HEALTHCARE ANTISEPTIC
TENTATIVE FINAL MONOGRAPH
[Docket FDA-2015-N-0101]

ETHYL ALCOHOL

INFORMATION PACKAGE

FDA MEETING JULY 30, 2015

GOJO®

Table of Contents

Product Names

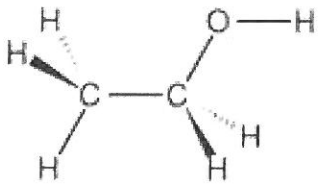
Alcohol Based Hand Rub (ABHR)

Alcohol Based Surgical Hand Rub (ABSHR)

Alcohol Based Hand Sanitizer (ABHS)

Alcohol Based Surgical Hand Sanitizer (ABSHS)

Chemical name and structure



Ethyl Alcohol (Ethanol)

CAS #64-17-5

Proposed Indications

Healthcare Personnel Hand Rub

Healthcare Personnel Surgical Hand Rub

Healthcare Personnel Hand Sanitizer

Healthcare Personnel Surgical Hand Sanitizer

Dosage Form, Route and Dosing Regimen

Leave-on topical gel, liquid, foam or wipe applied to hands as needed

Attendees

- Michael Dolan, Sr. Advisor, Science & Technology Vice President, GOJO Industries, Inc.
- Srini Venkatesh, PhD, Chief Science Officer and Vice President, GOJO Industries, Inc.
- Plinio De Goes, Regulatory Affairs & Product Safety Vice President, GOJO Industries, Inc.
- David Macinga, PhD, Research Fellow Microbiology and Clinical Science, GOJO Industries, Inc.
- Antonio Quiñones-Rivera, PhD, Product Safety Manager, GOJO Industries, Inc.
- Beth Glasgow, Sr. Global Regulatory Affairs Specialist, GOJO Industries, Inc.
- Professor Andrew Maier, MS, PhD, CIH, DABT. TERA/U. of Cincinnati, Consultant
- Bruce Stouch, PhD, President and Principal Biostatistician, BCS Statistical Solutions LLC, Consultant

Background

Brief history of the development program and events leading up to meeting

In the September 3, 2014, Briefing Document to the Nonprescription Drugs Advisory Committee (NDAC)[1] and May 1, 2015 Tentative Final Monograph "Safety and Effectiveness of Health Care Antiseptics; Topical Antimicrobial Drug Products for Over-the-Counter Human use"[2], FDA indicated that their administrative record for the safety of alcohol was incomplete with respect to: 1) human pharmacokinetic studies under maximal use conditions when applied topically and; 2) data to help define the effect of formulation on dermal absorption.

These stated gaps in the administrative record led GOJO Industries, Inc. to sponsor an investigation that included a critical review of the epidemiological and animal toxicity literature for ethanol, review of clinical PK studies with alcohol-based hand rubs, application of a physiologically-based pharmacokinetic (PBPK) model to a series of product use scenarios, and a risk assessment. This analysis was presented as a poster at the Society of Toxicology Meeting, March 2015 (Appendix A) as well as detailed in a manuscript (Maier et al., 2015 "Safety Assessment for Ethanol-Based Topical Antiseptic Use by Health Care Workers: Evaluation of Developmental Toxicity Potential") which was recently accepted for publication in the peer-reviewed journal, Regulatory Toxicology and Pharmacology. This paper will be submitted separately to FDA Docket No. FDA-2015-N-0101. Additionally, GOJO has assembled data related to formulation effects upon alcohol absorption that address this data gap.

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[1] FDA Briefing Document for a Meeting of the NDAC Healthcare Antiseptic Ingredients; Topic: Pre-market safety testing framework for over-the-counter healthcare antiseptic drugs; September 3, 2014 http://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/Drugs/NonprescriptionDrugsAdvisoryCommittee/UCM410289.pdf

[2] Topical Antimicrobial Drug Products for Over-the-Counter Human Use; Proposed Amendment of the Tentative Final Monograph; Reopening of Administrative Record

Status of product development (e.g., the target indication for use)

In the May 1, 2015 Tentative Final Monograph "Safety and Effectiveness of Health Care Antiseptics; Topical Antimicrobial Drug Products for Over-the-Counter Human use"[3], FDA indicated that their administrative record for the efficacy of ethyl alcohol safety was incomplete, and specified a number of data requirements and test protocol details to fill these gaps. GOJO initiated data reviews of past efficacy studies on alcohol-based skin disinfectants, as well as study designs to address the new efficacy requirements. These efforts have resulted in various proposals for FDA consideration prior to initiation of pilot and pivotal efficacy studies.

Ethyl Alcohol as an active ingredient in topical antiseptics has an established history of safe and effective use, and has been on the market for over 20 years without any safety signals when used as indicated. Products based on ethyl alcohol have become a standard of care in institutional healthcare settings.

GOJO intends to collaborate with FDA to ensure the safety and efficacy of alcohol-based skin antiseptics, a critical public health tool across healthcare and consumer settings.

[3] Topical Antimicrobial Drug Products for Over-the-Counter Human Use; Proposed Amendment of the Tentative Final Monograph; Reopening of Administrative Record

Purpose of meeting

The purpose of the meeting is to discuss and obtain feedback on the following:

a. Adequacy of available human PK data with ABHRs to establish the range of systemic exposure to ethanol from ABHRs

b. Application of PBPK modeling to evaluate the systemic exposure to ethanol from ABHRs at maximal use conditions

c. Adequacy of available data to demonstrate that product formulation has no clinically significant impact on ethanol absorption from ABHRs

d. *In vitro* and *in vivo* efficacy test requirements for ethyl alcohol

e. Proposed *in vitro* and *in vivo* test methods, protocols and study parameters

f. Applicability of safety and efficacy data across topical antiseptic monographs (i.e., Consumer and Healthcare)

Proposed agenda

- Introductions (5 minutes)
- GOJO Opening Remarks (5 minutes)
- Discussion (40 minutes)
- Summarize agreements and action items (10 minutes)

Clinical Pharmacology Questions (#1- #6)

In the 2015 Proposed Amendment of the 1994 Tentative Final Monograph for over-the-counter (OTC) antiseptic drug products[4], FDA indicated that their administrative record for the safety of alcohol is incomplete with respect to the following:

- Human pharmacokinetic studies under maximal use conditions when applied topically (MUsT) and
- Data to help define the effect of formulation on dermal absorption.

