protected Health Information Form, and List of Restricted Products prior to participating in the study. The above forms are provided as separate Informed Consent documents.

An Informed Consent Form, Authorization to Use and Disclose Protected Health Information Form, and List of Restricted Products will be provided to each subject prior to beginning the study. Trained personnel will explain the study to each participant and will be available to answer any questions that may arise.

9.3.1 Inclusion Criteria

- Subjects may be of either sex, at least 18 years of age, and of any race.
- Subjects must possess both hands and all ten digits.
- Subjects must be in good general health.
- Subjects must have read and signed an Informed Consent Form, Authorization to Use and Disclose Protected Health Information Form, and List of Restricted Products prior to participating in the study, all located in the separate Informed Consent documents.

9.3.2 Exclusion Criteria

- Known allergies to sunscreens, deodorants, laundry detergents, fragrances, latex (rubber), alcohols, to common antibacterial agents found in soaps or lotions, particularly Test Material active ingredients or chlorhexidine gluconate, or to topical antibiotic ointments (e.g., Neosporin® or Polysporin® [neomycin/bacitracin/polymyxin B]).
- Exposure of ungloved hands or forearms to antimicrobial agents, medicated soaps, medicated shampoos (e.g., anti-dandruff), hair mousses, or medicated lotions, during the 7-day pre-test conditioning period or on the single test day.
- Use of biocide-treated pools or hot tubs, or use of UV tanning beds or sunbathing during the 7-day pre-test conditioning period or on the single test day.
- Exposure of ungloved hands or forearms to strong detergents, solvents, or other irritants during the 7-day pre-test conditioning period or on the single test day.
- Use of systemic or topical antibiotic medications, during the 7-day pre-test conditioning period or on the single test day.
- Use of systemic or topical steroids other than for contraception or post-menopausal indications during the 7-day pre-test conditioning period or on the single test day. This includes steroid medications used to treat asthma.
- Application or presence of nail polish, artificial nails, or nail polish remover, or having undergone nail treatments during the 7-day pre-test conditioning period or on the single test day.
- A medical diagnosis of a physical condition, such as a current or recent severe illness, medicated or uncontrolled diabetes, hepatitis B, hepatitis C, an organ transplant, a heart murmur, mitral valve prolapse with heart murmur, congenital heart disease, an immunocompromised condition such as AIDS (or HIV positive), lupus, or medicated multiple sclerosis.
- Any prosthetic device in the neck or spine including pins, screws, plates, or rods.

- Any prosthetic joints (movable parts of the body).
- Any pins, screws, plates, or rods installed within the last 6 months.
- Any type of port (or portacath).
- Pregnancy, plans to become pregnant or impregnate a sexual partner within the pre-test or on the single test day, or nursing a child. Females must continue to take birth control precautions one month following the last day of testing. Males must continue to take birth control precautions through one week following the last day of testing.
- Any active skin rashes, dermatoses, hangnails, or breaks in the skin of the hands or forearms; skin blemishes such as dry scabs or warts may be permissible, with the specific approval of the Principal Investigator or consulting physician.
- An inflammatory skin condition, such as dermatitis, eczema, or psoriasis, anywhere on the body, that in the opinion of the Principal Investigator or consulting physician should preclude participation.
- Participation in a clinical study in the past 7 days or current participation in another clinical study.
- Any medical condition or use of any medications that, in the opinion of the Principal Investigator or consulting physician, should preclude participation.
- Unwillingness to fulfill the performance requirements of the study.

9.3.3 Subject Withdrawal

After admission to the study, the subject may withdraw at any time for any reason. If possible, the reason for withdrawal will be recorded. Any subject not adhering to Protocol requirements will be disqualified.

9.4 Test Methods

9.4.1 Equipment, Supplies, Test Solutions and Media

The equipment used during this study will be detailed on Clinical Trials Equipment Tracking Forms (Form No. 01-L-009), and the forms will be included in the Final Report.

The supplies used during this study will be detailed on Clinical Trials Supplies Tracking Forms (Form No. 01-L-008), and the forms will be included in the Final Report.

The Test Solutions and Media used for this study are listed below:

Sampling Solution

Stripping Suspending Fluid (SSF)

Stripping Suspending Fluid with product neutralizers (SSF++)

Neutralizing/Diluting Fluid

Butterfield's Phosphate Buffer Solution with Product Neutralizers (BBP++)

Media

Tryptic Soy Agar (TSA) for Inoculum Preparation and Neutralization Study

Tryptic Soy Agar with product neutralizers (TSA+)

Tryptic Soy Broth (TSB) for Inoculum Preparation

Phosphate Buffered Saline Solution (PBS) for Neutralization Study

Soft Soap (per ASTM E1174-13):

Ingredients:

Linseed oil (50 parts by weight)

Potassium hydroxide (9.5 parts)

Ethanol (7 parts)

Distilled or high purity water (as needed)

Linseed oil will be added to a solution of potassium hydroxide in 15 parts water and heated to approximately 70 °C while constantly stirring.

Ethanol will be added and heating continued while stirring until the saponification process is completed and a sample dissolves clearly in water and almost clearly in alcohol. The weight of the Soft Soap will then be brought up to 100 parts by addition of hot water. 200 g of the soft soap will be diluted in 1 L of water. The diluted Soft Soap will then be dispensed into appropriate containers and sterilized in an autoclave.

9.4.2 Identity of Test Materials

The test materials will be provided to the Testing Facility by the Study Sponsor, complete with appropriate documentation. The test materials will be received and stored by BioScience Laboratories, Inc. (BSLI) in accordance with instructions from the Sponsor and retained in secure quarantine when not being used in testing. BSLI will maintain an inventory of the test materials and a log of use. Responsibility for determination of the identity, strength, purity, composition, stability, and solubility of the test materials, as well as responsibility for retention of the test materials rests with the Sponsor. Unused, sealed test and control materials will be stored by BSLI until the Sponsor specifies their disposition. In the absence of a disposition request from the Sponsor within 1 year of their planned usage, the test materials will be returned to the Sponsor. No test materials or control materials will be destroyed unless so requested by the Sponsor. If the test material names and lot numbers are not stated below, they will be provided by the Sponsor prior to the start of testing.

Test Material #1: To Be Determined
Active Ingredient: _____
Lot Number: _____
Expiration Date: _____
Manufacture Date: _____

Test Material #2: To Be Determined
Active Ingredient: _____
Lot Number: _____
Expiration Date: _____
Manufacture Date: _____

Test Material #3: To Be Determined
Active Ingredient: _____
Lot Number: _____
Expiration Date: _____
Manufacture Date: _____

Positive Control: Avagard®
Active Ingredient: 61% w/w Ethyl Alcohol
Lot Number: _____
Expiration Date: _____
Manufacture Date: _____

Negative Control: Normal physiological saline
Active Ingredient: N/A
Lot Number: _____
Expiration Date: _____
Manufacture Date: _____

9.4.3 Method of Assigning Subjects to Treatment Groups

Subjects will be assigned randomly to test with one, and only one, of the five test materials.

9.4.4 Inoculum Preparation

Serratia marcescens (ATCC #14756) will be used to challenge the efficacy of the test materials.

A stock culture of *Serratia marcescens* (ATCC #14756) will be prepared by aseptically transferring a cryogenic stock culture or contents of a lyophilized vial to 5 mL of sterile Tryptic Soy Broth (TSB), which will then be incubated at 35 °C ± 2 °C for 25 hours ± 1 hour.

One or more 500-mL flasks, each containing approximately 125 mL of TSB, will be inoculated with approximately 1 mL of the 24-hour broth culture, placed on a platform shaker set at approximately 250 rpm, and incubated for 25 hours ± 1 hour at 35 °C ± 2°C.

10.0-mL aliquots from each flask will be dispensed into sterile graduated centrifuge tubes and centrifuged at 4750 rpm ± 50 rpm for 30 minutes or until sedimentation is complete. The supernatant will be decanted, and the pellet brought back to a volume of 1.0 mL using TSB. The tubes will be vortexed thoroughly and pooled into one container prior to use. The resulting population will be ~1 x 10^{10} CFU/mL

Prior to any withdrawal of culture, whether for hand contamination or for numbers assay, the suspension will be stirred or swirled. The suspension will be assayed for number of organisms at the beginning and end of the use period. A suspension will not be used for more than 8 hours.

9.4.5 Pre-Test Conditioning Period

The 7 days prior to the test portion of the study will constitute the pre-test conditioning period. During this time, subjects will avoid the use of medicated soaps, lotions, deodorants and shampoos, as well as skin contact with solvents, detergents, acids and bases, or any other products known to affect the normal microbial populations of the skin. Subjects will be supplied a personal hygiene kit containing nonmedicated soap, shampoo, lotion, and rubber gloves to be worn when contact with antimicrobials, solvents, detergents, acids, or bases cannot be avoided. Subjects will be instructed to use the contents of this kit exclusively during their participation in the study. Subjects must also avoid using UV tanning beds or sunbathing, and swimming or bathing in biocide-treated pools or hot tubs.

9.4.6 Test Period

Each subject will be in testing for 1 to 2 hours on a single day. Prior to being admitted into testing, subjects will be questioned regarding their adherence to the Protocol requirements. Subjects will clip their fingernails to a free edge of ≤ 1 mm, if they have not already done so. All jewelry will be removed from the hands and arms prior to washing.

Subjects will perform a practice hand-contamination, but using tap water instead of the challenge suspension and not performing an air-dry. A 0.2-mL aliquot of tap water will be transferred into each subject's cupped hands. The tap water will then be distributed evenly over both hands, not reaching above the wrists, via gentle continuous massage.

A 30-seconds ± 5 seconds handwash using 5 mL Soft Soap and a 30-second rinse will be performed to remove dirt and oil from the hands. The temperature of the water used for this and subsequent wash procedures will be controlled at 40 °C ± 2 °C. The technician will hand the subject a paper towel and instruct them to lightly pat their hands and forearms dry. The subject will wait at least five minutes before the next step of the procedure.

A 0.2 mL aliquot of the inoculum suspension containing approximately 1 x 10^{10} CFU/mL *Serratia marcescens* (ATCC #14756) will be transferred into the subject's cupped hands. The inoculum will be distributed evenly over both hands, not reaching above the wrists, via gentle continuous massage for 30 seconds ± 5 seconds.

After the timed 30 seconds ± 5 seconds massage, the Glove Juice Sampling Procedure (Section 9.4.6.2) will be performed. This first contamination cycle will provide the baseline population level. It will be followed by a 30 seconds ± 5 seconds handwash using 5 mL Soft Soap and a 30 seconds ± 5 seconds rinse, a paper towel dry and at least five minute wait before the next step of the procedure.

A 0.2 mL aliquot of the inoculum suspension will again be evenly distributed over both hands, and the subject will apply their randomly assigned test or control material according to one of the application procedures (Section 9.4.6.1).

The hands will be sampled for residual Serratia marcescens (ATCC #14756) after contamination/test material application, without drying the hands with paper towels. All samples will be performed using the Glove Juice Sampling Procedure (Section 9.4.6.2).

9.4.6.1 Application Procedure:

A volume of test material indicated in the table below will be dispensed into subject's cupped dry hands taking care to avoid loss of test material.

Test Material	**Test Material Identity**	**ApplicationVolume**
Test Material #1	To Be Determined	1.5 – 2.0 mL
Test Material #2	To Be Determined	1.5 – 2.0 mL
Test Material #3	To Be Determined	1.5 – 2.0 mL
Positive Control	Avagard®, 61% w/w Ethanol	1.5 – 2.0 mL
Negative Control	Normal saline	1.5 – 2.0 mL

Subjects will rub the test material over the entire surface of the hands and fingers paying special attention to nails and nail beds, and continue to rub into the skin until dry.

9.4.6.2 Glove Juice Sampling Procedures

Within 1 minute after contamination for baseline, the subject's hands will be placed into powder-free, sterile synthetic gloves while still wet from the inoculum, and 75.0 mL sterile SSF will be instilled into each of the gloves.

Within 1 minute after the test or control material application procedure the subject's hands will be placed into powder-free, sterile synthetic gloves, and 75.0 mL sterile SSF++ will be instilled into each of the gloves.

Within 1 minute of donning the gloves, the wrists will be secured, and technicians will massage the hands through the gloves in a standardized manner for 60 seconds. Within 1 minute of completing the massage following sampling for baseline and after test material application a 5.0 mL aliquot of the solution will be removed from each of the gloves and diluted separately in 5.0 mL BBP++ (dilution 10^0). The 10^0 dilutions will then be serially diluted in BBP++.

9.4.7 Blinding

In order to ensure blinded microbiologists, technicians who participate in test material application or the collection of samples from subjects during test material testing will not participate in plating samples and/or counting plates from samples collected from subjects after test material testing.

9.4.8 Subject Safety

Subjects will not be allowed to leave the laboratory for any reason once testing begins, except in an emergency. Additionally, subjects will be required to wear protective garments and not touch their clothing, faces, or any other body parts with their hands during the test period.

On completion of testing, subjects will be required to perform a 1-minute rinse with 70% ethanol and air-dry, followed by a supervised 4-minute wash with a 4% chlorhexidine gluconate solution. A topical antibiotic ointment will be applied to the hands and forearms following the decontamination procedure.

An antibiotic sensitivity profile for the *Serratia marcescens* (ATCC #14756) used in this study will be retained on file at BSLI.

9.4.9 Plating

Duplicate spiral and/or spread plates on TSA+ will be prepared from appropriate dilutions. The plates will be incubated at 25 °C ± 2 °C for approximately 48 hours, or until sufficient growth is observed. *Serratia marcescens* (ATCC #14756) will produce red colonies, and only those colonies will be counted. If colonies on one of the plates are uncountable, the count from the remaining plate will be used.

9.5 Efficacy and Safety Variables

9.5.1 Efficacy and Safety Measurements and Flow Chart

Mean $\log_{10}$ reductions of the indicator microorganism will be used to determine the antimicrobial effectiveness of the test materials.

Subject safety will be monitored by adverse event reporting (Section 11).

Flow Chart

Procedure	Day							
	-7 or more	-6	-5	-4	-3	-2	-1	0
Informed Consent Provided	X							
Pre-Test Conditioning Period	X	X	X	X	X	X	X	
Inclusion/Exclusion Criteria Reviewed								X
Begin Testing								X
Baseline Sample								X
Test Material Application								X
Post-Application Sample								X
Decontamination Procedure								X

9.5.2 Appropriateness of Measurements

The testing methods are based on the standardized test method ASTM E2755-15, *Standard Test Method for Determining the Bacteria-Eliminating Effectiveness of Healthcare Personnel Hand Rub Formulations Using Hands of Adults*, for use as a Health Care Personnel Handwash following a single test material application. The critical index is mean $\log_{10}$ reductions in microorganisms of ≥ 2.5 $\log_{10}$ within 5 minutes after one application, as recommended by the 2015 FDA TFM, 21 CFR Part 310, *Safety and Effectiveness of Health Care Antiseptics; Topical Antimicrobial Drug Products for Over-the-Counter Human Use; Proposed Amendment of the Tentative Final Monograph; Reopening of Administrative Record;* Proposed Rule.

9.5.2.1 Neutralization

A neutralization study will be performed to assure that the neutralizers used in the recovery medium quench the antimicrobial activity of each test material and are not toxic to the challenge species. Study procedures are based on ASTM E1054-08(13), *Standard Test Methods for Evaluation of Inactivators of Antimicrobial Agents*. *Serratia marcescens* (ATCC #14756) will be used as the challenge species in the neutralization study.

9.5.3 Primary Efficacy Variables

Mean $\log_{10}$ reductions of the indicator microorganism will be used to determine the antimicrobial effectiveness of the test materials. The critical index is mean $\log_{10}$ reductions in microorganisms of ≥ 2.5 $\log_{10}$ within 5 minutes after one application.

A non-antimicrobial negative control is included to show the contribution of the active ingredients to effectiveness. The test materials should be statistically superior to the negative control for the clinical simulation to be considered successful at showing that the test material is effective for use in health care antiseptic products.

A positive control is included to validate the study conduct to assure that the expected results are produced. For the results to be valid, the positive control should meet the appropriate log reduction criteria.

An analysis will be performed of the proportion of subjects who meet the log reduction criteria based on a two-sided statistical test for superiority to the negative control and a 95 percent confidence interval approach.

9.5.4 Data Collection and Microbial Recoveries

Colonies will be counted and data recorded using a computerized plate-counting system. If 10^0 spiral plates give an average count of zero, the average plate count will be expressed as 1.00×10^1.

The plate count data collected from this study will be evaluated using MiniTab® statistical computer software.

The estimated $\log_{10}$ number of viable microorganisms recovered from each hand will be designated the "*R*-value." It is the adjusted average $\log_{10}$ colony count measurement at each sampling time. Each *R*-value will be determined using the following formula:

$$R = \log_{10} [75 \times C_i \times 10^{-D} \times 2]$$

where:

75 = the amount (mL) of stripping solution instilled into each glove
C_i = the arithmetic average colony count of the two plate counts for each subject at a particular dilution level
D = the dilution factor
2 = the neutralization dilution

The $\log_{10}$ transformation is performed on these data to convert them to a linear scale. A linear scale, more appropriately a $\log_{10}$ linear scale, is a requirement of the statistical models to be used.