An extensive literature search was conducted to identify available data for use in assessing human pharmacokinetics of alcohol in ABHRs under maximal use conditions. A number of human studies were found that evaluated the absorption and systemic exposure to ethanol in a variety of ABHRs across a range of alcohol concentrations as well as excipient compositions. These studies provided significant information to address the human pharmacokinetics of ethanol in ABHRs.

The literature search included an extensive, critical review of published studies of the toxicology of ethanol. A physiologically-based pharmacokinetic (PBPK) model was applied to evaluate exposures of healthcare workers using alcohol-based handrubs (ABHRs). The methodology and findings were summarized in a manuscript (Maier et al., 2015 "*Safety Assessment for Ethanol-Based Topical Antiseptic Use by Health Care Workers: Evaluation of Developmental Toxicity Potential*") which was recently accepted for publication in Regulatory Toxicology and Pharmacology. This manuscript will be submitted separately to FDA Docket No. FDA-2015-N-0101.

In the sections below on Clinical Pharmacology and formulation effects is a discussion of how these data address the two safety data gaps identified by FDA.

[4] Federal Register / Vol. 80, No. 84 / Friday, May 1, 2015 / Proposed Rules.

Conclusions

- The human pharmacokinetics data for ethanol absorption from different ABHRs applied under a variety of conditions are extensive and sufficiently robust to support the conclusion that dermal absorption of ethanol from ABHRs is low and does not exceed 2-3% of the applied dose. Further, these data are representative of actual exposure in real world healthcare settings and inclusive of conditions that would be evaluated in a MUsT study design.

- Application of a PBPK model to evaluate absorption of ethanol under a variety of ABHR application scenarios further supports the conclusion that dermal absorption of ethanol is low. Additionally, this model demonstrated that ethanol exposure to healthcare workers via ABHRs is comparable to systemic exposure levels of ethanol from dietary sources generally deemed safe.

- The effect of formulation was evaluated via literature review, including assessment of dermal permeation enhancement by excipients used in ABHRs/ABSRs. Confirmatory in vitro studies further demonstrated insignificant formulation effects on extent of alcohol absorption.

- Collectively, the current data provide a *de facto* assessment of maximal use exposure. Therefore, a formal maximal use trial (MUsT) would not provide sufficient new information to materially improve the understanding of the human pharmacokinetics of ethanol absorbed from the dermal application of ABHRs/ABSHRs.

Data Gap #1 - Human pharmacokinetic studies under maximal use conditions when applied topically (MUsT)

Summary for Questions #1, #2 and #3 (Clin/Pharm - Human PK)

An evaluation of the absorption of ethanol from ABHRs and the resulting systemic exposure under conditions of maximal use is an important component of the safety assessment of ABHRs. A formal Maximal Use Trial (MUsT) is one way to generate the exposure data for this type of assessment and has been proposed by FDA[4].

Once the human exposure information is known (from MUsT or other data) it can be used in conjunction with the animal ADME and toxicology studies to assess the safety of the active ingredient. Specifically, in reference to the design elements of a MUsT, FDA highlighted that "The duration of dosing must mimic the planned or expected real world use...The goal is, as close as possible, to replicate in a controlled manner the maximal dosing that subjects could be exposed to under product labeling"[5]. Thus, the assessment of the maximum potential exposure to ethanol in ABHRs must be consistent with how a product is expected to be used, in particular as it relates to the following exposure elements:

- Frequency of dosing
- Duration of dosing
- Total involved surface area to be treated at one time and Method of application/site preparation
- Use of highest proposed strength
- Amount applied per square centimeter

All of these elements were incorporated into a systemic exposure assessment using a physiologically-based pharmacokinetic (PBPK) model. For details of the PBPK modeling, refer to Maier et al., 2015 (This paper will be submitted separately to FDA Docket No. FDA-2015-N-0101).

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[5] Overview of Bioavailability Testing for Topical Healthcare Antiseptics. Presentation at the September 2014 NDAC meeting. CAPT E. Dennis Bashaw, Pharm.D. Director, Division of Clinical Pharmacology-3. Office of Clinical Pharmacology, Office of Translational Sciences. http://www.fda.gov/AdvisoryCommittees/CommitteesMeetingMaterials/Drugs/NonprescriptionDrugsAdvisoryCommittee/ucm414580.htm

Briefly, a team of toxicologists and PBPK modeling experts from Toxicology Excellence in Risk Assessment (TERA) applied a published and broadly-used physiologically based pharmacokinetic (PBPK) model (Martin et al., 2012, 2014) to assess the pharmacokinetics of ethanol after use of ABHRs. The Martin et al. model has been developed by USEPA to support risk management decisions regarding environmental exposures to ethanol. To evaluate dermal absorption of ethanol from ABHRs applied chronically by health care workers, a dermal compartment was added, and absorption into the skin was described based on the permeability coefficient (Kp). Product use scenarios were designed to maximize product use rates in a manner consistent with the available evidence of product use. In addition, scenarios based on maximal use assumptions presented by FDA[5] were evaluated. The PBPK model was used to evaluate systemic exposures to ethanol in healthcare workers using ABHRs under the conditions described in the table below:

FDA has a strong history of using PBPK model to support their assessments and decisions. FDA has developed and applied PBPK models for methylmercury (Young et al., 2001), acrylamide (Doerge et al., 2008), bisphenol A (Fisher et al., 2011; Yang et al., 2013), formaldehyde (Mitkus et al., 2013), and methylphenidate (Yang et al., 2014). FDA has also used PBPK models to evaluate drug interactions (Vieira et al., 2012; Grillo et al., 2012; Wagner et al., 2014) and metabolism in pregnant women (Ke et al., 2012, 2013). Furthermore, FDA has recognized that it is not necessary to assess all conditions experimentally, and that PBPK models can be used to assess untested conditions in stating, "An important application of modeling and simulation in drug development is to assess various untested clinical situations" (Zhao et al., 2012; Sinha et al., 2014). Use of the PBPK model for ethanol to simulate MUsT conditions is an appropriate application of the model, and is analogous to the approach used by other scientists in using PBPK models to extrapolate across unusual workshifts for occupational exposures (Andersen et al., 1987; Goyal et al., 1992; Lapare et al. 2003).