9.6 Data Quality Assurance

The Sponsor's designated Quality Assurance Representative may conduct audits at the study site. Audits will include, but are not limited to test materials, presence of required documents, the informed consent process, and review of source documents. The Investigator agrees to participate with audits conducted at a reasonable time in a reasonable manner.

The study will be inspected by the Quality Assurance Unit at BioScience Laboratories, Inc., and reports will be submitted to the Principal Investigator and Management in accordance with Standard Operating Procedures.

9.7 Statistical Methods and Determination of Sample Size

9.7.1 Statistical and Analytical Plans

The MiniTab® 17 Statistical Computer Package will be used for all statistical calculations.

A one-factor Analysis of Variance (ANOVA) model will be used to determine differences. It will have the form:

$$\hat{y} = A + e$$

where:

$\hat{y}$ = Log_{10} reductions = Log_{10} baseline – Log_{10} recoveries

A = Product

1, if Test Material #1

2, if Test Material #2

3, if Test Material #3

4, if Positive Control (Avagard®)

5, if Negative Control (Normal Physiological Saline)

e = Error Term

The variances will be evaluated using a Dunnett's Test. The alpha (α) value will be set at 0.05.

The 95% confidence intervals will be calculated according to the Bonferroni method.

Descriptive statistics will be generated on the log_{10} microbial populations recovered in the samples and the reductions from the controls, including sample size, means, standard deviations, and ranges.

Mean log_{10} reductions of the indicator microorganism will be used to determine the antimicrobial effectiveness of the test materials. The critical index is mean log10 reductions in microorganisms of ≥ 2.5 log_{10} within five minutes after one application.

A non-antimicrobial negative control is included to show the contribution of the active ingredients to effectiveness. The test products should be statistically superior to the negative control for the clinical simulation to be considered successful at showing that the test product is effective for use in healthcare antiseptic products.

A positive control is included to validate the study conduct to assure that the expected results are produced. For the results to be valid, the positive control should meet the appropriate log reduction criteria.

9.7.2 Determination of Sample Size

The sample size for this study will be either 1) determined after performing a pilot study of lesser number of subjects that would provide data to better calculate the number of subjects needed for the pivotal study or 2) calculated using the following statistical method.

Sample size for this draft study protocol was determined using the following formula:

$$\mathbf{N} \geq \frac{5S^2\left(Z_{\alpha/2} + Z_{\mathrm{B}}\right)^2}{D^2}$$

Where:

N= Sample size per test material configuration arm

S= Estimate of variance =0.50

$Z_{\alpha/2}$= 0.05 level of significance (two-tail) = 1.96, Type I error (probability of stating a significant effect exists when one does not)

Z_{β}= 0.842 level of significance for Type II (beta) error (probability of stating no significant effect exists when one does)

D= Detectable difference (sensitivity) = 0.5

$$\mathbf{N} \geq \mathbf{5}\left(\frac{(0.50)^2\ (1.96 + 0.842)^2}{0.5^2}\right) = 39.25$$

A minimum of 39 subjects will be evaluated per test material for a total of at least 195 subjects tested in a randomized evaluation.

9.8 Changes in the Conduct of the Study or Planned Analyses

Neither the Investigators nor the Sponsor will modify or alter this protocol without first obtaining agreement from the other parties. All protocol modifications including, but not limited to, changes in the Principal Investigator, inclusion/exclusion criteria, number of subjects to be enrolled, study sites, or procedures must be submitted to the GIRB as a written amendment for review and approval prior to implementation.

10.0 STUDY SUBJECTS

10.1 Disposition of Subjects

A written consent form will be obtained from each subject and filed by the Investigator with the subject's records, in accordance with 21 CFR Parts 50 and 56.

The Investigator may discontinue individual subjects from the study at any time. Subjects may voluntarily withdraw from the study at any time. The Investigator will provide a written report on the appropriate study document describing the reason for discontinuance and the date of discontinuance. A subject who discontinues will be replaced with another qualified subject who will follow the same treatment (randomization) scheme as the discontinued subject. The discontinued or withdrawn subject who participated in testing may not re-enter the study.

In order to implement a valid revocation of authorization to use and disclose private health information, the subject or their representative **must** make the request in writing to BioScience Laboratories, Inc., 1765 South 19th Avenue, Bozeman, Montana 59718. The revocation cannot stop the use or disclosure of information that has been collected prior to the revocation, is needed to ensure complete and accurate study results, or is required by law or government regulation (e.g. reporting adverse events, etc.). Revocation of an authorization may not be used to withhold normal medical care from the subject, but will make the subject ineligible to receive the study treatment or care.

10.2 Protocol Deviations

This study will be conducted as described in this protocol, except for an emergency situation in which the protection, safety, and well-being of the subject requires immediate interventions, based on the judgment of the Principal Investigator. In the event of a significant deviation from the protocol due to an emergency, accident, or mistake, the Investigator or designee will document the details of the situation and any subsequent decisions. All deviations from the Protocol or approved amendments shall be documented by BSLI. Any deviation to the Protocol that may have an effect on the safety or rights of the subjects or the integrity of the study must be reported to the GIRB as soon as the deviation is identified.

The Sponsor or the Investigator has the right to discontinue the study at any time for medical and/or administrative reasons. As far as possible, this should occur after mutual consultation.

11.0 SAFETY EVALUATION

11.1 Safety Assessments

The subject's safety will be monitored by evaluations of reactions observed on the skin of the test sites and any adverse reactions. Adverse reactions will be fully documented, reported as an Adverse Event, and followed to resolution.

11.2 Evaluation of Test Sites

Prior to performing any test procedures, the test sites will be examined to ensure no evidence of injury, dermatosis, or dermatitis is present. On completion of testing, the subject's hands will be examined and scored for skin irritation using the Skin Irritation Scoring System (Draize).

SKIN IRRITATION SCORING SYSTEM (Draize)

Erythema	0	No reaction
	1	Mild and/or transient redness limited to sensitive area
	2	Moderate redness persisting over much of the test material-exposed area
	3 *	Severe redness extending over most or all of the test material-exposed area
Edema	0	No reaction
	1	Mild and/or transient swelling limited to sensitive area
	2	Moderate swelling persisting over much of the test material-exposed area
	3 *	Severe swelling extending over most or all of the test material-exposed area
Rash	0	No reaction
	1	Mild and/or transient rash limited to sensitive area
	2	Moderate rash persisting over much of the test material-exposed area
	3 *	Severe rash extending over most or all of the test material-exposed area
Dryness	0	No reaction
	1	Mild and/or transient dryness limited to sensitive area
	2	Moderate dryness persisting over much of the test material-exposed area
	3 *	Severe dryness extending over most or all of the test material-exposed area

* = A score of 3 in one or more of the conditions evaluated represents significant irritation and qualifies as an Adverse Event.

11.3 Adverse Events

Adverse events will be captured for all subjects from the time baseline samples are taken to the time of subject discharge from the study. Adverse events will be categorized in relationship to the test materials that were applied. Trained personnel and emergency treatment (e.g., for anaphylaxis) are available onsite in the laboratory facility, and medical facilities/personnel are in close proximity.

In the event that either the Principal Investigator or the Sponsor determines that continuation of the study poses a hazardous risk of serious injury or death to the subject, the study will be stopped.

11.3.1 Adverse Event/Experience

An Adverse Event/Experience is any unexpected or undesirable experience occurring to a subject during a study, which may or may not be related to the test materials. All adverse event/experiences will be recorded and reported using an Adverse Event Report Form according to the Standard Operating Procedures of the laboratory.

All adverse events, regardless of severity or the cause/effect relationship, are to be recorded. The severity of the effect will be noted as *"Mild," "Moderate,"* or *"Severe"* according to the following definitions:

Mild	Awareness of signs or symptom, but easily tolerated.
Moderate	Discomfort to a degree as to cause interference with normal daily life activities and /or requiring medication.
Severe	Incapacity with inability to work or do usual daily life activities and requiring medical attention/intervention.

11.3.2 Causal Relations of Adverse Event/Experience

When determining the causal/effect relationship to a test material, the relationship will be described as *"None,*" *"Possible,*" *"Probable,*" or *"Definite."* The following definitions will be utilized:

None	No association to the test materials. Related to other etiologies such as concomitant medications or conditions or subject's known clinical state.
Possible	Uncertain association. Other etiologies are also possible.
Probable	Clear-cut association with improvement upon withdrawal of the test materials. Not reasonably explained by the subject's known clinical state but not an anticipated event.
Definite	An adverse event with a clear-cut temporal association and laboratory confirmation if possible.

11.3.3 Serious Adverse Event/Experience – During this Study

A Serious Adverse Event/Experience is any adverse experience occurring that results in any of the following outcomes:

Death;
A life-threatening adverse drug experience;
Inpatient hospitalization or prolongation of existing hospitalization;
A persistent or significant disability/incapacity;
Congenital anomaly/birth defect;
An important medical event that may require medical or surgical intervention to prevent one of the previously listed outcomes.

11.3.4 Unexpected Adverse Event/Experience

An Unexpected Adverse Event/Experience is any adverse event/experience not listed in the current labeling for the test materials, the current investigator's brochure, or the Anticipated Reactions Section 11.7 of this Protocol. Where test material labeling or investigator's brochure is not available, anticipated experiences will be based on the known pharmacological/toxicological properties of the test materials or ingredients.

11.3.5 Follow-up

If an adverse event/experience occurs, the Sponsor will be monetarily responsible for all costs associated with the follow-up for said event including, but not limited to, medical visits and medication prescribed by a medical professional directly related to the adverse event along with an administration fee that covers the Principal Investigator's time resolving the Adverse Event. If it is determined by Test Facility Management that the adverse event is due to negligence on the part of the Test Facility, no cost will be passed through to the Sponsor. The subject under the direction of the Principal Investigator (or designee) may be referred to the nearest acute care facility for treatment. Serious or Unexpected Event/Experiences will be followed to resolution. Any adverse event will be documented on an Adverse Event Report Form.

11.3.6 Notification

The Sponsor and the reviewing IRB will be notified of all adverse events/experiences within 2 business days. Any Serious or Unexpected Adverse Event/Experience that occurs during the study must be reported immediately by the Principal Investigator to the Sponsor and the reviewing IRB, followed by written notification within three business days of the information being reported to the investigative study team.

The Principal Investigator is required to review all unanticipated problems involving risk to volunteers or others, serious adverse events, and all subject deaths associated with the protocol and provide an unbiased written report of the event. At a minimum, the Principal Investigator must comment on the outcomes of the event or problem, and in the case of a serious adverse event or death, comment on the relationship to participation in the study.

11.3.7 Anticipated Reactions

It is possible but not likely, that a rash, allergic reaction or infection will develop. The risks associated with this test are primarily related to application of the test materials and the indicator microorganism. Mild skin irritation is anticipated and in some cases mild to heavy erythema, swelling, itching, cracking, peeling, or in rare cases, blistering and/or an allergic reaction might occur.

12.0 EXCEPTIONAL CONDITIONS

The Sponsor will be notified within 24 hours by telephone, email, and/or letter of any exceptions encountered in this study. The exceptional conditions or occurrences will be detailed in full and formally recorded. Exceptional conditions that occur and are not addressed in this Protocol will be subject to Out-of-Scope charges (see Proposal/Contract).

13.0 REFERENCES

1994 FDA Tentative Final Monograph 21 CFR Parts 333 and 369, *Health-Care Antiseptic Drug Products*; *Proposed Rule* in *Effectiveness testing of an antiseptic handwash or health care personnel handwash products*. (FR59: No. 116, 17 June 94. pp 31448 to 31450)

2015 FDA TFM, 21 CFR Part 310, *Safety and Effectiveness of Health Care Antiseptics; Topical Antimicrobial Drug Products for Over-the-Counter Human Use; Proposed Amendment of the Tentative Final Monograph; Reopening of Administrative Record;* Proposed Rule. (FR80: No. 84, 1 May 2015. pp 25166 to 25205)

ASTM E2755-15, *Standard Test Method for Determining the Bacteria-Eliminating Effectiveness of Healthcare Personnel Hand Rub Formulations Using Hands of Adults.*

ASTM E1054-08(2013), *Standard Test Methods for Evaluation of Inactivators of Antimicrobial Agents*.

Code of Federal Regulations Title 21 Parts 50, 54, 56, 58 and 312.

ICH E6 Good Clinical Practice Guidelines.

14.0 FINAL REPORT

A Final Report will be prepared describing the methodology and results of the study in a clear and concise manner.

15.0 DOCUMENTATION AND RECORD KEEPING

All documentation and records will be compiled, analyzed, and retained by BioScience Laboratories, Incorporated, at its facility in Bozeman, Montana. All raw data for this study, as well as the Final Report, will be retained in safe storage by the Testing Facility for a period of at least 5 years. BioScience Laboratories, Inc. will notify the Study Sponsor before any documents or records are destroyed.

This Protocol has been approved by the GIRB on _____

15.1 Study Center File Management

It will be the responsibility of the Investigator to ensure that the Study File is maintained. The Study File for this protocol may contain, but will not be limited to, the information listed below:

- Investigational Brochure (if applicable) or other appropriate test material safety information
- GIRB Approved Signed Protocol
- Revised Protocol (if applicable)
- GIRB-Approved Informed Consent Form (blank)
- Copy of Signed Form(s) FDA-1572 (if applicable)
- Financial Disclosure for the Principal Investigator and Sub-investigators (if applicable)
- Curriculum Vitae of Principal Investigator and Sub-investigators
- DHHS Number for GIRB, or other documentation of IRB compliance with FDA regulation (Included in this Protocol)
- Documentation of GIRB approval of protocol, consent form, any protocol amendments and any consent form revisions
- All correspondence between the Principal Investigator, IRB, and Sponsor relating to study conduct
- Copies of information related to SAE and the information on Immediately Reported Adverse Events
- Copy of the Approval Letter from the GIRB
- Copy of Notification of Initiation of Clinical Testing (Initiation Letter to the GIRB)
- Copy of Notification of Completion of Clinical Testing (Completion Letter to the GIRB)
- Research Site Signature Log/Delegation of Duties
- FDA's Clinical Investigator Information Sheets (if applicable)

To protect privacy and maintain the confidentiality of data, each subject will be assigned a unique study number, all study samples and research records will be identified using the subject's study number, research records will be kept in a locked room with access limited to study personnel, and electronic databases will be maintained on password-protected computers.

16.0 LIABILITY AND INDEMNIFICATION

Test Facility's liability to Sponsor under this Protocol shall be limited to the price of this evaluation. Sponsor shall be responsible to Study Participants (when applicable) and to other third parties for the fitness of the test material for use as defined in the Study Protocol.

17.0 ACCEPTANCE

EVALUATION OF THE ANTIMICROBIAL EFFICACY OF THREE TEST MATERIALS WITH POSITIVE AND NEGATIVE CONTROLS BASED ON THE HEALTH CARE PERSONNEL HANDWASH PROCEDURE

BIOSCIENCE LABORATORIES, INC. (TESTING FACILITY)
1765 South 19th Avenue
Bozeman, Montana 59718

President
& CEO: _____ _____
Daryl S. Paulson, Ph.D. Date

Principal
Investigator: _____ _____
To Be Determined Date of Study Initiation

Sub-Investigator: _____ _____
To Be Determined Date

REVIEWED BY:

Manager of
Quality Assurance: _____ _____
Amy L. Juhnke, RQAP-GLP Date

ACCEPTED BY: **THE AMERICAN CLEANING INSTITUTE** (SPONSOR)
1331 L St. N.W Suite 650
Washington D.C. 20005

_____ _____
Representative Date

_____
Title

This Protocol has been approved by the GIRB on _____

ATTACHMENT 7: EVALUATION OF THE ANTIMICROBIAL EFFICACY OF FIVE TEST MATERIALS WITH POSITIVE AND NEGATIVE CONTROLS BASED ON THE SURGICAL SCRUB PROCEDURE

DRAFT PROTOCOL 150944-102

BIOSCIENCES LABORATORIES, INC.

BIOSCIENCE LABORATORIES, INC. DRAFT PROTOCOL 150944-102

EVALUATION OF THE ANTIMICROBIAL EFFICACY OF FIVE TEST MATERIALS WITH POSITIVE AND NEGATIVE CONTROLS BASED ON THE SURGICAL SCRUB PROCEDURE

1.0 TITLE PAGE

Test Materials:	Test Material #1 To Be Determined Test Material #2 To Be Determined Test Material #3 To Be Determined Test Material #4 To Be Determined Test Material #5 To Be Determined
Sponsor:	The American Cleaning Institute 1331 L Street N.W. Suite 650 Washington D.C. 20005
Study Number:	150944-102
Sponsor Representative:	Francis H. Kruszewski
Principal Investigator:	To Be Determined
Sub-Investigator:	To Be Determined
Clinical Site:	BioScience Laboratories, Inc. 1765 South 19th Avenue Bozeman, Montana 59718 Telephone: (406) 587-5735
Date:	October 23, 2015

Confidentiality Statement

This document contains the confidential information of The American Cleaning Institute and BioScience Laboratories, Inc. It is intended solely for the guidance of the clinical investigation. This protocol may not be disclosed to parties not associated with the clinical investigation or used for any purpose without the prior written consent of The American Cleaning Institute and BioScience Laboratories, Inc. Permission to release the Protocol and Study Results to the United States Food and Drug Administration (FDA) or other regulatory agency to which this study will be submitted is explicitly granted.

This Protocol has been approved by the GIRB on _____

2.0 PROTOCOL SYNOPSIS

Name of Sponsor: The American Cleaning Institute	
Name of Test Materials and Active Ingredients: Test Material #1: To Be Determined Test Material #2: To Be Determined Test Material #3: To Be Determined Test Material #4: To Be Determined Test Material #5: To Be Determined Positive Control: Hibiclens®, Chlorhexidine gluconate solution, 4% (w/v) Positive Control: Alcohol: Avagard® 61% w/w Ethanol Negative Control: Normal Saline	**Protocol Number** **150944-102**

Title of Study:	**EVALUATION OF THE ANTIMICROBIAL EFFICACY OF FIVE TEST MATERIALS WITH POSITIVE AND NEGATIVE CONTROLS BASED ON THE SURGICAL SCRUB PROCEDURE DESCRIBED IN THE STANDARDIZED ASTM E1115-11 TEST METHOD**
Principal Investigator:	To Be Determined
Sub-Investigator:	To Be Determined
Study Center:	BioScience Laboratories, Inc.
Publications (References)	ASTM E1115-11, *Standard Test Method for Evaluation of Surgical Hand Scrub Formulations* 1994 FDA TFM, 21 CFR Parts 333 and 369, *Health-Care Antiseptic Drug Products*; *Effectiveness testing of an antiseptic handwash or health-care personnel handwash;* Proposed Rule. (FR59: No. 116, 17 June 94. pp 31448 to 31450) 2015 FDA TFM, 21 CFR Part 310, *Safety and Effectiveness of Health Care Antiseptics; Topical Antimicrobial Drug Products for Over-the-Counter Human Use; Proposed Amendment of the Tentative Final Monograph; Reopening of Administrative Record;* Proposed Rule. (FR80: No. 84, 1 May 2015. pp 25166 to 25205)
Study Duration:	A pre-test period of 7 days and a baseline day of 1 day and a test day of 1 day.

This Protocol has been approved by the GIRB on _____

Name of Sponsor: The American Cleaning Institute	**Protocol Number** **150944-102**
Name of Test Materials and Active Ingredients: Test Material #1: To Be Determined Test Material #2: To Be Determined Test Material #3: To Be Determined Test Material #4: To Be Determined Test Material #5: To Be Determined Positive Control: Hibiclens®, Chlorhexidine gluconate solution, 4% (w/v) Positive Control: Alcohol: Avagard® 61% w/w Ethanol Negative Control: Normal Saline	
Objectives:	The purpose of this study is to evaluate the antimicrobial efficacy of four test materials with positive and negative controls, for use as a Surgical Scrubs following single test material applications.
Methodology:	The testing methods are based on the standardized test method ASTM E1115-11, *Standard Test Method for Evaluation of Surgical Hand Scrub Formulations*, for use as Surgical Scrubs following a single test material application. Subjects will be required to complete a 7-day pre-test conditioning period, a 1-day baseline period consisting of glove juice sampling to establish that the subject meets the baseline criteria of ≥ 5 $\log_{10}$ number of viable microorganisms recovered from each hand. Subjects will complete a 1-day test period, during which time subjects will use the assigned test material once. Glove juice sampling for microbial populations will be performed immediately and 6-hours post-test material-application on Test Day.
Number of Subjects:	At least 47 subjects will be evaluated per test material for a total of at least 282 subjects tested in a randomized evaluation.
Main Criteria for Inclusion:	A sufficient number of overtly healthy subjects at least 18 years of age will be admitted into the study. Insofar as possible, the groups of subjects selected will be of mixed sex, age, and race. All subjects' hands will be free from clinically evident dermatoses, injuries to the hands or forearms, hangnails, or any other disorders that may compromise the

This Protocol has been approved by the GIRB on _____

Name of Sponsor: The American Cleaning Institute	**Protocol Number** **150944-102**
Name of Test Materials and Active Ingredients: Test Material #1: To Be Determined Test Material #2: To Be Determined Test Material #3: To Be Determined Test Material #4: To Be Determined Test Material #5: To Be Determined Positive Control: Hibiclens®, Chlorhexidine gluconate solution, 4% (w/v) Positive Control: Alcohol: Avagard® 61% w/w Ethanol Negative Control: Normal Saline	

	subject or the study.
Duration of treatment:	One of the test materials will be applied by subjects one time.
Criteria for Evaluation:	**Efficacy:** The critical indices are mean $\log_{10}$ reductions in microorganisms: ≥ 2 $\log_{10}$ within 1 minute after a single scrub and that microbial populations recovered in the 6-hour samples are less than baseline populations. A non-antimicrobial negative control is included to show the contribution of the active ingredients to effectiveness. The test materials should be statistically superior to the negative control for the clinical simulation to be considered successful at showing that the test material is effective for use as a surgical scrub. A positive control is included to validate the study conduct to assure that the expected results are produced. For the results to be valid, the positive control should meet the appropriate log reduction criteria.
	Safety: Evaluation for safety of use of the Test Materials will consist of Adverse Event-reporting.
Statistical Methods:	Log_{10} reductions from baseline population recovered from each of a subject's hands will be calculated by subtracting the $\log_{10}$ number of viable recovered following test material application from the $\log_{10}$ baseline population recovered from that hand. Log_{10} microbial data and population reductions

This Protocol has been approved by the GIRB on _____

Name of Sponsor: The American Cleaning Institute	**Protocol Number** **150944-102**
Name of Test Materials and Active Ingredients: Test Material #1: To Be Determined Test Material #2: To Be Determined Test Material #3: To Be Determined Test Material #4: To Be Determined Test Material #5: To Be Determined Positive Control: Hibiclens®, Chlorhexidine gluconate solution, 4% (w/v) Positive Control: Alcohol: Avagard® 61% w/w Ethanol Negative Control: Normal Saline	

	from each of a subject's hands will be presented in tabular form. Statistical calculations of mean and standard deviation will be generated on the $\log_{10}$ data from baseline samples, post- test material-application samples, and the reductions from baseline. The statistical analysis will determine the portion of subjects who meet the $\log_{10}$ reduction criteria based on a two-sided statistical test for superiority to the negative control and a 95 percent confidence interval approach.

This Protocol has been approved by the GIRB on _____

3.0 TABLE OF CONTENTS

SECTION **PAGE**

This Protocol has been approved by the GIRB on _____

This Protocol has been approved by the GIRB on _____

4.0 LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

AIDS	Acquired immune deficiency syndrome
ASTM	American Society for Testing and Materials
ATCC	American Type Culture Collection
BBP++	Butterfield's Phosphate Buffer Solution with product neutralizers
BSLI	BioScience Laboratories, Inc.
CFR	Code of Federal Regulations
CFU	Colony Forming Units
DHHS	Department of Health and Human Services
FR	Federal Register
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GIRB	Gallatin Institutional Review Board
HIPAA	Health Insurance Portability and Accountability Act
HIV	Human immunodeficiency virus
ICH	International Conference on Harmonization
PBS	Phosphate Buffered Saline
SAE	Serious Adverse Event
SSF	Stripping Suspending Fluid
SFF++	Stripping Suspending Fluid with neutralizers / 10% Tween
TFM	Tentative Final Monograph
TSA	Tryptic Soy Agar
TSA+	Tryptic Soy Agar with neutralizers
TSB	Tryptic Soy Broth
UV	Ultraviolet
Glove Juice Sampling Procedure	A procedure used to sample bacteria from the hands using a sterile glove and a specified volume of sampling fluid.
Mean $\log_{10}$ reductions	Average of the differences between the baseline microbial populations expressed as $\log_{10}$ CFU/hand and the populations in $\log_{10}$ CFU/Hand recorded

	from the post-application samples.
Source Documents	Recorded results of original observations and activities of a clinical investigation.
Subjects	Healthy human paid participant that has consented to test in the study.
Test Material	The test material, negative control or positive control that are to be tested according to procedures in this protocol.
Negative Control	A material that is to be tested according to procedures in this protocol with no active ingredient.
Positive Control	A product that is to be tested according to procedures in this protocol in order to validate the testing procedures and to be used as a control.

5.0 ETHICS

5.1 Institutional Review Board

Informed Consent Forms and any other supportive material relevant to the safety of the subjects will be supplied to the Gallatin Institutional Review Board (GIRB) for their review and approval. The primary purpose of the GIRB is the protection of the rights and welfare of the subjects involved (reference CFR 21, Parts 50, 56, 312, and 314). This study will begin only after GIRB approval has been obtained.

5.2 Ethical Conduct of Study

The study will be conducted in compliance with the Good Laboratory Practice standards (21 CFR Part 58) and Good Clinical Practice standards (21 CFR Parts 50, 56, 312, and 314, and ICH E6), the United States Food and Drug Administration regulations, Standard Operating Procedures of BioScience Laboratories, Inc., the study protocol, and any protocol amendments.

5.3 Subject Information and Consent

The Informed Consent Form, Authorization to Use and Disclose Protected Health Information Form, and List of Restricted Products will be provided to each subject prior to beginning the study. Trained personnel will explain the study to each subject and will be available to answer any questions that may arise.

This Protocol has been approved by the GIRB on _____

6.0 INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

BioScience Laboratories, Inc.
1765 S 19th Ave.
Bozeman, Montana 59715

Contact: To Be Determined
Phone: (406) 587-5735 Ext. XXX
Fax: (406) 586-7930

Principal Investigator: To Be Determined

Sub-Investigator: To Be Determined

Quality Assurance Monitor: Amy L. Juhnke, RQAP-GLP

Statistical Consultant: Daryl S. Paulson, Ph.D.

Subject Recruitment and Consenting: Chelsey Allison

Consulting Medical Experts: Gabor Benda, M.D. and David McLaughlin, M.D.

Gallatin Institutional Review Board (GIRB)
3006 Secor Avenue
Bozeman, Montana 59715
Phone: (406) 581-8559
DHHS Number: IRB00005939

6.1 Monitoring

The American Cleaning Institute, as Sponsor of this study, is responsible for ensuring the proper conduct of the study with regard to protocol adherence and validity of the data recorded on the study documents. The American Cleaning Institute has therefore assigned a study monitor to this study. The progress of the study may be monitored by:

- Periodic on-site review
- Telephone communications
- Review of sample data sheets and source documents

The Investigator will give The American Cleaning Institute, study monitor direct access to source documents that support data on the study documents and make available such records to authorized The American Cleaning Institute, quality assurance, IRB, and regulatory personnel for inspection and/or copying.

Note: The Federal Privacy rule (HIPAA) specifically permits the use and disclosure of protected health information "to a person subject to the jurisdiction of the Food and Drug Administration (FDA) [e.g. study sponsor] with respect to an FDA-related product or activity for which that person has responsibility, for the purpose of activities related to the quality, safety, or effectiveness of such FDA-regulated product or activity" [45 CFR 164.512(b)(1)(iii)].

This Protocol has been approved by the GIRB on _____

7.0 INTRODUCTION

Healthcare Personnel, as a standard of care, perform a surgical scrub procedure prior to surgery using formulations that contain antiseptic ingredients, in order to reduce the microbial populations on their skin and protect patients from nosocomial infection. The proposed Tentative Final Monograph (TFM) for *Health-Care Antiseptic Drug Products* (Vol. 59, No. 116, June 17, 1994) describes an *in-vivo* procedure for evaluating these types of materials, as well as expected performance criteria. The 2015 FDA TFM, 21 CFR Part 310, *Safety and Effectiveness of Health Care Antiseptics; Topical Antimicrobial Drug Products for Over-the-Counter Human Use; Proposed Amendment of the Tentative Final Monograph; Reopening of Administrative Record;* Proposed Rule now proposes that additional safety data are necessary to support the safety of antiseptic active ingredients for these uses. The new effectiveness criteria described in the 2015 proposed rule include mean $\log_{10}$ reductions in microorganisms: ≥ 2 $\log_{10}$ on each hand within 1 minute after a single application and that microbial populations recovered in the 6-hour samples are less than baseline populations.

8.0 STUDY OBJECTIVES

The purpose of this study is to evaluate the antimicrobial efficacy of four test materials with positive and negative controls, for use as Surgical Scrubs following single test material applications.

9.0 INVESTIGATIONAL PLAN

9.1 Overall Study Design and Plan

This study is designed to determine the antimicrobial effectiveness of four test materials (Test Materials #1, #2, #3,#4, and #5), and positive and negative controls intended for use as Surgical Scrubs. The testing methods are based on ASTM E1115-11, *Standard Test Method for Evaluation of Surgical Hand Scrub Formulations,* and based on the methodology specified by the Food and Drug Administration Tentative Final Monograph (TFM) for *Effectiveness Testing of a Surgical Hand Scrub* and the Food and Drug Administration Proposed Rule *Safety and Effectiveness of Health Care Antiseptics; Topical Antimicrobial Drug Products for Over-the-Counter Human Use; Proposed Amendment of the Tentative Final Monograph; Reopening of Administrative Record*. The immediate and cumulative antimicrobial effects will be measured to evaluate the efficacy.

At least 47 subjects will be evaluated per test material for a total of at least 282 subjects tested in a randomized evaluation. Subjects will be required to complete a 7-day pre-test conditioning period, a 1-day baseline period consisting of glove juice sampling to establish that the subject meets the baseline criteria of ≥ 5 $\log_{10}$ number of viable microorganisms recovered from each hand. Subjects will complete a 1-day test period,

during which time subjects will use the assigned test material once. Glove juice sampling for microbial populations will be performed immediately and 6-hours post-test material-application on Test Day.

The critical indices are mean $\log_{10}$ reductions in microorganisms: $\geq 2 \log_{10}$ within 1 minute after a single scrub and that microbial populations recovered in the 6-hour samples are less than baseline populations.

9.2 Discussion of Study Design, Including Choice of Sample Size

The sample size for this study was determined using the following formula:

$$\mathbf{N} \geq \frac{6S^2\left(Z_{\alpha/2} + Z_{\mathrm{B}}\right)^2}{D^2}$$

Where:

- N= Sample size per test material configuration arm
- S= Estimate of variance =0.50
- $Z_{\alpha/2}$= 0.05 level of significance (two-tail) = 1.96, Type I error (probability of stating a significant effect exists when one does not)
- Z_{β}= 0.842 level of significance for Type II (beta) error (probability of stating no significant effect exists when one does)
- D= Detectable difference (sensitivity) = 0.5

$$\mathbf{N} \geq \mathbf{6}\left(\frac{(0.50)^2\ (1.96 + 0.842)^2}{0.5^2}\right) = 47.10$$

At least 47 subjects will be evaluated per test material for a total of at least 282 subjects tested in a randomized evaluation.

9.3 Selection of Study Population

A sufficient number of overtly healthy subjects at least 18 years of age will be admitted into the study to ensure that at least 282 subjects, complete the study. Insofar as possible, the groups of subjects selected will be of mixed sex, age, and race. All subjects' hands will be free from clinically evident dermatoses, injuries to the hands or forearms, open wounds, hangnails, and/or any other disorders that may compromise the subject and the study. All subjects will sign the Informed Consent Form, Authorization to Use and Disclose Protected Health Information Form, and List of Restricted Products prior to participating in the study. The above forms are provided as separate Informed Consent documents.

An Informed Consent Form, Authorization to Use and Disclose Protected Health Information Form, and List of Restricted Products will be provided to each subject prior to beginning the study. Trained personnel will explain the study to each participant and will be available to answer any questions that may arise.

9.3.1 Inclusion Criteria

- Subjects may be of either sex, at least 18 years of age, and of any race.
- Subjects must possess both hands and all ten digits.
- Subjects must have an average baseline population of $\geq 5 \times 10^5$ colony forming units (CFU) per hand.
- Subjects must be in good general health.
- Subjects must have read and signed an Informed Consent Form, Authorization to Use and Disclose Protected Health Information Form, and List of Restricted Products prior to participating in the study, all located in the separate Informed Consent documents.

9.3.2 Exclusion Criteria

- Washing the hands or applying lotion within the 2-hour period prior to testing.
- Known allergies to sunscreens, deodorants, laundry detergents, fragrances, latex (rubber), alcohols, to common antibacterial agents found in soaps or lotions, particularly Test Material active ingredients or chlorhexidine gluconate, or to topical antibiotic ointments (e.g., Neosporin® or Polysporin® [neomycin/bacitracin/polymyxin B]).
- Exposure of ungloved hands or forearms to antimicrobial agents, medicated soaps, medicated shampoos (e.g., anti-dandruff), hair mousses, or medicated lotions, during the 7-day pre-test conditioning period or through the last test day.
- Use of biocide-treated pools or hot tubs, or use of UV tanning beds or sunbathing during the 7-day pre-test conditioning period or through the last test day.
- Exposure of ungloved hands or forearms to strong detergents, solvents, or other irritants during the 7-day pre-test conditioning period or through the last test day.
- Use of systemic or topical antibiotic medications, during the 7-day pre-test conditioning period or through the last test day.
- Use of systemic or topical steroids other than for contraception or post-menopausal indications during the 7-day pre-test conditioning period or through the last test day. This includes steroid medications used to treat asthma.
- Application or presence of nail polish, artificial nails, or nail polish remover, or having undergone nail treatments during the 7-day pre-test conditioning period or through the last test day.
- A medical diagnosis of a physical condition, such as a current or recent severe illness, medicated or uncontrolled diabetes, hepatitis B, hepatitis C, an organ transplant, a heart murmur, mitral valve prolapse with heart murmur, congenital heart disease, or

an immunocompromised condition such as AIDS (or HIV positive), lupus, or medicated multiple sclerosis.