ABHR Use Scenarios for Healthcare Workers					
Product Use Scenario Elements	**Hand Hygiene Scenarios**			**Surgical Use Scenarios**	
	Average Use Rate	**High Use Rate**	**Intensive Use Rate**	**Typical Use Rate**	**Intensive Use Rate**
Frequency of dosing	7 times/hr	22 times/hr	30 times/hr	Every 4 hours	Every 4 hours
Duration of dosing	12-hr shift	12-hr shift	12-hr shift	12-hr shift	12-hr shift
Treated surface area/Method of application	To front and back of hands	To front and back of hands	To front and back of hands	Applied to hands and forearms	Applied to hands and forearms
Use of highest proposed strength	90% (w/w)	90% (w/w)	90% (w/w)	61% (w/w)	90% (w/w)
Amount applied	1.3 ml	1.3 ml	1.3 ml	6 ml	20 ml

For all the exposure scenarios considered, the peak BACs were < 1 mg/dL (see Table below).

BACs and AUCs for Use Scenarios for Healthcare Workers					
Predicted Internal Doses	**Hand Hygiene Scenarios**			**Surgical Use Scenarios**	
	Average Use Rate	**High Use Rate**	**Intensive Use Rate**	**Typical Use Rate**	**Intensive Use Rate**
Peak (mg/dL)	0.39	0.75	0.94	0.22	0.33
AUC (mg/dL*hr)	2.3	7.4	10.1	0.17	0.24

Based on the estimated peak BACs, and a NOAEL-LOAEL boundary of 150 mg/dL from animal toxicology studies (see detailed explanation in Maier et al. 2015) the estimated Margins of Exposure (MOE) for all scenarios were >100 (Table below).

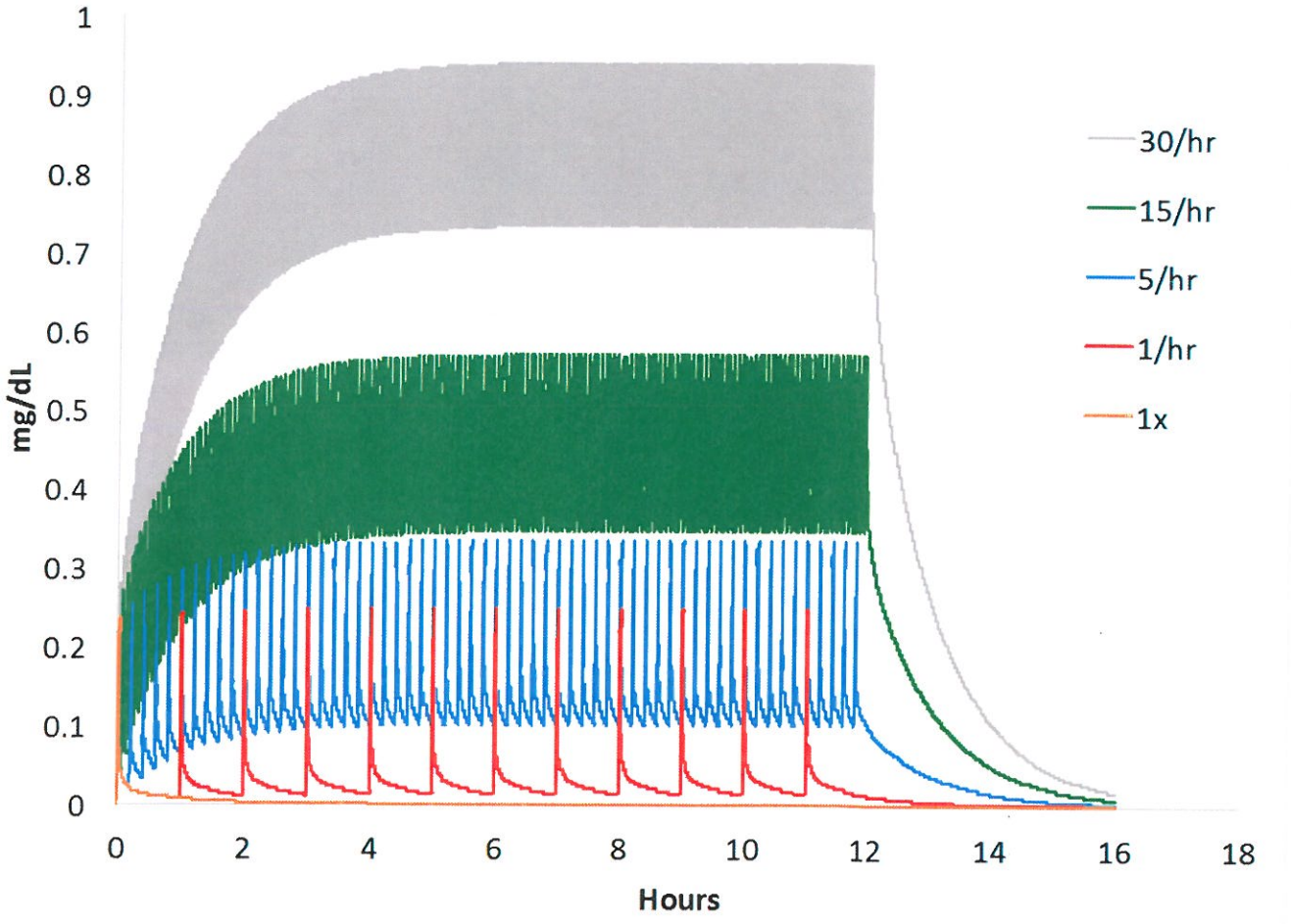
MOEs for Use Scenarios for Healthcare Workers Based on Estimated Peak BACs					
	Hand Hygiene Scenarios			**Surgical Use Scenarios**	
	Average Use Rate	**High Use Rate**	**Intensive Use Rate**	**Typical Use Rate**	**Intensive Use Rate**
MOE NOAEL-LOAEL boundary = 150 mg/dL	380	200	160	680	450

The systemic ethanol exposures associated with the intensive use of ABHRs considered in the PBPK model are in the range of those associated with the safe consumption of many dietary sources that contain ethanol, including flavored

water, soft drinks, fruits, and juices (Logan and Distefano, 1998; Musshoff et al., 2010; Obenland et al., 2008). As an additional point of reference, these exposures measured on a peak and AUC basis are either within or below the range of current OSHA occupational exposure guidelines (Maier et al., 2015).