- Any type of port (or portacath).
- Pregnancy, plans to become pregnant or impregnate a sexual partner within the pre-test or through the last test day, or nursing a child. Females must continue to take birth control precautions one month following the last day of testing. Males must continue to take birth control precautions through one week following the last day of testing.
- Any active skin rashes, dermatoses, hangnails, or breaks in the skin of the hands or forearms; skin blemishes such as dry scabs or warts may be permissible, with the specific approval of the Principal Investigator or consulting physician.
- An inflammatory skin condition, such as dermatitis, eczema, or psoriasis, anywhere on the body, that in the opinion of the Principal Investigator or consulting physician should preclude participation.
- Participation in a clinical study in the past 7 days or current participation in another clinical study.
- Any medical condition or use of any medications that, in the opinion of the Principal Investigator or Consulting Physician, should preclude participation.
- Unwillingness to fulfill the performance requirements of the study.

9.3.3 Subject Withdrawal

After admission to the study, the subject may withdraw at any time for any reason. If possible, the reason for withdrawal will be recorded. Any subject not adhering to Protocol requirements will be disqualified.

9.4 Test Methods

9.4.1 Equipment, Supplies, Test Solutions and Media

The equipment used during this study will be detailed on Clinical Trials Equipment Tracking Forms (Form No. 01-L-009), and the forms will be included in the Final Report.

The supplies used during this study will be detailed on Clinical Trials Supplies Tracking Forms (Form No. 01-L-008), and the forms will be included in the Final Report.

The Test Solutions and Media used for this study are listed below:

Sampling Solution

Stripping Suspending Fluid with product neutralizers (SSF++)

Neutralizing/Diluting Fluid

Butterfield's Phosphate Buffer Solution with Product Neutralizers (BBP++)

Media

Tryptic Soy Agar (TSA) for Inoculum Preparation

Tryptic Soy Agar with product neutralizers (TSA+)

Tryptic Soy Broth (TSB) for Inoculum Preparation

Phosphate Buffered Saline Solution (PBS) for Neutralization Study

Normal Saline

Johnson & Johnson Head to Toe

Soft Soap (per ASTM E1174-13):

Ingredients:

Linseed oil (50 parts by weight)

Potassium hydroxide (9.5 parts)

Ethanol (7 parts)

Distilled or high purity water as needed

Linseed oil will be added to a solution of potassium hydroxide in 15 parts water and heated to approximately 70 °C while constantly stirring.

Ethanol will be added and heating continued while stirring until the saponification process is completed and a sample dissolves clearly in water and almost clearly in alcohol. The weight of the Soft Soap will then be brought up to 100 parts by addition of hot water and 200 g of the Soft Soap transferred into 1 L of water. The diluted Soft Soap will then be dispensed into appropriate containers and sterilized in an autoclave.

9.4.2 Identity of Test Materials

The test materials will be provided to the Testing Facility by the Study Sponsor, complete with appropriate documentation. The test materials will be received and stored by BioScience Laboratories, Inc. (BSLI) in accordance with instructions from the Sponsor and retained in secure quarantine when not being used in testing. BSLI will maintain an inventory of the test materials and a log of use. Responsibility for determination of the identity, strength, purity, composition, stability, and solubility of the test materials, as well as responsibility for retention of the test materials rests with the Sponsor. Unused, sealed test and control test materials will be stored by BSLI until the Sponsor specifies their disposition. In the absence of a disposition request from the Sponsor within 1 year of their planned usage, the test materials will be returned to the Sponsor. No test material or control materials will be destroyed unless so requested by the Sponsor. If the test material names and lot numbers are not stated below, they will be presented in the Final Report, if provided by the Sponsor.

Test Material #1: To Be Determined
Active Ingredient: _____
Lot Number: _____
Expiration Date: _____
Manufacture Date: _____

Test Material #2: To Be Determined
Active Ingredient: _____
Lot Number: _____
Expiration Date: _____
Manufacture Date: _____

Test Material #3: To Be Determined
Active Ingredient: _____
Lot Number: _____
Expiration Date: _____
Manufacture Date: _____

Test Material #4: To Be Determined
Active Ingredient: _____
Lot Number: _____
Expiration Date: _____
Manufacture Date: _____

Test Material #5: To Be Determined
Active Ingredient: _____
Lot Number: _____
Expiration Date: _____
Manufacture Date: _____

Positive Control: Hibiclens®
Active Ingredient: Chlorhexidine gluconate solution 4% (w/v)
Lot Number: _____
Expiration Date: _____
Manufacture Date: _____

Positive Control: Avagard®
Active Ingredient: Ethanol 61% wt/wt
Lot Number: _____
Expiration Date: _____
Manufacture Date: _____

This Protocol has been approved by the GIRB on _____

Negative Control: Normal Saline
Active Ingredient: N/A
Lot Number: _____
Expiration Date: _____
Manufacture Date: _____

9.4.3 Pre-Test Conditioning Period

The 7 days prior to the baseline portion of the study will constitute the pre-test conditioning period. During this time, subjects will avoid the use of medicated soaps, lotions, and shampoos, as well as skin contact with solvents, detergents, acids, and bases. Non-antimicrobial personal hygiene products will be supplied to the subjects, who will be instructed to use them exclusively throughout the period of the study. During this period, subjects also must not sunbathe or use tanning beds or biocide-treated pools or hot tubs. This regimen will allow for stabilization of the normal microbial populations residing on the hands. Following the pre-test period, prospective subjects will proceed into the Baseline Week.

9.4.4 Method of Assigning Subjects to Treatment Groups

Baseline evaluations will be conducted on one day to establish microbial baseline population values for each subject. Each subject will be in testing for 0.5 to 1 hour on baseline day. For a subject to continue into the Experimental Period, baseline population of each hand must be $\geq 5 \times 10^5$ CFU/hand.

Prior to being sampled, subjects will be questioned regarding their adherence to Protocol requirements. Subjects also will be questioned as to whether they have complied with a requirement not to wash their hands or apply any type of lotion in the 2 hours prior to sampling.

For the baseline determination, the hands will be washed under technician supervision with Soft Soap and according to the procedures below.

- Subjects will clip fingernails to ≤ 1 mm free-edge, if necessary. All jewelry will be removed from hands and arms.
- Subjects will clean under the fingernails with a nail cleaner.
- All washes/rinses will be performed in tap water regulated at 40 °C ± 2 °C. Hands will be positioned higher than the elbows throughout this procedure.
- Subjects will rinse hands, including the lower two-thirds of forearms, under running tap water for at least 30 seconds.
- Subjects will then wash hands and forearms with 5.0 mL of Negative Control for 30 seconds, using water as required to develop lather.

- Subjects will rinse hands and forearms thoroughly for 30 seconds under running tap water to remove all lather.
- The wash with Negative Control will be followed by performance of the Glove Juice Procedure (Section 9.4.5) while the hands are still wet, to determine baseline populations.

All subjects accepted into the Experimental Day will be randomly assigned to use one of the six test materials listed below. The immediate sample will be randomized to the right or left hand.

Test Material	**Test Material Identity**	**Product Type**
Test Material #1	To Be Determined	To Be Determined
Test Material #2	To Be Determined	To Be Determined
Test Material #3	To Be Determined	To Be Determined
Test Material #4	To Be Determined	To Be Determined
Test Material #5	To Be Determined	To Be Determined
Positive Control Scrub	Hibiclens®	Water-aided
Positive Control Surgical Rub	Avagard®	Waterless Alcohol based
Negative Control	Normal Saline	Water-aided

9.4.5 Experimental Period

Each subject will be in testing for 6 to 7 hours on the test day. Subjects will have been instructed to come to the laboratory with clean, dry hands and nails, but to not have washed their hands or applied lotion in the 2 hours prior to test material application. Prior to being admitted into testing, subjects will be questioned regarding their adherence to the Protocol requirements.

Prior to test material application, subjects will clip fingernails to ≤ 1 mm free-edge, if necessary. All jewelry will be removed from hands and arms.

On the test day, the test material will be used once by subjects according to the following directions for either water-aided or waterless alcohol based surgical scrubs:

9.4.5.1 Test Material Application Procedures for Water-aided Surgical Scrubs

- The tap water used for all hand scrub procedures will be maintained at 40 °C ± 2 °C. Hands will be positioned higher than the elbows throughout this entire application procedure.
- Hands, including two-thirds of forearm, will be wet thoroughly for at least 30 seconds under running tap water maintained at 40 °C ± 2 °C. During this rinse, fingernails and cuticles will be cleaned with a nail pick. Additional time will be allowed to ensure all fingernails and cuticles are cleaned.

- 4.0 mL of test material will be dispensed into the subjects' cupped hands, and they will spread the product over the hands and forearms. Subjects will alternately scrub right hand and lower two-thirds of forearm and left hand and lower two-thirds of forearm, using the sponge for 1.5 minutes each. Water will be added to the sponge as needed, to help develop lather.
- The scrub should concentrate on the interdigit areas and nail cuticle area. A timer will be used to guide the approximately 1 minute 30 seconds ± 15 seconds scrub performed per hand using a scrub sponge.
- After completing this sequence, the sponge will be placed in a sterile dish within easy reach. Both hands and the lower two-thirds of forearms will be rinsed for 30 seconds ± 5 seconds. The scrub sponge will not be rinsed.
- 4.0 mL of Test Product will again be dispensed into the subjects' cupped hands, and spread over the hands and forearms.
- Subjects will alternately scrub right hand and lower two-thirds of forearm and left hand and lower two-thirds of forearm, using the scrub sponge. Water will be added to the sponge, as needed, to help develop lather.
- Subject will rinse each hand and forearm separately in running water such that each hand and forearm is rinsed for 1 minute ± 5 seconds.
- Following the final rinse, the subjects will dry their hands with sterile paper towels, drying from the fingertips down the arms.

9.4.5.2 Application instructions for Waterless Alcohol based Surgical Scrubs:

- Apply to clean, dry hands, use no brushes or water
- Clean under nails with pick, wash hands with 3 ml of Soft Soap for 30 seconds. Rinse 30 seconds. Dry thoroughly with paper towels.
- Apply one to two pumps (2 ml) of alcohol surgical rub into the palm of one hand. Dip fingertips of the opposite hand into the hand prep and work under fingernails, concentrating on interdigit areas, spread remaining hand prep over the hand and up to mid forearm, below the elbow.
- Apply one pump (1-2 ml) and repeat procedure with opposite hand.
- Apply final pump (1-2 ml) of hand prep into either hand and reapply to all aspects of both hands up to the wrists. Allow to dry.

9.4.5.3 Glove Juice Sampling Procedures

Following the baseline day final wash or the test material application procedure the subject's hands will be placed into powder-free, sterile synthetic gloves.

The glove on the hand assigned for sampling at least 6 hours but within 6 hours 10

minutes following the test material application procedure will be secured at the wrist using masking tape. A second glove will be donned over the first glove, and also secured at the wrist to protect the synthetic glove during the 6-hour wait.

Within 1 minute of donning the gloves for baseline and immediate samples, and at the 6-hour sample time, 75.0 mL Stripping Suspending Fluid (SSF++) will be instilled into the glove. The wrist will be secured, and a technician will massage the hand through the gloves in a standardized manner for 60 seconds. A 5-mL aliquot of the glove juice will be removed from the glove and placed in a sterile test tube. The sample will be serially diluted in Butterfield's Phosphate Buffer Solution with product neutralizers (BBP++) as appropriate.

9.4.6 Blinding

In order to ensure blinded microbiologists, technicians who participate in test material application or the collection of samples from subjects during test material testing will not participate in plating samples and/or counting plates from samples collected from subjects after test material testing.

9.4.7 Subject Safety

Subjects will not be allowed to leave the laboratory for any reason during the sampling periods of testing, except in an emergency.

9.4.8 Plating

Duplicate spiral plates and/or duplicate spread plates will be prepared from each of the dilutions on Tryptic Soy Agar with product neutralizers (TSA+) and incubated at 30 °C ± 2 °C for 72 hours or until sufficient growth is observed. Colonies will be counted and data recorded using a computerized plate-counting system. If 10^0 spread plates give an average count of zero, the average plate count will be expressed as 0.50 for duplicate plating.

9.5 Efficacy and Safety Variables

9.5.1 Efficacy and Safety Measurements and Flow Chart

The immediate and residual antimicrobial effectiveness of the test materials will be measured to evaluate the efficacy. The critical indices are mean $\log_{10}$ reductions in microorganisms: ≥ 2 $\log_{10}$ within 1 minute after a single scrub and that microbial populations recovered in the 6-hour samples are less than baseline populations.

Evaluation for safety of use of the Test Materials will consist of Adverse Event-reporting (refer to Section 11) and assessment for skin reactions following testing.

Flow Chart

Procedure	Day -7 or more	Day -6 to 0	Day Baseline Day 0	Day Test day
Informed Consent Provided	X			
Pre-Test Conditioning Period	X	X	X	X
Inclusion/Exclusion Criteria Reviewed	X		X	X
Baseline Sample			X	
Test Material Application				X
Post-Application Sample				X

9.5.2 Appropriateness of Measurements

The testing methods are based on the standardized test method ASTM E1115-11, *Standard Test Method for Evaluation of Surgical Hand Scrub Formulations,* for use as Surgical Scrubs following a single test material application. The critical indices are mean $\log_{10}$ reductions in microorganisms: ≥ 2 $\log_{10}$ within 1 minute after a single scrub and that microbial populations recovered in the 6-hour samples are less than baseline populations, as recommended by the 2015 FDA TFM, 21 CFR Part 310, *Safety and Effectiveness of Health Care Antiseptics; Topical Antimicrobial Drug Products for Over-the-Counter Human Use; Proposed Amendment of the Tentative Final Monograph; Reopening of Administrative Record;* Proposed Rule.

9.5.3 Primary Efficacy Variables

The immediate and cumulative antimicrobial effectiveness of the test materials will be measured to evaluate the efficacy. The critical indices are mean $\log_{10}$ reductions in microorganisms: ≥ 2 $\log_{10}$ within 1 minute after a single scrub and that microbial populations recovered in the 6-hour samples are less than baseline populations.

A non-antimicrobial negative control is included to show the contribution of the active ingredients to effectiveness. The test material should be statistically superior to the negative control for the clinical simulation to be considered successful at showing that the test material is effective for use in health care antiseptic products.

A positive control is included to validate the study conduct to assure that the expected results are produced. For the results to be valid, the positive control should meet the critical indices.

9.5.4 Data Collection and Microbial Recoveries

Colonies will be counted and data recorded using a computerized plate-counting system. If 10^0 spread plates give an average count of zero, the average plate count will be expressed as 0.50 for duplicate plating, and 0.25 for quadruplicate plating.

The plate count data collected from this study will be evaluated using MiniTab® 17 statistical computer software.

The estimated $\log_{10}$ number of viable microorganisms recovered from each hand will be designated the "*R*-value." It is the adjusted average $\log_{10}$ colony count measurement at each sampling time. Each *R*-value will be determined using the following formula:

$$R = \log_{10} [75 \times C_i \times 10^{-D}]$$

where:

75 = the amount (mL) of stripping solution instilled into each glove

C_i = the arithmetic average colony count of the two plate counts for each subject at a particular dilution level

D = the dilution factor

The $\log_{10}$ transformation is performed on these data to convert them to a linear scale. A linear scale, more appropriately a $\log_{10}$ linear scale, is a requirement of the statistical models to be used.

9.6 Data Quality Assurance

The Sponsor's designated Quality Assurance Representative may conduct audits at the study site. Audits will include, but are not limited to test materials, presence of required documents, the informed consent process, and review of source documents. The Investigator agrees to participate with audits conducted at a reasonable time in a reasonable manner.

The study will be inspected by the Quality Assurance Unit at BioScience Laboratories, Inc., and reports will be submitted to the Principal Investigator and Management in accordance with Standard Operating Procedures.

9.7 Statistical Methods and Determination of Sample Size

9.7.1 Statistical and Analytical Plans

The MiniTab® Statistical Computer Package will be used for all statistical calculations.

A one-factor Analysis of Variance will be applied to the data. The model will have the form:

$$\hat{y} = A + e$$

where:

$\hat{y}$ = Log_{10} Reductions = Baseline minus Wash

A = Test Material

1, if Test Material #1

2, if Test Material #2

3, if Test Material #3

4, if Test Material #4

5, if Test Material #5

6, if Positive Control (Hibiclens for scrubs, Avagard for Ethanol)

7, if Negative Control (Normal Saline)

e = Error Term

A Dunnett's Test will be used to compare the controls to the other test materials.

The 95% confidence intervals will be compared to the 2.0 log_{10} level.

Descriptive statistics will be provided, including the sample size, means, standard deviations, and ranges.

The immediate and cumulative antimicrobial effectiveness of the test materials will be measured to evaluate the efficacy. The critical indices are mean log_{10} reductions in microorganisms: ≥ 2 log_{10} on each hand within 1 minute after a single scrub and that microbial populations recovered in the 6-hour samples are less than baseline populations.

A non-antimicrobial negative control is included to show the contribution of the active ingredients to effectiveness. The test material should be statistically superior to the negative control for the clinical simulation to be considered successful at showing that the test material is effective for use in health care antiseptic products.

A positive control is included to validate the study conduct to assure that the expected results are produced. For the results to be valid, the positive control should meet the critical indices.

9.7.2 Determination of Sample Size

The sample size for this study will be either 1) calculated using the following statsistical method, or 2) may be determined after performing a pilot study that would provide data to better calculate the number of subjects needed for the pivotal study.

Sample size may be determined using the following formula:

$$\mathbf{N} \geq \frac{6S^2\left(Z_{\alpha/2} + Z_{\mathrm{B}}\right)^2}{D^2}$$

Where:

N= Sample size per test material configuration arm

S= Estimate of variance =0.50

$Z_{\alpha/2}$= 0.05 level of significance (two-tail) = 1.96, Type I error (probability of stating a significant effect exists when one does not)

Z_{β}= 0.842 level of significance for Type II (beta) error (probability of stating no significant effect exists when one does)

D= Detectable difference (sensitivity) = 0.5

$$\mathbf{N} \geq \mathbf{6}\left(\frac{(0.50)^2\ (1.96 + 0.842)^2}{0.5^2}\right) = 47.10$$

At least 47 subjects will be evaluated per test material for a total of at least 282 subjects tested in a randomized evaluation.

9.8 Changes in the Conduct of the Study or Planned Analyses

Neither the Investigators nor the Sponsor will modify or alter this protocol without first obtaining agreement from the other parties. The protocol including modifications must be submitted to the GIRB as a written amendment for review and approval prior to implementation.

10.0 STUDY SUBJECTS

10.1 Disposition of Subjects

A written consent form will be obtained from each subject and filed by the Investigator with the subject's records, in accordance with 21 CFR Parts 50 and 56.

The Investigator may discontinue individual subjects from the study at any time. Subjects may voluntarily withdraw from the study at any time. The Investigator will provide a written report on the appropriate study document describing the reason for discontinuance and the date of discontinuance. A subject who discontinues will be replaced with another qualified subject who will follow the same treatment (randomization) scheme as the discontinued subject. The discontinued or withdrawn subject who participated in testing may not re-enter the study.

In order to implement a valid revocation of authorization to use and disclose private health information, the subject or their representative must make the request in writing to BioScience Laboratories, Inc., 1765 South 19th Avenue, Bozeman, Montana 59718. The revocation cannot stop the use or disclosure of information that has been collected prior to the revocation, is needed to ensure complete and accurate study results, or is required by law or government regulation (e.g. reporting adverse events, etc.). Revocation of an authorization may not be used to withhold normal medical care from the subject, but will make the subject ineligible to receive the study treatment or care.

10.2 Protocol Deviations

This study will be conducted as described in this protocol, except for an emergency situation in which the protection, safety, and well-being of the subject requires immediate interventions, based on the judgment of the Principal Investigator. In the event of a significant deviation from the protocol due to an emergency, accident, or mistake, the Investigator or designee will document the details of the situation and any subsequent decisions. All deviations from the Protocol or approved amendments shall be documented by BSLI. Any deviation to the Protocol that may have an effect on the safety or rights of the subjects or the integrity of the study must be reported to the GIRB as soon as the deviation is identified. The Sponsor or the Investigator has the right to discontinue the study at any time for medical and/or administrative reasons. As far as possible, this should occur after mutual consultation.

11.0 SAFETY EVALUATION

11.1 Safety Assessments

The subject's safety will be monitored by evaluations of any adverse reactions. Adverse reactions will be fully documented, reported as an Adverse Event, and followed to resolution.

11.2 Evaluation of Test Sites

Prior to performing any test procedures, the test sites will be examined to ensure no evidence of injury, dermatosis, or dermatitis is present.

11.3 Adverse Events

Adverse events will be captured for all subjects from the time baseline samples are taken to the time of subject discharge from the study. Adverse events will be categorized in relationship to the test materials that were applied. Trained personnel and emergency treatment (e.g., for anaphylaxis) are available onsite in the laboratory facility, and medical facilities/personnel are in close proximity. In the event that either the Principal Investigator or the Sponsor determines that continuation of the study poses a hazardous risk of serious injury or death to the subject, the study will be stopped.

11.3.1 Adverse Event/Experience

An Adverse Event/Experience is any unexpected or undesirable experience occurring to a subject during a study, which may or may not be related to the test materials. All adverse event/experiences will be recorded and reported using an Adverse Event Report Form according to the Standard Operating Procedures of the laboratory.

All adverse events, regardless of severity or the cause/effect relationship, are to be recorded. The severity of the effect will be noted as *"Mild," "Moderate,"* or *"Severe"* according to the following definitions:

Mild	Awareness of signs or symptom, but easily tolerated.
Moderate	Discomfort to a degree as to cause interference with normal daily life activities and /or requiring medication.
Severe	Incapacity with inability to work or do usual daily life activities and requiring medical attention/intervention.

11.3.2 Causal Relations of Adverse Event/Experience

When determining the causal/effect relationship to a test material, the relationship will be described as *"None,*" *"Possible,*" *"Probable,*" or *"Definite."* The following definitions will be utilized:

None	No association to the test materials. Related to other etiologies such as concomitant medications or conditions or subject's known clinical state.
Possible	Uncertain association. Other etiologies are also possible.
Probable	Clear-cut association with improvement upon withdrawal of the test materials. Not reasonably explained by the subject's known clinical state but not an anticipated event.
Definite	An adverse event with a clear-cut temporal association and laboratory confirmation if possible.

11.3.3 Serious Adverse Event/Experience – During this Study

A Serious Adverse Event/Experience is any adverse experience occurring that results in any of the following outcomes:

Death;
A life-threatening adverse drug experience;
Inpatient hospitalization or prolongation of existing hospitalization;
A persistent or significant disability/incapacity;
Congenital anomaly/birth defect;
An important medical event that may require medical or surgical intervention to prevent one of the previously listed outcomes.

11.3.4 Unexpected Adverse Event/Experience

An Unexpected Adverse Event/Experience is any adverse event/experience not listed in the current labeling for the test materials, the current investigator's brochure, or the Anticipated Reactions Section 11.3.7 of this Protocol. Where test material labeling or investigator's brochure is not available, anticipated experiences will be based on the known pharmacological/toxicological properties of the test materials or ingredients.

11.3.5 Follow-up

If an adverse event/experience occurs, the Sponsor will be monetarily responsible for all costs associated with the follow-up for said event including, but not limited to, medical visits and medication prescribed by a medical professional directly related to the adverse event along with an administration fee that covers the Principal Investigator's time resolving the Adverse Event. If it is determined by Test Facility Management that the adverse event is due to negligence on the part of the Test Facility, no cost will be passed through to the Sponsor. The subject under the direction of the Principal Investigator (or designee) may be referred to the nearest acute care facility for treatment. Serious or Unexpected Event/Experiences will be followed to resolution. Any adverse event will be documented on an Adverse Event Report Form.

11.3.6 Notification

The Sponsor and the reviewing IRB will be notified of all adverse events/experiences within 2 business days. Any Serious or Unexpected Adverse Event/Experience that occurs during the study must be reported immediately by the Principal Investigator to the Sponsor and the reviewing IRB, followed by written notification within three business days of the information being reported to the investigative study team.

The Principal Investigator is required to review all unanticipated problems involving risk to volunteers or others, serious adverse events, and all subject deaths associated with the protocol and provide an unbiased written report of the event. At a minimum, the Principal Investigator must comment on the outcomes of the event or problem, and in the case of a serious adverse event or death, comment on the relationship to participation in the study.

11.3.7 Anticipated Reactions

The risks associated with this test are primarily related to application of the test materials. Mild skin irritation is anticipated and in some cases mild to heavy erythema, swelling, itching, cracking, peeling, or in rare cases, blistering and/or an allergic reaction might occur.

12.0 EXCEPTIONAL CONDITIONS

The Sponsor will be notified within 24 hours by telephone, email, and/or letter of any exceptions encountered in this study. The exceptional conditions or occurrences will be detailed in full and formally recorded. Exceptional conditions that occur and are not addressed in this Protocol will be subject to Out-of-Scope charges (see Proposal/Contract).

13.0 REFERENCES

1994 FDA Tentative Final Monograph 21 CFR Parts 333 and 369, *Health-Care Antiseptic Drug Products*; *Proposed Rule* in *Effectiveness testing of an antiseptic handwash or health care personnel handwash products*. (FR59: No. 116, 17 June 94. pp 31448 to 31450)

2015 FDA TFM, 21 CFR Part 310, *Safety and Effectiveness of Health Care Antiseptics; Topical Antimicrobial Drug Products for Over-the-Counter Human Use; Proposed Amendment of the Tentative Final Monograph; Reopening of Administrative Record;* Proposed Rule. (FR80: No. 84, 1 May 2015. pp 25166 to 25205)

ASTM E1115-11, *Standard Test Method for Evaluation of Surgical Hand Scrub Formulations*

ASTM E1054-08(13), *Standard Test Methods for Evaluation of Inactivators of Antimicrobial Agents*.

Code of Federal Regulations Title 21 Parts 50, 54, 56, 58 and 312.

ICH E6 Good Clinical Practice Guidelines.

14.0 FINAL REPORT

A Final Report will be prepared describing the methodology and results of the study in a clear and concise manner.

15.0 DOCUMENTATION AND RECORD KEEPING

All documentation and records will be compiled, analyzed, and retained by BioScience Laboratories, Incorporated, at its facility in Bozeman, Montana. All raw data for this study, as well as the Final Report, will be retained in safe storage by the Testing Facility for a period of at least 5 years. BioScience Laboratories, Inc. will notify the Study Sponsor before any documents or records are destroyed.

This Protocol has been approved by the GIRB on _____

15.1 Study Center File Management

It will be the responsibility of the Investigator to ensure that the Study File is maintained. The Study File for this protocol may contain, but will not be limited to, the information listed below:

- Investigational Brochure (if applicable) or other appropriate test material safety information
- GIRB Approved Signed Protocol
- Revised Protocol (if applicable)
- GIRB-Approved Informed Consent Form (blank)
- Copy of Signed Form(s) FDA-1572 (if applicable)
- Financial Disclosure for the Principal Investigator and Sub-investigators (if applicable)
- Curriculum Vitae of Principal Investigator and Sub-investigators
- DHHS Number for GIRB, or other documentation of IRB compliance with FDA regulation (Included in this Protocol)
- Documentation of GIRB approval of protocol, consent form, any protocol amendments and any consent form revisions
- All correspondence between the Principal Investigator, IRB, and Sponsor relating to study conduct
- Copies of information related to SAE and the information on Immediately Reported Adverse Events
- Copy of the Approval Letter from the GIRB
- Copy of Notification of Initiation of Clinical Testing (Initiation Letter to the GIRB)
- Copy of Notification of Completion of Clinical Testing (Completion Letter to the GIRB)
- Research Site Signature Log/Delegation of Duties

FDA's Clinical Investigator Information Sheets (if applicable)To protect privacy and maintain the confidentiality of data, each subject will be assigned a unique study number, all study samples and research records will be identified using the subject's study number, research records will be kept in a locked room with access limited to study personnel, and electronic databases will be maintained on password-protected computers.

16.0 LIABILITY AND INDEMNIFICATION

Test Facility's liability to Sponsor under this Protocol shall be limited to the price of this evaluation. Sponsor shall be responsible to Study Participants (when applicable) and to other third parties for the fitness of the test material for use as defined in the Study Protocol.

17.0 ACCEPTANCE

EVALUATION OF THE ANTIMICROBIAL EFFICACY OF FIVE TEST MATERIALS WITH POSITIVE AND NEGATIVE CONTROLS BASED ON THE HEALTH CARE PERSONNEL HANDWASH PROCEDURE

BIOSCIENCE LABORATORIES, INC. (TESTING FACILITY)
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Daryl S. Paulson, Ph.D. Date

Principal
Investigator: _____ _____
To Be Determined Date of Study Initiation

Sub-Investigator: _____ _____
To Be Determined Date

REVIEWED BY:

Manager of
Quality Assurance: _____ _____
Amy L. Juhnke, RQAP-GLP Date

ACCEPTED BY: **THE AMERICAN CLEANING INSTITUTE** (SPONSOR)
1331 L St. N.W Suite 650
Washington D.C. 20005

_____ _____
Representative Date

_____
Title

This Protocol has been approved by the GIRB on _____

ATTACHMENT 8: ASTM METHOD E1173-15, STANDARD TEST METHOD FOR EVALUATION OF PREOPERATIVE, PRECATHETERIZATION, OR PREINJECTION SKIN PREPARATIONS

Designation: E1173 – 15

Standard Test Method for Evaluation of Preoperative, Precatheterization, or Preinjection Skin Preparations[1]

This standard is issued under the fixed designation E1173; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 The test method is designed to measure the reduction of the microflora of the skin.

1.2 A knowledge of microbiological techniques is required for these procedures.

1.3 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.3.1 *Exception*—In this test method, metric units are used for all applications except for linear measure, in which case inches are used, and metric units follow in parentheses.

1.4 Performance of this procedure requires a knowledge of regulations pertaining to the protection of human subjects (**1**).[2]

1.5 *This standard does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

2. Referenced Documents

2.1 *ASTM Standards:*[3]

E1054 Test Methods for Evaluation of Inactivators of Antimicrobial Agents

E1874 Test Method for Recovery of Microorganisms From Skin using the Cup Scrub Technique

E2756 Terminology Relating to Antimicrobial and Antiviral Agents

3. Terminology

3.1 Terms used in this standard are defined in E2756, Standard Terminology Relating to Antimicrobial and Antiviral Agents. Others defined below are specific to their use in this document.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *active ingredient, n*—a substance added to a formulation specifically for the inhibition or inactivation of microorganisms.

3.2.2 *inguen, n*—groin: the junctional region between the abdomen and thigh; pl. *inguina*.

3.2.3 *inguinal crease*—the discrete region of flexure between the abdomen and the thigh.

3.2.4 *sampling fluid*—a recovery fluid that contains a neutralizer demonstrated to inactivate or quench the active ingredient(s) in test and reference control formulations. See Test Method E1054.

3.2.5 *test formulation*—a formulation containing an active ingredient(s).

4. Summary of Test Method

4.1 These test methods are conducted on human subjects selected randomly from a group of volunteers who, after refraining voluntarily from using topical and oral antimicrobials for at least two weeks (14 days), exhibit acceptably high normal flora counts on the skin sites to be used in testing (see Section 8).

4.2 The antimicrobial activity of preoperative, vascular precatheterization, or preinjection skin preparations is measured by comparing microbial counts, obtained at various time intervals after application of a test formulation to skin sites, to counts obtained from those same sites prior to application of the test formulation. Skin sites recommended for use in testing are: *(1)* the inguinal region and the abdomen for preoperative skin preparations; *(2)* the inguinal region, the subclavian (clavicular) region, or the median cubital region of the arm for vascular precatheterization preparations, or both; and *(3)* the median cubital region of the arm for preinjection skin preparations.

4.2.1 *Preoperative Skin Preparation*—Microbial samples are collected from the test sites a minimum of three (3) times

[1] This test method is under the jurisdiction of ASTM Committee E35 on Pesticides, Antimicrobials, and Alternative Control Agents and is the direct responsibility of Subcommittee E35.15 on Antimicrobial Agents.

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[2] The boldface numbers in parentheses refer to the list of references at the end of this standard.

[3] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

after treatment application on both moist and dry skin sites. The recommended sample times are 10 min, 30 min, and 6 h post-treatment, but other relevant times may be selected.

4.2.2 *Vascular Precatheterization Preparation*—Microbial samples are collected from the test sites a minimum of three (3) times after treatment application on both moist and dry skin sites. The recommended sample times are "immediate," 12 h, and 24 h post-treatment, but other relevant times may be selected. The immediate sample may be 30 s to 10 min, depending on the test material evaluated.

4.2.3 *Preinjection Preparation*—A microbial sample is collected from the test site 30 s post-treatment.

4.3 The fluid used for sampling the test sites must effectively quench (neutralize) the antimicrobial action of all formulations tested. The effectiveness of the inactivator must be demonstrated prior to initiation of product-testing, as described in Test Method E1054, and using in-vivo techniques consistent with the cup-scrub technique (see Section 10).

4.4 To ensure the internal validity of the test, a reference control formulation having performance characteristics known to the laboratory should be tested in parallel with the test formulation.

5. Significance and Use

5.1 These procedures should be used to test topical antimicrobial-containing preparations that are intended to be fast-acting in reducing significantly the number of organisms on intact skin immediately and, for preoperative and vascular precatheterization preparations, to maintain reductions for an extended time.

6. Apparatus

6.1 *Colony Counter*—Any of several types may be used; for example, Quebec colony counters and similar devices, or automated, computerized plater/counter systems.

6.2 *Incubator*—Any incubator that can maintain a temperature of 30° ± 2°C may be used.

6.3 *Sterilizer*—Any steam sterilizer that can produce the conditions of sterilization is acceptable.

6.4 *Timer (stopwatch)*—One that displays hours, minutes, and seconds.