The PBPK modeling shows that, following a single application of ABHR, the peak concentration of ethanol in blood is predicted to be approximately 0.24 mg/dL. If this exposure is repeated once every hour for 12 hours, the predicted peak blood concentration does not increase by 12-fold, but instead only increases by 5%. More importantly, if this single exposure is repeated 360 times (30 applications/hour for 12 hours) the predicted peak blood concentration does not increase by 360-fold but, instead, by a more modest 4-fold.

Figure 1. Impact of Frequency of ABHR Use Over a 12-Hour Shift on BAC



Rationale for Questions #1, #2 and #3 (Clin/Pharm - Human PK)

- The human pharmacokinetics data for ethanol absorption from different ABHRs applied under a variety of conditions are extensive and sufficiently robust to support the conclusion that dermal absorption of ethanol from ABHRs is low and does not exceed 2-3% of the applied dose. Further, these data are representative of actual exposure in real world healthcare settings and inclusive of conditions that would be evaluated in a MUsT study design.

- A PBPK model was applied to determine the exposure of healthcare workers using ABHRs at rates up to 30 times per hour for a full shift. The predicted peak BACs were less than 1 mg/dL for all scenarios (2 orders of magnitude below the NOAEL-LOAEL boundary) and therefore of no safety concern.

Question #1 (Clin/Pharm Human PK)

Does FDA agree that available human PK studies across various formulations of ABHRs applied under a variety of conditions, including conditions of intensive use, establish the range of the systemic exposure to ethanol from ABHRs?

Question #2 (Clin/Pharm Human PK)

Does FDA agree that the pharmacokinetic parameters, including BAC, AUC, and Tmax, that were obtained by applying a PBPK model to several ABHR use scenarios are appropriate and sufficient to evaluate systemic exposure to ethanol from ABHRs?

Question #3 (Clin/Pharm Human PK)

Does FDA agree that the published human studies of systemic absorption of ethanol from ABHRs and the results of the PBPK modeling for healthcare workers address the safety issues associated with maximal systemic exposure to ethanol and thus, a MUsT in humans is not necessary?

Rationale for Question #4 (General)

In the April 30, 2015 FDA Media Briefing related to release of the May 1, 2015 TFM for healthcare antiseptics it was stated "The FDA does not believe that these ingredients are ineffective or unsafe, but is requesting additional data to establish the safety of long-term, daily repeated exposure to these ingredients in the health care setting. The FDA recommends that health care personnel continue to use these products consistent with infection control guidelines while

additional data are gathered. Health care antiseptics are an important component of infection control strategies in hospitals, clinics, and other health care settings, and they remain a standard of care to prevent illness and the spread of infection."

However, contrary to FDA's stated intent the reclassification of alcohol from Category I in 1994 TFM to Category III in 2015 TFM may be causing some confusion in the health care community and affecting acceptance and use of ABHRs (See Medscape article http://www.healthleadersmedia.com/page-2/QUA-315924/FDA-Seeks-More-Safety-Data-on-Hospital-Antiseptics-Proposes-Rule).

FDA has announced its intent to issue a final monograph for Healthcare Antiseptics in 2018. This schedule results in a potential multi-year scenario of confusion and changes in use patterns for ABHRs. In the interest of public safety FDA should consider completing the safety assessment of alcohol and reclassification to Category I as soon as possible.

Question #4 (General)
Would FDA undertake the safety determination for alcohol and subsequently change the category status from III to IE prior to issuance of the Final Monograph for Healthcare Antiseptics?

Summary for Question #5 (General)

FDA introduced a model for antiseptic hand hygiene products with three distinct user categories; Consumer Hand Wash, Healthcare Antiseptics and Consumer Hand Rubs. The Dec 2013 TFM for Consumer Hand Wash and the 2015 Healthcare Antiseptic TFM both utilized the same safety framework to outline data required to demonstrate active ingredients can be considered GRAS

In the NRDC related Consent Decree filed November 2013, FDA indicated that a TFM for Consumer Hand Rub products would be introduced on June 30, 2016. In a June 5, 2015 Defendants Status Report under the Consent Decree, FDA indicated "FDA anticipates that its work on both the TFM for Consumer Antiseptic Hand Wash Products and TFM for Healthcare Antiseptic Products will also inform the development of the TFM for Consumer Antiseptic Hand Rub Products and FDA has begun drafting the TFM for those hand rub products."

Rationale for Question #5 (General)

The use of ABHRs in the healthcare setting represents the most intensive exposure scenario. FDA recognizes that real-world exposure from health care personnel hand wash and rub and surgical hand scrub and rub use is likely to be greater than from patient preoperative skin preparation use and has thus concluded that MUsT data on an active ingredient for either of these indications also would be sufficient to fulfill the MUsT requirement for a patient preoperative skin preparation4. Consistent with FDA approach this rationale can be applied to the safety assessment of ABHRs used by consumers. Consequently the human safety of hand sanitizers used in consumer settings should be covered by the same data sets examined for healthcare settings.

Thus safety data that supports a GRAS determination for alcohol in healthcare settings should be sufficient for a similar GRAS determination in the Consumer category.

Question #5 (General)

If FDA determines that the submitted alcohol safety data are sufficient to support a GRAS determination for alcohol for healthcare use settings, would FDA also consider the data sufficient for a GRAS determination in the upcoming Proposed Rule for Consumer "Leave-On" products?

Data Gap #2 - Effect of formulation on dermal absorption

Summary for Question #6 (Clin/Pharm - Formulation Effects)

In the May 2015 Proposed Amendment to the 1994 Tentative Final Monograph for over-the-counter (OTC) antiseptic drug products[4], FDA indicated that their administrative record for the safety of alcohol was incomplete with respect to data to help define the effect of formulation on dermal absorption.

The human pharmacokinetics using ABHRs has been well studied to evaluate the systemic exposure to ethanol (Table 1).

	Table 1: Human studies of Ethanol Absorption Using a Variety of ABHRs					
Product Tested	**Ethanol Conc. (%, w/w)**	**Application Regime**	**n**	**Mass Ethanol Applied (grams)**	**Absorption (%)** [6,7]	**Reference**
Sterillium® Virugard	95%	10 20-ml applications in 80 minutes	12	149	0.7	Kramer et al., 2007
Sterillium® Gel	85%			140	1.1	
Manorapid Synergy®	55% (+ 10% 1-propanol)			99	0.5	
Sterillium® Virugard	95%	20 4-ml applications in 30 minutes		60	2.3	
Sterillium® Gel	85%			56	1.1	
Manorapid Synergy®	55% (+ 10% 1-propanol)			40	0.9	
Avagard ®	70%	30 1.2-ml applications in 1 hour	20	21	2.5	Brown et al., 2007
Softasept® N	74.1% (+ 10% 2-propanol)	20 ml for 10 minutes	14	12	0.6	Kirschner et al., 2009
Anisogel 85®	70%	Several 3-ml applications in 4-hr shift	86	5.5	0.2[8]	Ahmed-Lecheheb et al., 2012
Purell™ hand sanitizer	62%	12 1-ml applications/ hr for 10 hrs for 3 days	11	61.8[9]	Not detected in urine. No blood samples collected	Reisfield et al., 2011

[6] The percent absorption is calculated as the absorbed mass of ethanol divided by the total mass of applied ethanol.
[7] When not provided by the authors, the absorbed mass of ethanol was estimated based on an assumed body weight of 72 kg, the maximum blood alcohol concentration reported in the study, and a volume of distribution of 0.6 L/kg body weight.
[8] Ethanol detected in only one subject
[9] grams per day.

These studies demonstrate that ethanol absorption is low, with the highest dermal absorption in the range of 2% of the total dose applied. Moreover, these studies provide evidence that differences in product formulation and application rates do not result in any discernible, clinically-meaningful differences in ethanol absorption.

Kirschner et al. (2009) treated volunteers with patches containing 74.1% ethanol either in an aqueous solution or in a commercial, alcohol based handrub that also contained isopropanol at 10%. After a 10-minute application period, there were no statistically significant differences in pharmacokinetic parameters (Cmax, tmax, AUC) measured up to 60 minutes after application (Kirschner et al., 2009). The highest BAC in this study (0.175 mg/dL) results in an estimated ethanol absorption of 0.6%. The applied volume of product (20 ml) is about 12 times larger than is normally applied in many hand hygiene use scenarios. The exposure time of 10 minutes greatly exceeded the duration of a typical single application of ABHR (less than 30 to 40 seconds). Thus, no differences in absorption based on formulation were manifested in this study.

In a study by Kramer et al. (2007), volunteers applied alcohol based handrubs containing 55%, 85%, or 95% (w/w) ethanol using a hand hygiene scenario or a surgical scenario. Under the hand hygiene scenario, 4 mL of product were applied 20 times to both hands over a 30-minute period. Under the surgical disinfection scenario, a total of 20 mL of product was applied to hands and forearms 10 times in an 80-minute period. In addition to the differences in ethanol concentration, one of these products contained 1-propanol at a relatively high concentration. Under the hand hygiene application scenario, ethanol absorption (calculated as the amount of ethanol absorbed relative to the amount applied onto skin) from ABHRs containing 55%, 85%, or 95% was 0.9%, 1.1%, and 2.3%, respectively. Application of the products using the surgical disinfection use scenario resulted in ethanol absorption values of 0.5, 1.1, and 0.7%. The study by Ahmed-Lecheheb et al. (2012) is notably important because it demonstrates the low absorption of ethanol under real-world conditions even when the product used in this study contained bisabolol, a terpene with known, moderate dermal penetration enhancement activity (Williams and Barry, 2012). Reisfield et al. (2011) conducted a MUsT-type study and examined the urine of 11 subjects who applied ABHRs every 5 minutes for 10 hours on three consecutive days. In all subjects, urine ethanol concentrations at the end of the day were below the quantitation limit (LOQ = 20 mg/dL).

Another perspective for penetration enhancement is the overall concentration of the active ingredient in the formulated product. Although the dermal absorption of

an active ingredient present at <5% in a product could be affected by the remaining 95% of excipients, it is less likely that the dermal absorption of ethanol, generally present in ABHRs at concentrations ranging from 62 to 95%, would be affected by the small balance of excipients. This distinguishes ABHRs from other OTC antiseptics in the evaluation of the potential for the enhanced dermal absorption of the active ingredient.

The stratum corneum (SC) provides skin with its barrier function (Suhonen et al., 1999) and dermal penetration enhancers (DPEs) can interact with the SC in a manner that reduces its barrier capacity (Karande et al., 2005; Williams and Barry, 2015). The proposed modes of action (MOA) and chemical properties of dermal penetration enhancers (DPEs) have been discussed (Engelbrecht et al., 2011; Karande et al., 2005; Suhonen et al., 1999; Williams and Barry, 2015). DPEs generally tend to affect dermal permeability by extraction or fluidization of the lipid bilayers of the stratum corneum (SC) (Karande et al., 2005) or general alterations of the lipid bilayer structure and packing in the SC (Williams and Barry, 2015). Furthermore, effective DPEs tend to share some physical-chemical features (Karande et al., 2005; Williams and Barry, 2015). There is no evidence to indicate that the excipients commonly used in most ABHRs have the physical-chemical properties that would allow them to interact with the SC in a manner that would enhance the dermal absorption of ethanol.

Virtually all U.S. alcohol-based handrubs (ABHRs) contain ethanol at concentrations above 62% and, in a very limited number of products, can be as high as 95%. The remaining balance of the formula is typically composed of water, thickeners, foaming agents, skin care agents, fragrances, and pH adjusters (Table 2). After ethanol, water is the most abundant ingredient in most products, present at approximately 33% in the lower alcohol concentrations. Isopropyl Alcohol, a denaturant, is present at up to 4% and does not impact the absorption of alcohol (Kirschner 2009). Individually, each of the other excipients is present at <1%.