6.5 *Examining Table*—Any elevated surface, such as a 3-by-6-ft (0.9-by-1.8-meter) table with mattress or similar padding to allow the subject to recline.

7. Reagents and Materials

7.1 *Bacteriological Pipettes*—10.0 and 2.2-mL or 1.1-mL capacity, available from most laboratory supply houses.

7.2 *Petri Dishes*—100 mm by 15 mm, for performing standard plate counts, available from most laboratory supply houses.

7.3 *Scrubbing Cups*—Autoclavable cylinders, height approximately 1 in (2.5 cm), inside diameter of a size convenient to placement on the skin of the anatomical area to be sampled. Useful diameters range from approximately 0.5 to 1.5 in (1.3 to 3.8 cm), depending on sites to be sampled.

7.4 *Rubber Policeman, TFE-fluorocarbon Scrubbers, or other appropriate advice*—Can be fashioned in the laboratory or purchased from most laboratory supply houses. Whichever type is selected, it should be used throughout the course of testing.

7.5 *Testing Formulation, including directions for use.*

7.6 *Sterile Gauge Pads*—Used to cover treated skin sites.

7.7 *Sterile Dressings*[4]—Used to cover treated skin sites.

7.8 *Sampling Fluid*—Dissolve 0.4 g KH_2PO_4, 10.1 g Na_2HPO_4, and 1.0 g isooctylphenoxypolyethoxyethanol in 1 L of distilled water. Inactivator(s) specific for the antimicrobial active(s) in the test and reference control formulations must be included (See Test Method E1054). Adjust to pH 7.8. Dispense in appropriate volumes and sterilize.

7.9 *Dilution Fluid*—Butterfield's **(2)** phosphate-buffered water adjusted to pH 7.2, or other suitable diluent, which must contain antimicrobial inactivators specific for the test and reference control formulations (see Test Method E1054).

7.10 *Plating Medium*—Soybean-casein digest agar **(3)**, with or without antimicrobial inactivators.

7.11 *Sterile Template Material*—Used to demarcate the skin sites; made from paper, plastic, or cloth, for example.

7.12 *Surgical Skin Marker*—Used to delineate mark the skin sites to be used in testing.

NOTE 1—Because some markers contain crystal violet or other fluids that are inhibitory to many skin microflora, a marker should be proven non-antimicrobial prior to use in testing.

8. Skin Sites to be Used in Testing

8.1 *Preoperative Skin Preparations:*

8.1.1 The skin sites selected for evaluation of the effectiveness of preoperative skin preparations should include both moist and dry skin areas. Bacterial baseline populations should be at least 3.0 $\log_{10}/cm^2$ greater on moist skin sites than the detection limit of the sampling procedure, and at least 2.0 $\log_{10}/cm^2$ greater than the detection limit on dry skin sites. The preferred moist-skin areas are the inguina, in which skin-to-skin contact results in a moist environment conducive to higher populations of microflora. The preferred dry-skin area is the lower abdomen below the umbilicus. These areas are illustrated in Fig. 1.

8.1.2 Using a 1.5-by-5-in (3.8-by-12.7-cm) sterile template (for example, paper, plastic, cloth), treatment sites in the inguina are delineated on the uppermost inner aspects of both thighs, centering the long axis of the template along the inguinal crease, and marking the corners using a surgical skin marker. If, due to a subject's anatomy, the treatment site cannot be centered along the inguinal crease, the site should be

[4] The sole source of supply of the apparatus (TELFA non-adherent dressing, No. 3279) known to the committee at this time is Kendall Co.; Hospital Products; Boston, MA 02101. This product is not sterile, but can be steam-sterilized prior to use. If you are aware of alternative suppliers of appropriate dressings, please provide this information to ASTM International Headquarters. Your comments will receive careful consideration at a meeting of the responsible technical committee,[1] which you may attend.

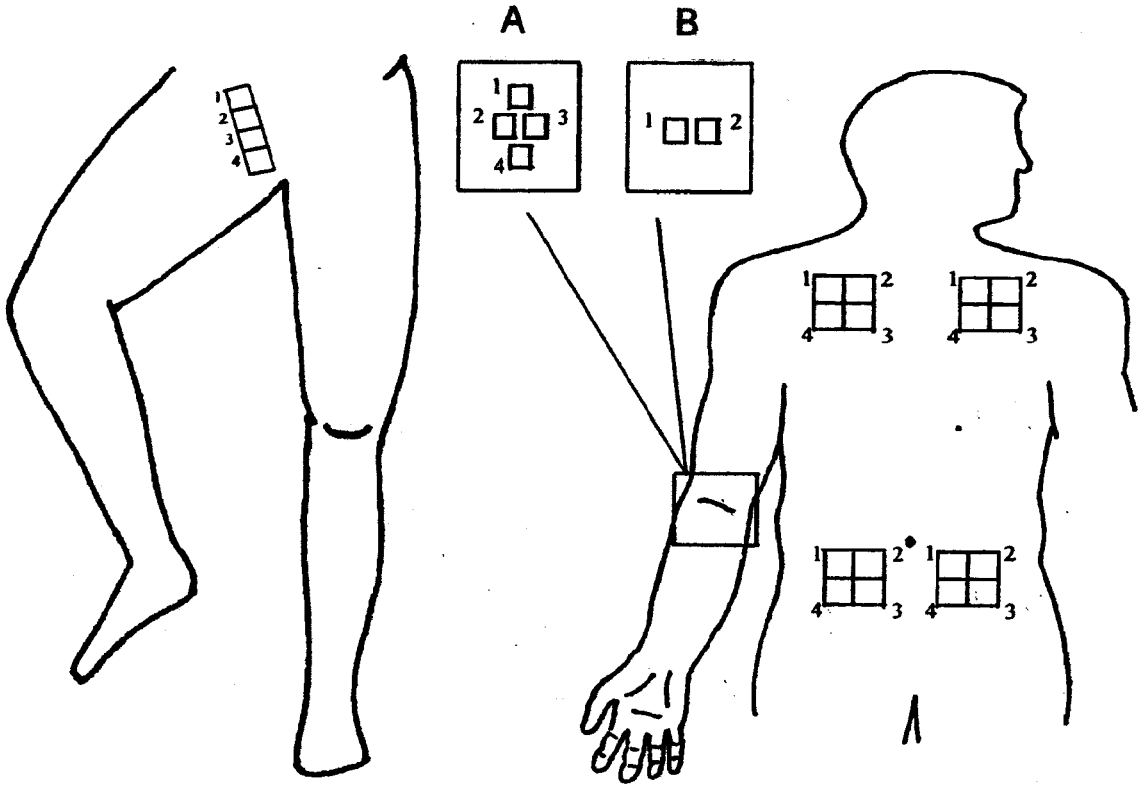

FIG. 1 Illustration of Approximate Sampling Locations on Treatment Sites: Inguen, Abdomen, Clavicular Region, and Median Cubital Region of Arm

positioned on the upper, inner thigh as close to the crease as possible. In no instance should testing be performed on areas not having skin-to-skin contact. The site is then divided on the long axis into 1-by-1.5-in (2.5-by-3.8-cm) sampling areas, allowing for spaces of about 0.25 (about 0.6 cm) between each of the four areas.

8.1.2.1 Sampling areas are numbered from anterior to posterior, beginning with 1 and proceeding perineally to 4, and then are randomized to sampling for baseline and the three post-treatment sampling times (see Note 2).

NOTE 2—Bacterial populations in the inguina are known to be heterogeneous, with counts tending to increase proceeding from the upper reaches of the inguinal crease perineally toward convergence of the inguina at the gluteal fold, and to decrease proceeding laterally from the inguinal crease onto the (dry) surface of the upper thigh. Hence, sampling areas must be confined to skin immediately adjacent to the inguinal crease where skin-to-skin contact provides the moist environment conducive to bacterial growth. Note that the large variance in the count data that results from randomization of the sampling areas likely will require testing of a relatively large number of subjects in order to demonstrate statistical significance of post-treatment reductions.

8.1.2.2 Because of constraints imposed by the anatomical area, sampling cylinders used for the inguinal sites must be ≤ 1 in (≤ 2.54 cm) in diameter.

8.1.2.3 The test formulation and reference control material are then randomized bilaterally to the treatment sites.

8.1.3 Abdominal treatment sites are to be located within 5-by-5-in (12.7-by-12.7-cm) sites below and to the right or left of the umbilicus, approximately midway between the umbilicus and the pubis. Using a 5-by-5-in (12.7-by-12.7-cm) sterile template (for example, paper, plastic, cloth), the corners of each site are numbered 1, 2, 3, and 4 directly on the skin, using a surgical skin marker. Numbering is to be the same for all abdominal sites: number 1 is placed at the top corner to the subject's right, and numbers 2, 3, and 4 are assigned in order clockwise from 1. Three quadrants of each site are used for the three different treatment exposure times, and the remaining quadrant is used for a baseline count. The test formulation and reference control material are then randomized to the treatment sites, right and left, and baseline and the three post-treatment sampling times are randomized to the four sampling areas within each site.

8.2 *Vascular Precatheterization Skin Preparations:*

8.2.1 The skin sites selected for evaluation of the effectiveness of vascular precatheterization skin preparations should include body areas that may be catheterization sites and should include both moist and dry skin areas. Bacterial baseline populations should be at least 3.0 $\log_{10}/cm^2$ greater on moist skin sites than the detection limit of the sampling procedure, and at least 1.0 $\log_{10}/cm^2$ greater than the detection limit on dry skin sites. The preferred moist-skin areas are the inguina, and the preferred dry-skin areas are the clavicular region and the median cubital region of the arm.

8.2.2 Test sites in the inguina are to be located and evaluated as specified for testing of preoperative skin preparations (see 8.1.2.1, Note 2, and Fig. 1).

8.2.3 The dry skin sites and sampling configurations used in testing vascular precatheterization preparations are illustrated

in Fig. 1 and Fig. 1 Detail A. Sterile templates (for example, paper, plastic, cloth) are fashioned for the sampling configuration such that they accommodate the diameter of the sampling cylinder, plus at least 0.5 in (1.25 cm) between the 4 sampling areas. The template is applied to the treatment site, and a surgical skin marker is used to demarcate the sampling areas. These are numbered 1 through four at outside corners, beginning at the subject's upper right and proceeding clockwise in the clavicular region, and beginning proximally and proceeding distally on the arm. Three sampling areas of the site are used for different treatment exposure times of "immediate" (30 s to 10 min, depending on test product), 12 h, or 24 h, and the remaining sampling area is used for a baseline count. The test formulation and reference material should be randomized to the treatment sites, right or left, and exposure times and baseline should be randomized to the four quadrants of each site.

8.3 *Preinjection Skin Preparations:*

8.3.1 The skin site selected for use in evaluating the effectiveness of preinjection skin preparations should represent a body area that is commonly used for transepidermal injection or phlebotomy. Bacterial baseline populations should be at least 1.0 $\log_{10}/cm^2$ greater than the detection limit of the sampling procedure. A suitable dry-skin area is the median cubital region of the arm.

8.3.2 The dry-skin site and sampling configuration used in testing preinjection preparations are illustrated in Fig. 1 Detail B. Sterile templates (for example, paper, plastic, cloth) are fashioned for the sampling configuration, such that they accommodate the diameter of the sampling cylinder, plus at least 0.5 in (1.25 cm) between the two sampling areas. The template is applied to the treatment site, and a surgical marker is used to demarcate the sampling areas. These are numbered 1 and 2 at outside corners. One sampling area of the site is used for the treatment exposure of 30 s, and the remaining sampling area is used for a baseline count

8.3.3 The test formulation and reference control material are then randomized to the treatment sites, right and left, and baseline and post-treatment or baseline are randomized to the two sampling areas.

9. Procedure

9.1 *Number of Subjects*—Because the purpose of the study is to demonstrate efficacy (defined as a significant reduction from baseline counts), sample size calculations should be done to determine the number of subjects per treatment group necessary to find statistically significant differences (reductions) from baseline. The number of subjects required depends on the statistical confidence required for the expected results, the variability encountered in the data collection (for example, variability in reductions from baseline), and the expected efficacy of the test product (for example, approximate reductions from baseline expected). This number of subjects per treatment group (n) can be estimated from the following equation (4):

$$n \geq S^2 \left[\frac{(Z_{a/2} + Z_{\beta})^2}{D^2} \right]$$

where:

S^2 = estimate of variance (of reductions from baseline based on in-house data pool);
$Z_{a/2}$ = cumulative probability of the standard normal distribution = 1.96 for $\alpha = 0.05$;
Z_{β} = power of the test = 0.842 for $\beta = 0.80$; and
D = expected efficacy (expected reduction from baseline).

9.2 Recruit a sufficient number of healthy adult volunteers who have no visual evidence of dermatoses, open wounds, or other skin disorders that may affect the test.

9.3 *Pretest Period (14 days)*—Instruct volunteers selected as test subjects to avoid contact with antimicrobials (other than test formulations) for the duration of the pretest and test periods. This restriction includes antiperspirants, deodorants, shampoos, lotions, bathing soaps, body powders, other hygienic products that contain antimicrobials, and such materials as acids, bases, and solvents. Subjects also are to refrain from wearing clothes that have been treated with antimicrobials or fabric softeners, and from bathing in biocide-treated pools, hot tubs, or spas.

9.3.1 Provide test subjects with a kit of nonantimicrobial personal hygiene products for exclusive use during the pretest and test periods. Subjects are not to shower or tub-bathe during the 24-h period prior to the application of test material or microbial sampling. The bathing restriction period may be lengthened, if desired, to increase bacterial populations.

9.3.2 If the skin sites selected for testing include areas that would require clipping of hair prior to surgery (for example, the abdominal and inguinal regions), hair from these sites should be clipped to reduce difficulties bandaging them. Clipping must be performed at least 48 h prior to microbial sampling for first baseline.

9.4 *Test Period*—After subjects have refrained from using antimicrobials for at least two weeks, obtain at least one estimate of baseline bacterial population from the anatomical regions to be evaluated. Collect these samples at least 72 h prior to initiation of product-testing to permit selection of only those subjects whose baseline counts meet the minimum criteria for the treatment site (see 8.1.1, 8.2.1, or 8.3.1). Sampling and enumeration techniques described in Sections 10 and 11 should be applied.

9.4.1 A baseline sample is to be collected at the time of testing. The sample should be taken from a sampling area predetermined by design (for example, on an inguinal site) or by random assignment to sampling areas within a treatment site.

9.4.2 *Treatment Application Procedure*—Immediately after taking the baseline sample, the treatment is applied according to label directions or as stated in the proposed directions.

9.4.3 *Sampling Schedule*—According to the predetermined sampling design or randomization, samples of the prepped site are taken from the sampling areas when the specified post-treatment exposure times have elapsed.

NOTE 3—Between the time of treatment application and final sampling, subjects should avoid activities or positions that would cause untreated skin sites or clothing to contact treated sites. To allow the subjects some degree of mobility between the time of treatment and final sampling, the treated skin areas should be covered with a sterile semi-occlusive dressing

(7.6 and 7.7). This material is applied in a manner so as to protect the treated skin site from contact with untreated skin, and such that air circulation is not restricted.

10. Microbiological Sampling Methods

10.1 Microbial samples are obtained using the cup scrub technique. See (**5**) and Test Method E1874. At the designated sampling time, a sterile cylinder (glass or stainless steel) is held firmly onto the area to be sampled. A known volume of sterile sampling fluid containing appropriate product inactivators is instilled into the cylinder. The known volume selected will depend on the size of the cylinder and the total amount of fluid required for subsequent dilution. The skin area inside the cylinder is then massaged in a circular manner for 1 min with a sterile rubber policeman, Teflon scrubber, or other appropriate device. At the end of the timed procedure, the fluid is removed and placed in a sterile test tube.

10.2 A second known volume of sterile sampling fluid containing appropriate product inactivators is instilled into the cylinder, and the skin area inside the cylinder is massaged again in a circular manner for 1 min with the scrubbing device. At the end of the timed procedure, the fluid is removed and placed in the test tube with the first aliquot, pooling the samples.

10.3 1.0 mL aliquots of this suspension of microorganisms (10^0 dilution) are removed and serially diluted in Butterfield's[3] phosphate-buffered water, or other suitable diluent containing product inactivators. Serial diluting and plating should be completed within 30 min.

11. Enumeration of Bacteria in Sampling Solution

11.1 The bacteria in the sampling fluid are enumerated using standard procedures such as those described in Wehr and Frank (**6**), but using soybean-casein digest agar (7.10) and a suitable inactivator for the antimicrobial, where necessary. Sample dilutions are analyzed in duplicate and are incubated at 30° ± 2°C for 48 to 72 h before enumeration.

11.2 In order to convert this volumetric measurement into the number of colony-forming units per square centimeter (cm^2), the following formula is employed:

$$R = \log_{10}\left[\frac{F \times \left[\frac{\sum c_i}{n}\right] \times 10^{-D}}{A}\right]$$

where:

R = the average colony-forming unit count in $\log_{10}$ scale per cm^2 of sampling surface;
F = total number of mL of stripping fluid added to the sampling cylinder (refer to Section 10);
$\Sigma c_i/n$ = average of the duplicate plate counts used for each sample collected;
D = dilution factor of the plate counts; and
A = inside area of the cylinder in cm^2.