Table 2: Representative Ingredients by Function		
Function	**Ingredient**	**Concentrations in Product**
Antimicrobial agent	Ethyl Alcohol (64-17-5)	~ 63%
Vehicle / Denaturant	Purified Water	~ 33%
	Isopropyl alcohol (67-63-0)	< 4%
Thickeners	Polyquaternium□37 (26161-33-1)	< 1%
	Acrylates/C10□30 Alkyl Acrylate Crosspolymer (Proprietary)	< 1%
Foaming Agents	PEG□12 Dimethicone (68937-54-2)	< 1%
Skin Care Agents	Isopropyl Myristate (110-27-0)	< 1%
	Hydroxyethyl Urea (1320-51-0)	< 1%
	Niacinamide (98-92-0)	< 1%
	Glycerin (56-81-5)	< 1%
	Diisopropyl Sebacate (7491-02-3)	< 1%
	Tocopheryl acetate (7695-91-2)	< 1%
	Caprylyl glycol (1117-86-8)	< 1%
Scent	Fragrance (Proprietary)	< 1%
pH Adjuster	Aminomethyl Propanol (124-68-5)	< 1%

Gajjar et.al. (2014) performed *in vitro* human skin absorption studies using Franz diffusion cells with a range of dosages from 5 to 40 mg/cm^2 at 32° C utilizing a modified version of the OECD GD 428 standard reference method. The cumulative percent of dose absorbed over one hour ranged from 0.20% (10.1 µg/cm^2) for the 5.0 mg/cm^2 dose to 0.12% (47.8 µg/cm^2) for the 40.0 mg/cm^2 dose. For the 24-hour period it ranged from 0.25% (12.6 µg/cm^2) for the 5.0 mg/cm^2 dose to 0.17% (66.3 µg/cm^2) for the 40.0 mg/cm^2 dose. Absorption did not increase significantly over the 24-hour period.

GOJO performed similar Franz diffusion (OECD GD 28) studies using human skin. The test apparatus (Figure 1) used jacketed Franz permeation cells, 11.28 mm, 6 mL chamber volume, with a 1.00 cm^2 exposure area (PermeGear Incorporated, Hellertown, PA). Testing was completed at 32 ± 1° C. The substrate was also human skin (ZenBio Incorporated, Research Triangle Park, NC). The collection media consisted of 5% buffered saline to simulate physiological conditions. The experimental dosage range was higher than Gajjar at 5.0 to 100.0 mg/cm^2 dose.

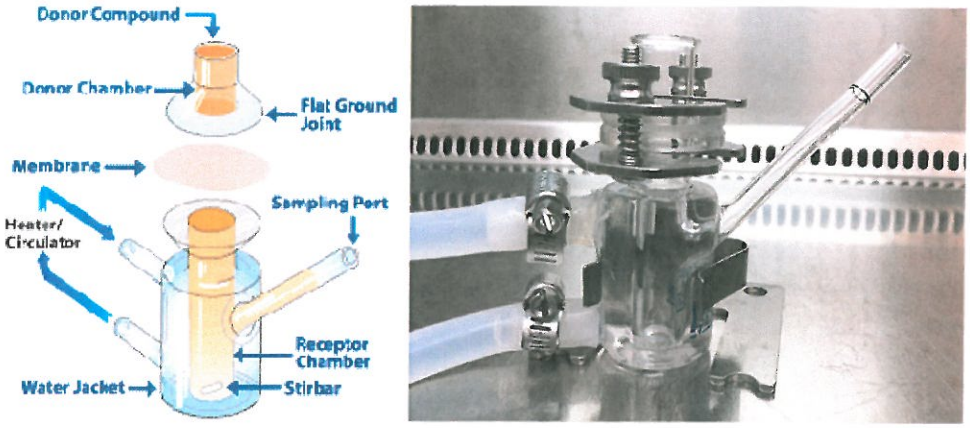


Figure 1. Franz permeation cell and apparatus

The cumulative percent of ethanol dose absorbed over a one-hour interval ranged from 0.48% (24.1µg/cm^2) for the 5.0 mg/cm^2 dose to 0.17% (168 µg/cm^2) for the 100.0 mg/cm^2 dose. A similar study was completed with PURELL® Advanced Gel, a benchmark formulated product. Results ranged from 0.54% (27.2 µg/cm^2) for the 5.0 mg/cm^2 dose to 0.15% (146 µg/cm^2) for the 100.0 mg/cm^2 dose. Although the ethanol content was identical, no difference was noted in absorption through human skin between the unformulated ethanol and the benchmark formulated product

Based on the standard OECD method, the published data, and GOJO data show less than one percent absorption, and similar results between ethanol alone and formulated product. Absorption ranged from 0.12 to 0.54% depending on the dosage and time test period. The results of these multiple empirical studies are consistent, and lower than statistical modeling data that yield results of less than three percent.

Rationale for Questions #6 (Clin Pharm - Formulation Effects)

1. Human studies conducted with ABHRs of different compositions demonstrate that the absorption is in the range of 2% and variations in product formulation do not produce discernible, clinically-relevant differences in dermal absorption of ethanol.

2. There is no evidence to indicate that the excipients commonly used in ABHRs have the physical-chemical properties that would allow them to interact with the stratum corneal in a manner that would enhance the dermal absorption of ethanol.

3. The standard OECD method, published data, and *in vitro* studies conducted by GOJO consistently demonstrate less than one percent ethanol absorption across a variety of formulations.

Taken collectively these findings confirm that variations in product composition will not have a significant, clinically-meaningful effect on the dermal absorption of ethanol.

Question #6 (Clin/Pharm - Formulation Effects)

Does FDA agree that the body of evidence discussed above demonstrate that product formulation has no clinically-significant impact on the absorption of ethanol from ABHRs? If not, what additional *in vitro* studies does FDA recommend be conducted to address the effect of product formulation?

Microbiology Questions (#7 - #11)

Summary for Questions #7 and #8 (Microbiology)

In the Healthcare Antiseptic TFM released May 1, 2015, FDA states that "a GRAE determination for a health care antiseptic active ingredient should be supported by adequate *in vitro* characterization of the antimicrobial activity of the ingredient." Acknowledging that "the ability of an antiseptic to kill microorganisms, rather than inhibit them, is more relevant for a topical product" FDA has requested the following *in vitro* efficacy study to demonstrate the spectrum of activity of the antiseptic:

> *MIC or MBC testing of 25 representative clinical isolates and 25 reference (e.g., American Type Culture Collection) strains of each of the 23 microorganisms listed in the 1994 TFM (59 FR 31402 at 31444).*

Rationale for Question #7 and #8 (Microbiology)

In Appendix B, we propose a study synopsis in which ethanol will be evaluated by both MIC and MBC using standardized methods against one thousand one-hundred (1,100) unique microorganism strains. The microoganisms will consist of twenty-five (25) American Type Culture Collection [ATCC] strains and twenty-five (25) clinical Isolates of each of the twenty-two (22) microorganisms listed in the Tentative Final Monograph, Federal Register, 17 June 1994, vol. 59:116, p. 31444.