12. Study Design

12.1 The basic study design for these testing procedures is a pre- to post-treatment comparative structure. The microbial population determined prior to treatment with test formulation (baseline population) is compared with the population remaining at a specified time after treatment (**7**).

NOTE 4—Baseline microbial population counts for the left and right test sites should be pooled, but only if they are shown to be statistically equivalent at a ≤ 0.05. This permits direct comparison of formulations on the basis of a single mean baseline value.

12.2 The basic design for a study of two formulations is illustrated as follows (**8**):

Pre-Treatment			Post-Treatment				
R (1)	O_{BL1}	O_{BL2}	A (1)	$O_{1,1}$	$O_{1,2}$	...	$O_{1,n}$
R (2)	O_{BL1}	O_{BL2}	A (2)	$O_{2,1}$	$O_{2,2}$	...	$O_{2,n}$

where:

R = test sites, right and left, assigned randomly to the test and reference formulations: where I = 1, if formulation 1; or 2, if formulation 2.
A = Independent variables: test and reference formulations.
O_{ij} = Dependent variables: microbial counts.
i = BL, if Baseline 1, 2, ...n; 1, if formulation 1; and 2, if formulation 2; and
j = exposure times 1, 2, ...n

12.3 This design will accommodate statistical evaluation by means of either parametric or nonparametric models.

13. Statistical Analysis

13.1 Regardless of the analytical model selected, it is preferable that the level of significance be set at α = 0.05 for rejection of H_o, the hypothesis of no significant difference between microbial populations, pre- to post-treatment.

13.2 Student's *t* test is an appropriate parametric approach to the analysis of the changes from baseline (pre- to post-treatment). It may be appropriate to adjust the alpha level due to multiple *t* tests being conducted (**9**). One choice for an adjusted value of alpha (α*) is calculated as follows:

$$\alpha^* = 1 - (1 - \alpha)^k$$

where:

k = the number of comparisons to be made;
α = the level of significance desired (= 0.05, preferably); and
α* = true level of significance for each of the multiple tests on H_o.

13.3 If data do not conform to a normal distribution, a nonparametric statistical model, such as the Mann-Whitney U Test, may be applied.

14. Precision and Bias

14.1 A precision and bias statement cannot be made for this test method at this time.

15. Keywords

15.1 antimicrobial; cup scrub; efficacy; precatheterization; preinjection; preoperative; skin preparation

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ATTACHMENT 9: ESTIMATION OF CHARGES ASSOCIATED WITH PREVENTABLE HEALTHCARE ACQUIRED INFECTIONS IF ANTIBACTERIAL HANDWASH PRODUCTS WERE UNAVAILABLE

PREPARED FOR ACI BY EXPONENT

FEBRUARY 25, 2015

Exponent®

Estimation of Charges Associated with Preventable Healthcare Acquired Infections if Antibacterial Handwash Products Were Unavailable

Exponent®

Estimation of Charges Associated with Preventable Healthcare Acquired Infections if Antibacterial Handwash Products Were Unavailable

Prepared for

American Cleaning Institute
1331 L Street, N.W., Suite 650
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Prepared by

Exponent
Maynard, MA
Alexandria, VA

February 24, 2015

Doc. no. 1401739.000 - 1053

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Acronyms and Abbreviations

C diff	clostridium difficile
CAUTI	catheter-associated urinary tract infection
CHG	chlorhexidine gluconate
CI	confidence interval
CLABSI	central line-associated bloodstream infection
GI	gastrointestinal
HAI	hospital-associated/acquired infection
HAP	hospital-acquired pneumonia
ICU	intensive care unit
IV	intravenous
LOS	length of stay
MDR	multi-drug resistant
MeSH	medical subject heading
MRSA	methicillin-resistant staphylococcus aureus
OTC	over-the-counter
PCMX	chloroxylenol
RIA	regulatory impact analysis
SSI	surgical site infection
VAP	ventilator-associated pneumonia
VRE	vancomycin-resistant enterococcae

1 Introduction

Exponent previously examined the *Regulatory Impact Analysis (RIA) for the FDA Proposed Rule, Safety and Effectiveness of Consumer Antiseptics; Topical Antimicrobial Drug Products for Over-the-Counter Human Use; Proposed Amendment of the Tentative Final Monograph* for the purpose of developing comments on the proposed rule RIA. One of the components of that evaluation was to assess the estimated costs and benefits associated with the rule, specifically the costs and benefits relevant to potentially preventable gastrointestinal illness, and the underlying assumptions. Exponent developed estimates for the costs and benefits, using published data augmented by expert opinion, that demonstrate the range of estimates of potentially reduced benefits that could be associated with decreased product availability. Along similar lines, the American Cleaning Institute (ACI) has asked Exponent to consider costs and benefits associated with over-the-counter (OTC) healthcare antiseptic products.

Specifically, the goal of this task is to identify the incremental medical expenditures associated with preventable illnesses that may no longer be prevented, if healthcare antiseptics were to be removed from the market. A total national estimate of the potential lost benefits of the proposed regulation is estimated based on national estimates of the number of cases of hospital-acquired infections, assumptions about the proportion that are associated with hand hygiene, and published literature on the cost of these illnesses (specific to each pathogen/infection). The end product of the effort is a spreadsheet model that incorporates various input parameters and can be used to test and explore potential outcomes of limiting OTC healthcare antiseptic products. To account for the various sources of uncertainty in each input to the model, a range of estimates is presented. The inputs, including values and sources, and outputs of the model are described in this document.

Brown and colleagues (1) point out that some HAI are unavoidable and further that lack of reimbursement for some of these infections may encourage proactive antibiotic treatment and the unintended negative consequence of contributing to development of resistance. It is important to recognize that this analysis does not challenge the idea that some hospital-acquired infections are not preventable. Even the most conservative estimates from the model assume that some HAI cannot be prevented. Another consideration is that in their efforts to minimize rates of HAI, hospitals may conduct more screening at admission to understand whether patients are already colonized at admission to identify whether infections should be considered hospital-acquired (1). As patterns of reimbursement change, they may affect the proportion of infections considered to be HAI but the model does not speculate about how this may affect expenditures. The underlying framework of this model assumes that there is some proportion of HAI that are currently avoided as a result of the use of healthcare antiseptics, and that limiting availability of these types of products would be associated with an increase in the rate of these types of infections.

2 Methods

2.1 Definition of Terms

For the purpose of this analysis and report, the term "healthcare antiseptics" shall refer to the following categories of products and specific antiseptics (provided in parentheses):

- Healthcare personnel handwashes and rubs (benzalkonium chloride, benzethonium chloride, chlorhexidine gluconate (CHG), chloroxylenol (PCMX), iodine, triclosan, alcohol (ethanol/isopropanol))
- Surgical hand scrubs and rubs (CHG, PCMX, iodine, ethyl alcohol with or without CHG)
- Patient preoperative skin preparations and pre-injection skin preparations (CHG, isopropanol, povidine iodine, alcohol, iodine)

Whenever possible, we attempted to limit findings to these specific products and categories of products. This was largely impossible, so sensitivity analyses and ranges were used.

In this model, HAI is used as generally described by the CDC and its reports: central-line associated bloodstream infections (CLABSI), catheter-associated urinary tract infection (CAUTI), surgical site infections (SSI), and ventilator-associated pneumonia (VAP). As per reports from the CDC (see Scott (2)), the category for pneumonia in this report also includes hospital-acquired pneumonia (HAP) and another category, gastrointestinal infections (GI), is included.

While the words "costs" and "charges" are often used interchangeably, they have distinct meanings in the health economic literature. "Charges" refers to the amounts requested on claims submitted to insurers by healthcare providers, while "costs" are the amounts reimbursed. The literature on medical expenditures associated with HAI that were included in this model typically included hospital charges rather than costs.

2.2 Literature Search

Exponent initially conducted a literature search of PubMed (a database maintained by the National Library of Medicine that provides access to more than 24 million citations for biomedical literature from MEDLINE, life science journals, and online books; accessible at http://www.ncbi.nlm.nih.gov/pubmed) for publications through January 21, 2015. The literature search had dual goals: a) to identify publications reporting on the relationship, number, and/or proportion of hospital-acquired infections (HAI) that could be attributed to hand hygiene and healthcare OTC antiseptics and b) to identify publications reporting on the charges associated with these HAI.

2.2.1 Literature Search: Clinical Publications

For the clinical component of the search, search terms (MeSH, keywords, and text fields) included "handwash," "healthcare," "hospital," and "rate" to identify papers in the past 25 years published in English with human subjects. No country or region limitations were used, as it was determined that these should not be decided *a priori* but rather reviewed on a case by case basis. This search was essentially an update of the search conducted by Exponent in July 2014 for the memo created for ACI entitled "Preliminary Findings of Literature Review on Effectiveness of Healthcare Antiseptics."

In addition to identifying published estimates of the number of HAI and the proportion that may be attributable to healthcare antiseptics, this search also identified a number of papers on surveillance, cost-effectiveness of various mitigation strategies (such as quarantine for a ward), and reviews. While these studies could not be used as a primary resource for this search, reference lists of these papers were reviewed for evidence of primary reports of data required to populate the model. In addition, reference lists of identified papers were reviewed. As MeSH terms are revised over time and journals are added to PubMed, it was decided to explore the literature by expanding upon the systematic search of PubMed exlusively in order to be comprehensive rather than to limit the search to publications identified directly from the initial literature search alone.

2.2.2 Literature Search: Charge Publications

For the economic component of the search, search terms (MeSH, keywords, and text fields) included "healthcare," "hospital," "infection," "costs and cost analysis" and related sub-headings suggested by PubMed with filters applied to identify papers in the past 10 years published in English with human subjects. As treatment patterns change for the infections of interest, a smaller time frame was selected to minimize variation that would occur with a longer time frame. It should be noted that one of the most useful economic MeSH terms is "costs and cost analysis" although the articles of interest typically provided information on hospital charges rather than costs (i.e., the charges submitted to insurers rather than the reimbursed amounts provided to them). While the terms "costs" and "charges" are often used interchangeably in the literature, the data included in the analysis are on charges. Papers on charges were limited to those providing estimates for the United States. As with the clinical search, reference lists of identified papers were reviewed.

In addition to charge estimates identified in the literature search, the reference list of a recent meta-analysis of charges associated with HAI in the United States (3) was reviewed for papers that might not have been identified in the initial search that could be relevant for this model.

Studies were considered eligible for the data abstraction process if they presented charge per case, rather than per household or total expenditures associated with an outbreak. Further, papers were included if they reported on a broad mix of patients and infections. For example, articles reporting only on post-partum women or only on spinal surgery were excluded. This exclusion criterion was applied to limit the need to introduce assumptions for the economic estimates. It was believed that the charges for infections could vary greatly across patient types

(i.e., immunocompromised, very young or elderly populations) and the inclusion of studies looking only at very specific populations would limit the generalizability of the model.

2.3 Data Abstraction

The data abstraction process for both types of papers included a review of titles and abstracts, requests for full text when titles and abstracts were not conclusive, and review of full text for all papers that appeared to be relevant. In general, publications that provided only reviews of findings presented in other papers, but not primary analyses, were not retained for review, although their reference lists were reviewed. Every attempt was made to ensure that the same patient populations were not represented in multiple studies to avoid double-counting or overweighting individual estimates.

2.3.1 Data Abstraction: Clinical Publications

For the clinical papers, we first, we revisited the papers discussed in the memo previously submitted to the ACI. Second, we reviewed papers published in 2014 after the previous literature search. All papers that were abstracted are included in the reference section of this report.

2.3.2 Data Abstraction: Charge Publications

Multiple key elements of information were abstracted from each relevant publication. First, the charge estimate per case was identified, along with details about the derivation of the estimate including the setting and type of payer (i.e., commercial vs public payer). For example, some of the analyses present a single value that is already weighted based on the distribution of severity of illnesses, while others present a range. Second, the fiscal year in which charges are presented was recorded. These data points allow for the selection of the appropriate base case and extreme (minimum and maximum) charges for each pathogen-induced HAI, as well as the ability to inflate each to 2014 U.S. values. Charges were inflated to 2014 US$ using the Consumer Price Index for medical care, published by the Bureau of Labor Statistics (series ID CUUR0000SAM).

2.4 Findings

Establishing the total number of cases of HAI in the United States annually, and the proportion of those that are preventable requires careful consideration.

As there are multiple estimates available in the literature for the total number of HAI, the distribution of HAI, or the proportion of HAI that are preventable, the spreadsheet model includes multiple potential values, implemented as pull-down menus, from which the user can select. The following sections describe the manner in which the user can manipulate each of these variables and what is used for default options in the model.

2.4.1 Epidemiology of HAI: Number of Cases and Type

Several studies provide estimates of the number of HAI annually, with varying levels of segmentation by infection or pathogen or population. There are three prominent papers on the topic (2, 4, 5) and they include the most recent estimates about the number of HAI annually in the United States. These estimates can be seen in Table 1.

Table 1. Number of HAI Cases by Type and Source

	All	VAP/HAP	SSI	GI	CAUTI	CLABSI	Other
Scott (2)	1,737,125	52,543	290,485	178,000	449,334	92,011	674,752
Magill (5)	721,800	157,500	157,500	123,100	93,300	71,900	118,500
Klevens (4)	1,737,125	250,205	290,485	Not reported; included in "other"	561,667	248,678	386,090

Because these studies estimate the number of cases of various infections but do not attempt to link infections to pathogens, there are insufficient data to include the pathogen type in the model. The model simplifies by not including a distribution of pathogens for each condition. For example, VAP can be caused by any number of pathogens, including *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, and *Acinetobacter*, among others. Similarly, each type of HAI included in the model can be caused by one (or a combination of) pathogen. There are not at this point sufficient data to accurately estimate how the distribution of pathogens at a facility affects the prevalence of HAI and therefore the proportion that are preventable by pathogen, nor are there data on the differences in cost, if any, by condition based on the causal pathogen. Should these data become available, the model could be enhanced to address this issue.

2.4.2 Epidemiology of HAI: Number of Preventable Cases

There are various estimates in the literature for the proportion of cases that are preventable (1, 6). Cases that are not preventable are eliminated from this analysis, as the use of healthcare antiseptics would not have an effect on these infections. To be conservative, the choice was made to eliminate these infections from the denominator at this point in the analysis rather than to adjust the proportion attributable. For example, Umscheid estimates that 65-70% of CLABSI and CAUTI and 55% of cases of VAP and SSI are preventable. These values may vary across settings, populations, and based on the distribution of pathogens present at a facility, but they provide a starting point for the analysis. In their comprehensive review of the impact of various interventions, Harbarth and colleagues also found wide variation across settings and patient types, but they suggest that 20% is a reasonable proportion of HAI that are preventable, with a range of 10-70% being plausible in certain circumstances (7). Based on the wide range of values in the literature, the model includes multiple options for the proportion of HAIs that are

preventable (20%, 35%, 50%, and 70%). The value chosen can be applied to all infection types although the user can also specify different values for each type of infection. There is also an option for the user to customize the model by introducing a value not already provided as an existing option.

2.4.3 Clinical Findings: Number of Cases Attributable to Healthcare Antiseptics

It is important to note that there appears to be a trend towards increasingly complex hand hygiene interventions. In their meta-analysis, Schweizer and colleagues point out that more than three-fourths of interventions included bundles with multiple components rather than the single-intervention studies that have been observed in previous reviews (8).

Due to challenges in linking specific pathogens to infections, the model makes simplifying assumptions. Consideration was given to whether a single value for the reduction of MRSA-related infections, for example, could be aggregated from the literature and then applied across infections for which MRSA may be causal. This would require having a systematic way to identify the reduction for all possible organisms of interest (or at least a majority of them) for the infections of greater interest (i.e., those highlighted by the CDC in its reports on HAI), and knowing the distribution of causal organisms for each infection of interest. As this is likely highly variable across facilities, it was deemed not feasible and determined that while it would increase precision, it would not yield greater accuracy than using a more qualitative method. Thus, the studies listed in Table 2 provide a set of maximum values upon which to base calculations rather than contributing to a single point estimate for the reduction of cases associated with healthcare antiseptic use.

Table 2. Papers Used to Estimate Number of Cases Attributable to Healthcare Antiseptics

Citation	Condition/Pathogens
Gordin et al., 2005 (9)	MRSA, VRE, C diff
Hayden et al., 2006 (10)	VRE
Lai et al., 2006 (11)	MRSA
Harrington et al., 2007 (12)	MRSA
Pittet et al., 2000 (13)	MRSA
Zoabi et al., 2011(14)	MRSA

Based on the findings reported in the papers listed in Table 2, the model allows the user to select from among the following possible values representing the proportion of cases that could be prevented with healthcare antiseptic use: 10%, 20%, and 30%. The same value can be applied to all types of infections; the model can be modified to allow for different values for each infection type.

2.4.4 Economic Findings: Charges Associated with HAI

A number of reviews and summary papers were found that help guide the search to primary data sources. For example, Scott published a national estimate of HAI counts that also estimated charges in the United States (presented in 2007 $US) (2). As did most of the studies included here, this paper presented a range of values, since there were multiple sources of uncertainty and a lack of consensus on the most appropriate analytic approach. The studies from which charges for each infection of interest were derived appear in Table 3. Other studies were identified in the search, however, they either aggregated infections and adverse events rather than presenting the infections of interest separately, or the study included a very specific population (e.g., only pediatric or only elderly) or a small set of surgical interventions or settings, or they did not present the year in which charge were presented. Therefore, those studies were not included in this analysis.