Question #7 (Microbiology)

We identified 22 unique microorganisms in the 1994 TFM (Appendix B). Is there an additional microorganism listed in the 1994 TFM (59 FR 31402 at 31444) that FDA has identified and would like us to test?

Question #8 (Microbiology)

Does FDA agree with the proposed in vitro efficacy testing plan for MIC/MBC (Appendix B)?

Summary for Question #9 (Microbiology)

In the Healthcare Antiseptic TFM released May 1, 2015, FDA requests the following in vitro efficacy study to evaluate kinetics of antimicrobial activity:

- Time-kill testing of each of the microorganisms listed in the 1994 TFM (59 FR 31402 at 31444) to assess how rapidly the antiseptic active ingredient produces its effect. The dilutions and time points tested should be relevant to the actual use pattern of the final product.

Rationale for Question #9 (Microbiology)

In Appendix C, we propose a study synopsis in which Time-Kill testing of ethanol will be carried out according to ASTM E2783-11. We propose to test 3 concentrations of ethanol (60%, 70%, and 95% v/v), which span the range at which ethanol is proposed for each of the the three antiseptic uses in the 2015 TFM *(80 FR 25166 at 25171).* The evaluation will be carried out at a single exposure time, 15 s, which is relevant to the use pattern of Health care personnel hand rubs, which has the shortest use time of the three proposed uses for ethanol. In our experience, products containing ethanol consistently reduces the proposed microorganisms to below detection limits in 15 s and therefore do not believe additional information would be gained by testing at longer contact times (Appendix D).

Question #9 (Microbiology)

Does FDA agree with the proposed *in vitro* efficacy testing plan for Time-Kill (See Appendix C)?

Summary for Question #10 (Microbiology)

In GOJO's Pre-Investigational New Drug Application (PIND 110270) file, FDA recommended that an *in vitro* study include the following ATCC strains:

Gram-positive organisms
1. Enterococcus faecalis (ATCC 19433 and ATCC 29212)
2. Vancomycin-Resistant Enterococcus faecalis (VRE) (ATCC 51299 and ATCC 51575)
3. Enterococcus faecium (ATCC 19434 and ATCC 51559)
4. Staphylococcus aureus (ATCC 6538 and ATCC 29213)
5. Methicillin-Resistant Staphylococcus aureus (MRSA) (ATCC 33591 and ATCC 33592)
6. Staphylococcus epidermidis (ATCC 12228 and 1 additional reference strain)
7. Methicillin-Resistant Staphylococcus epidermidis (MRSE) (ATCC 51625)
8. Streptococcus pneumoniae (ATCC 6303 and ATCC 49619)
9. Streptococcus pyogenes (ATCC 14289 and ATCC 19615)

Gram-negative organisms
10. Burkholderia cepacia (ATCC 25416 and ATCC 25608)
11. Escherichia coli (ATCC 11775 and ATCC 25922)
12. Klebsiella pneumoniae (ATCC 13883 and ATCC 27736)
13. Pseudomonas aeruginosa (ATCC 15442 and ATCC 27853)
14. Serratia marcescens (ATCC 8100 and ATCC 14756)

Yeast
15. Candida albicans (ATCC 18804 and ATCC 66027)

Rationale for Question #10 (Microbiology)

GOJO believes these strains are more relevant in health care settings than the organisms listed in the 1994 TFM, and that using this updated list of microorganisms is more aligned with recent NDAs for this category. Therefore, GOJO would recommend allowing testing using this list of microorganisms in place of the 1994 TFM microorganism list.

Question #10 (Microbiology)

Does FDA believe it would be more appropriate to use the updated list of microorganisms above which is more representative of organisms of concern in healthcare settings today instead of the list of microorganisms contained in the 1994 TFM?

Summary for Question #11 (Microbiology)

In the Healthcare Antiseptic TFM released May 1, 2015, FDA states that they "agree that the in vitro testing proposed in the 1994 TFM is overly burdensome for testing every final formulation of an antiseptic product that contains a GRAE ingredient." (80 FR 25166 at 25177).

Rationale for Question #11 (Microbiology)

In our extensive experience, products containing ethanol consistently reduce the proposed microorganisms to below detection limits in 15 s in Time-Kill studies. Further, healthcare antiseptic products containing alcohol are used at full strength and not diluted. We therefore do not believe that testing of final products containing alcohol by MIC/MBC or Time-Kill provides critical acceptability information. Therefore, this testing should not be mandatory for ethanol-containing products in the future final healthcare antiseptic monograph.

Question #11 (Microbiology)

Does FDA agree that if ethanol is established as GRAE and is included in the final healthcare antiseptic monograph, *in vitro* testing of ethanol-containing products should not be a mandatory requirement?

Clinical Questions (#12- #20)

The Healthcare Antiseptic TFM released May 1, 2015, FDA states that "clinical simulation testing when adequately controlled also can be used to demonstrate that an active ingredient is GRAE for use in a health care antiseptic product" (80 FR 25166 at 25178) and recommends the use of test methods proposed in the 1994 TFM to conduct this testing.

Surgical Hand Rub

Summary for Questions #12-13 (Surgical Scrub)

According to the Healthcare Antiseptic TFM released May 1, 2015, FDA is recommending that the bacterial log reduction studies used to demonstrate that an active ingredient is GRAE for use in Surgical hand rubs include the following:

- A negative (or vehicle) control
- Log reduction of 2.5 $\log_{10}$ on each hand within 5 minutes after a single rub
- An active control that meets the appropriate log reduction criteria.
- Threshold of at least a 70 percent success (responder) rate (80 FR 25166 at 25178)

Rationale for Question #12 (Surgical Scrub)

The Healthcare Antiseptic TFM released May 1, 2015, proposes ethanol at concentrations of 60%-95% v/v for each of the three antiseptic uses (80 FR 25166 at 25171). GOJO has not evaluated ethanol by ASTM E 1115 (Appendix E) using the success criteria proposed in the 2015 TFM and must therefore conduct a pilot study to identify the most appropriate ethanol concentration to evaluate in the pivotal study. The initial pilot test of approximately 3-5 test arms will be used to identify the minimum concentration of ethanol needed to meet the proposed efficacy requirement.