After inflating charge values to 2014 US$, estimates were aggregated by infection type by taking the average of available estimates in each category. The minimum and maximum attributable charges per infection, after using the value presented in studies from Table 3 and inflating as appropriate, are shown in Table 4. Also provided are estimates inflated from the 2012 values presented in the Zimlichman et al. meta-analysis (3). Zimlichman's meta-analysis, while including papers from very small or specific populations that were rejected based on our methods for identifying charge papers, is the most recent attempt to identify the economic impact of HAI in the US and so its values are highlighted specifically. Although the estimates are based on papers prior to the period of interest for this analysis and some include very narrow populations, these values are provided for reference. Other estimates are also included in the model; these come from papers that provided only a point estimate. They are listed below in Table 3 (which includes papers that were used to develop the high, low, and middle charge estimates used in the model) but are not listed in Table 4 (which includes only the meta-analysis and the papers contributing to the high and low scenarios). Table 5 contains the complete set of charges available for selection in the spreadsheet model.

Table 3. Papers Used to Estimate Charges per HAI

Citation	Condition(s)
Anderson et al. 2009 (15)	SSI (point estimate)
Anderson et al., 2013 (16)	CAUTI, CLABSI, GI, SSI, HAP/VAP
de Lissovoy et al., 2009 (17)	SSI (point estimate)
Eber et al., 2010 (18)	CLABSI, HAP/VAP
Goudie et al., 2014 (19)	CLABSI (point estimate)
Kandilov et al., 2014 (20)	CAUTI, SSI (point estimate)
Kollef et al., 2013 (21)	HAP/VAP (point estimate)
Lipp et al., 2012 (22)	GI (point estimate)
McGlone et al., 2012 (23)	GI
Scott et al., 2009 **(2)**	CAUTI, CLABSI, GI, SSI, HAP/VAP
Warren et al. 2006 (24)	CLABSI (point estimate)
Yi et al., 2014 (25)	CAUTI (point estimate)

Table 4. Charges per Case by HAI: Meta-analysis, Low and High

Condition	Charge per Case (in US $2014)	Sources
CAUTI	$937 $995 to $1,136	Zimlichman et al. (3) Anderson et al. 2013, Scott et al. 2009 (2, 16)
CLABSI	$47,901 $8,158 to $36,808	Zimlichman et al. (3) Anderson et al. 2013, Eber et al. 2010, Scott et al. 2009 (2, 16, 18)
GI	$11,799 $8,305 to $11,438	Zimlichman et al. (3) Anderson et al. 2013, McGlone et al. 2012, Scott et al. 2009 (2, 16, 23)
SSI	$21,732 $14,187 to $39,613	Zimlichman et al. (3) Anderson et al. 2009, Anderson et al. 2013, Scott et al. 2009 (2, 15, 16)
VAP/HAP	$41,973 $18,960 to $43,036	Zimlichman et al. (3) Anderson et al. 2013, Eber et al. 2010, Scott et al. 2009 (2, 16, 18)

Note: The first value listed in the "charge per case" column is the value from the Zimlichman et al meta-analysis. The second and third values are the low and high values derived from averaging the values presented in the papers listed.

Table 5. Charges Available for Selection in Model

	VAP/HAP	SSI	GI	CAUTI	CLABSI
High estimate	$43,036	$39,613	$11,438	$1,136	$36,808
Middle estimate	$30,998	$26,900	$9,872	$1,066	$22,483
Low estimate	$18,960	$14,187	$8,305	$995	$8,158
Zimlichman meta-analysis	$41,973	$21,732	$11,799	$937	$47,901

3 Model and Results

A spreadsheet model designed for this analysis incorporated findings from the literature review, along with the capacity for users to modify inputs to reflect specific papers, trends, or to explore uncertainty. Figure 1 shows a screenshot of the user input selections for the "low" or minimum impact scenario; Figure 2 shows output corresponding output.

Figure 1. Model Inputs – "Low" Scenario

Model Input Parameter	User Selections		
Number of HAI, United States, annual	Magill (10-state survey, extrapolated)		
	All Infections		Individually
Proportion of HAI that are preventable	20%	Pneumonia	The value selected to the left is being
	Please choose to set one rate for	Surgical site infection	The value selected to the left is being
	all infections or each infection individually.	Gastrointestinal infection	The value selected to the left is being
		Catheter-associated UTI	The value selected to the left is being
		Bloodstream infection	The value selected to the left is being
	All Infections		Individually
Proportion of HAI prevention attributable to use of healthcare antiseptics	10%	Pneumonia	The value selected to the left is being
	Please choose to set one rate for	Surgical site infection	The value selected to the left is being
	all infections or each infection individually.	Gastrointestinal infection	The value selected to the left is being
		Catheter-associated UTI	The value selected to the left is being
		Bloodstream infection	The value selected to the left is being
Pneumonia charge (per case)	Low estimate		
Surgical site infection charge (per case)	Low estimate		
Gastrointestinal infection charge (per case)	Low estimate		
Urinary tract infection charge (per case)	Low estimate		
Bloodstream infection charge (per case)	Low estimate		

Figure 2. Model Outputs – "Low" Scenario

Outputs: Current cost of HAI and portion attributable to healthcare antiseptic use

	Number of cases (annual, US)	Number of preventable cases	Charges per case	Charges (current)	Cases currently prevented by antiseptics	Charges currently prevented by antiseptics	Charges (projected) without antiseptic use
Pneumonia	157,500	31,500	$ 18,960	$ 597,239,603	3,150	$ 59,723,960	$ 656,963,564
Surgical site infection	157,500	31,500	$ 14,187	$ 446,878,064	3,150	$ 44,687,806	$ 491,565,870
Gastrointestinal infection	123,100	24,620	$ 8,305	$ 204,474,032	2,462	$ 20,447,403	$ 224,921,435
Catheter-associated UTI	93,300	18,660	$ 995	$ 18,573,548	1,866	$ 1,857,355	$ 20,430,902
Bloodstream infection	71,900	14,380	$ 8,158	$ 117,312,203	1,438	$ 11,731,220	$ 129,043,423
TOTAL	**603,300**	**120,660**		**$ 1,384,477,449**	**12,066**	**$ 138,447,745**	**$ 1,522,925,194**

Two scenarios are presented in this report to provide a range of estimates for the healthcare expenditures currently being averted based on the use of healthcare antiseptics. The "low" and "high" estimates use the following inputs, as shown in Table 6. Some published estimates for the number of HAI contained lower or higher counts for specific infection types than the estimates used in these scenarios. However, the selected estimates result in the lowest and

highest total expenditures when totaled across infection types. That is, by manipulating individual inputs, one could arrive at higher or lower inputs, but the low and high scenarios selected here use all the estimates for the conditions of interest from a single published paper (Magill for the low, Scott for the high) for consistency.

Table 6. Low and High Scenario Input Parameters

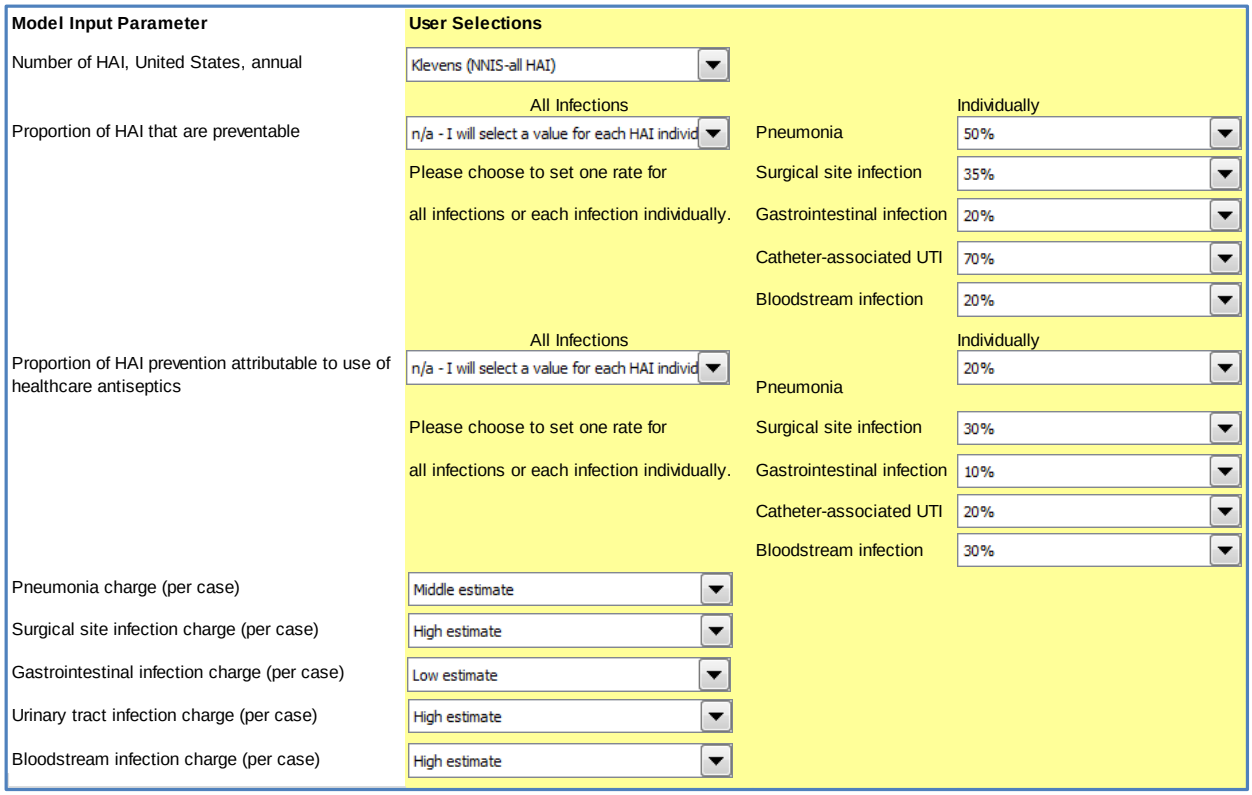
Parameter	Low	High
Number of HAI, United States, annual	Magill et al. (5)	Scott et al. (2)
Proportion of HAI that are preventable	20%	70%
Proportion of HAI prevention attributable to use of healthcare antiseptics	10%	30%
Charges per HAI	Average low estimate from literature review	Average high estimate from literature review

Based on the findings, the potential additional burden of hospital-acquired infections avoided by the use of healthcare antiseptics is $175 million to $4 billion annually in the United States. These results are presented in Table 7.

Other scenarios not detailed in this table include intermediary values for the number of HAI in the United States annually, the proportion of these HAI that are preventable, the proportion preventable through the use of healthcare antiseptics and the charge per case for the above-listed infection types. For example, the distribution of types of HAI varies among the estimates provided as possible input parameters. Depending on the distribution, the use of the high or low charge estimates from the literature can vary the total potential charges averted by different amounts. However, while modifying individual input parameters can vary the findings, the approach taken here – to use a consistent set of values (i.e., not to vary charges or the proportion attributable by condition) – provides a reasonable baseline for discussion.

In order to illustrate how the model's inputs can be varied, a third figure appears below. In this version of the model input sheet, the user has created a model in which the proportion of preventable HAI varies by infection rather than using a single value across all HAI. Similarly, the user has chosen to select different values for the proportion of HAI attributable to healthcare antiseptics. Finally, the user has chosen a variety of values to use for charges (all of which can be viewed directly in a supporting worksheet of the spreadsheet model). The input parameters provided in Figure 3 demonstrate the flexibility that the user has in specifying inputs; the specific inputs shown in the image are not endorsed.

Figure 3. Model Inputs – Illustrative Only



Given the uncertainty around many of the estimates in this model, typical sensitivity analyses are not necessary. The results presented here instead provide a minimum and maximum estimate of the potential increase in cases and medical expenditures associated with elimination of healthcare antiseptic use. It is expected that actual potential increases would fall somewhere between these minimum and maximum estimates. However, should alternatives be proposed, such as limiting use to certain settings, for example, the model can be re-run with a different set of input parameters. In that case, it may be more reasonable to enter a lower number of HAI cases in a particular setting, with a higher or lower likelihood of prevention associated with HAI. These types of "what if" scenarios can be modeled upon request.

Table 7. Estimate of Potential Additional National Economic Burden

	Low estimates				High estimates			
Condition	**Current charges**	**Number of current cases**	**Charges prevented**	**Number of cases prevented**	**Current charges**	**Number of current cases**	**Charges prevented**	**Number of cases prevented**
CAUTI	$19M	93.3K	$2M	1.9K	$357M	449.3K	$107M	94.4K
CLABSI	$117M	71.9K	$12M	1.4K	$2,370M	92.0K	$711M	19.3K
GI	$205M	123.1K	$21M	2.5K	$1,425M	178.0K	$428M	37.4K
SSI	$447M	157.5K	$45M	3.2K	$8,055M	290.5K	$2,416M	61.0K
VAP/HAP	$597M	157.5K	$60M	3.2K	$1,582M	52.5K	$475M	11.0K
Total	**$1,385M**	**603.3K**	**$175M**	**12.1K**	**$13,791M**	**1,062.4K**	**$4,137M**	**223.1K**

1. Charges are presented in millions of dollars. The numbers of cases are presented in thousands.
2. "Charges prevented" and "Cases prevented" refer to the cases and associated charges that are estimated to be currently prevented by the use of antiseptics.
3. Totals may differ from the expected value due to rounding.
4. As the number of cases in the low and high scenarios differ for each condition, the relationships between low and high estimates are influenced by factors other than the portion attritutable and proportion associated with antiseptics and results are not linear.

4 Discussion

4.1 Uncertainty and Limitations

There are multiple sources of uncertainty associated with each model input parameter. To address this uncertainty and corresponding lack of consensus regarding input parameter estimates, the model provides the user with the ability to select input parameter values from a range of plausible values.

Attribution of an infection to acquisition in a hospital setting (i.e., a "true" HAI) is complicated by the fact that patients may be colonized upon admission to the hospital. For example, they may have a community-acquired infection, or an infection acquired in a skilled nursing facility, among other sources. Thus, the number of HAI may be overestimated although the numbers offered in the model are derived from published, peer-reviewed studies that describe limitations of their methods (2, 4, 5).

Healthcare antiseptics, particularly alcohol-based hand rubs and gels, may have beneficial effects in preventing both bacterial and viral infections. Currently the model only accounts for bacterial infections. Thus, the benefits of healthcare antiseptics may be underestimated. However, at this point there is insufficient data in the literature to support the inclusion of benefits for viral infections.

Attribution of healthcare antiseptics to prevention rates is impossible because of the multi-part strategy to address hygiene (World Health Organization guidance (26)) and the fact that the isolated effect of healthcare antiseptics on prevention rates is not addressed in the literature for infection types of interest. For this reason, the model requires the user to specify the proportion of cases that are preventable for a particular type of infection.

There is uncertainty about the financial impact of HAI. A variety of studies with different methods and approaches have been used to develop estimates for the model, relying on averages of charge estimates to minimize the influence of individual studies. Furthermore, actual costs and reimbursements may differ and continue to diverge as policies that deny reimbursements for HAI are being implemented. As a result, the financial impact may depend on the stakeholder's perspective (e.g., provider, payer).

Financial impact estimates may be influenced by variation in hospital specific characteristics (e.g., average patient age, comorbidities) and geographic characteristics (e.g., regional variation of prevalence of pathogens). The model is designed to reflect an average hospital and region in the United States. To assist providers and payers with applying the model findings to their unique situation, sensitivity analysis has been conducted to obtain the most and least conservative financial impact estimates ($175 million to $4 billion). However, the model cannot be readily applied to a specific hospital and/or region without a detailed review and adjustment of model assumptions.

The model estimate for the currently averted expense by the use of antiseptics is based on an estimate of the number of preventable infections from proper antiseptic use. There may be difference between the numbers of HAI prevented versus HAI preventable. Thus, there may be an overestimate of averted expenses by using preventable versus prevented HAI.

The model does not include a number of additional costs that may be relevant to calculating the impact of removing antiseptic products from the market. These costs include but are not limited to: hospital readmissions, short-term rehabilitation, long-term follow-up care, lost wages, lost productivity and transportation. General estimates for these elements are available in the published literature and could be combined with the HAI-specific inputs to this model to produce a more comprehensive scope of costs. For example, published studies have explored the societal costs associated with antimicrobial resistant infections (27) or with infections identified in this model, although not specific to hospital-acquired cases (23). The research supporting these values is sparse; it was determined that it would add too much uncertainty to the model results to include a module with other types of HAI-related costs.

4.2 Conclusions

The purpose of a model is to help guide decision-making in the face of uncertainty. This model is a simplification of a complex real-world relationship between changing rates of infection, charges, and the potential impact of healthcare antiseptics. The model reflects the current state of knowledge while providing the opportunity to explore uncertainty. The minimum and maximum estimates presented in this report can be used to understand the potential economic impact of a change in availability of healthcare antiseptics on human health in the United States but it should be noted that the values presented here are restricted to direct medical charges and do not include follow-up care or indirect costs of any sort. These non-medical costs are currently less well-documented but likely to increase the economic impact of limiting healthcare antiseptic availability.

5 References

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