Table: Example Pilot E1115 Study

Test Product	Application Volume*
Ethanol 60% v/v	3 applications of 2mL
Ethanol 70% v/v	3 applications of 2mL
Ethanol 80% v/v	3 applications of 2mL

*Per Directions for use as Surgical Hand Rub

In the pivotal study, we propose to test a single concentration of ethanol (equal to or greater than 60% volume/volume) at one application volume, which would establish the minimum concentration of ethanol for a Surgical Rub indication. We propose that by establishing the lower range of the effective dose for ethanol, it will not be necessary to test additional concentrations of ethanol to establish 95% volume/volume as the upper range of a GRAE.

Question #12 (Surgical Scrub)

We proposed to conduct a pilot study(ies) (Appendix F) to evaluate multiple concentrations of ethanol at multiple application volume and a pivotal study evaluating a single concentration of alcohol at one application volume as determined by the pilot study. Does FDA agree with the proposed approach?

Rationale for Question #13 (Surgical Scrub)

We are aware of only one FDA approved, alcohol-based product with a Surgical Hand Rub indication (Avagard, NDA 21-074). Based on the data provided in the NDA for this product, (see excerpts below), it is highly unlikely that Avagard would meet the proposed FDA success criteria using E 1115 (*Standard Test Method for Evaluation of Surgical Hand Scrub Formulations*). Therefore, GOJO is not aware of any FDA approved product that has been shown to meet the new requirements for active ingredients outlined in the 2015 TFM and proposes that no active control should be required for inclusion in the study. Instead, we propose that the data provided for ethanol at test conditions used in our proposed study be used to establish ethanol as the active control in future studies for products seeking a Surgical Hand Rub indication.

Excerpt from Avagard NDA 21-074

The following table is taken from the Avagard Summary Basis of Approval (SBA) Microbiology Review (Pg. 17).

Table 10. Post-treatment log reductions achieved and descriptive statistics calculated for the test and control products Hibiclens, HPD-5a vehicle (HPD-5b) and HPD-5a (Avagard) for as-treated subjects.*

Day/Time point for enumeration	Treatment groups		
	HDP-5a (N=34)	HDP-5b (N=31)	Hibiclens (N=20)
Day 1 Log Reductions			
N	21	21	13
1 minute± SD (95% CI)***	2.6± 1.53 (1.9, 3.3)	1.1± 1.61 (0.4, 1.8)	1.6± 1.47 (0.7, 2.5)
N	23	21	14
3 Hours± SD (95% CI)	3.1± 0.94 (2.7, 3.6)	1.4± 0.83 (1.0, 1.8)	1.8± 0.98 (1.2, 2.4)
N	24	20	13
6 hours± SD (95% CI)	2.8± 1.06 (2.3, 3.2)	0.5± 0.69 (0.2, 0.8)	1.4± 0.90 (0.8, 1.9)

The following table is taken from the Avagard SBA Microbiology Review (Pg. 20).

Table 13. Post-treatment log reductions achieved and descriptive statistics calculated for HPD-5a and Hibiclens, for intent-to-treated subjects (LIMS 7957).

Day/Time point for enumeration	Treatment groups	
	HDP-5a (N=27)	Hibiclens (N=25)
Day 1 Log Reductions		
N	18	17
1 minute± SD (95% CI)***	2.5±0.82(2.1, 2.9)	1.8±0.55(1.5, 2.1)
N	19	16
3 Hours± SD (95% CI)	2.6±0.92(2.0, 3.0)	1.8±0.8(1.3, 2.1)
N	17	17
6 hours± SD (95% CI)	2.2±1.04(1.6, 2.7)	1.9±0.66(1.6, 2.3)

Question #13 (Surgical Scrub)

Does FDA agree that the proposed *in vivo* study synopsis using ASTM E1115 (Appendix E), which we propose be conducted without an active control is acceptable for efficacy evaluation for a surgical hand rub indication (Appendix G)?

Healthcare Personnel Hand Rub

Summary for Questions #14-18 (HCPHR)

According to the Healthcare Antiseptic TFM released May 1, 2015, FDA is recommending that the bacterial log reduction studies used to demonstrate that an active ingredient is GRAE for use in health care personnel hand rubs (HCPHR) include the following:

- A negative (or vehicle) control
- Log reduction of 2.5 $\log_{10}$ on each hand within 5 minutes after a single rub
- An active control that meets the appropriate log reduction criteria.
- Threshold of at least a 70 percent success (responder) rate (80 FR 25166 at 25178)

Rationale for Question #14 (HCPHR)

GOJO previously proposed use of ASTM E2755 to support a hand rub indication (PIND 110270), and received FDA agreement that the method is suitable for this purpose. The following is an excerpt from the 2011 briefing document that explains the rationale for ASTM E 2755 being a more appropriate method for evaluation of hand rubs than the method outlined in the 1994 TFM.

> "Rationale for use of ASTM E2755-10 in place of the Healthcare Personnel Handwash standard (ASTM E1174-06): The HCPHW method (ASTM E1174) was designed to evaluate antimicrobial hand washing agents, which are lathered with the aid of water and then rinsed off. In the absence of a method specifically designed to evaluate leave-on products, E1174 has become the default method to evaluate their clinical efficacy. However, E1174 presents several technical issues when evaluating leave-on products. Most importantly, the bacterial challenge suspension typically remains wet on the hands when the test product is applied thus diluting the active ingredient, and compromising activity. Furthermore, as multiple contamination/product application cycles are conducted, excessive soil load from the bacterial challenge builds up and the hand wetness is exacerbated. These test conditions are counter to the recommended use conditions for leave-on products (i.e. dry, unsoiled hands). In 2010, ASTM International approved a new standard test method specifically developed to evaluate hand sanitizers which more closely mimics in use conditions. This method designated E2755-10, follows the same overall design of E1174 with the exception that hands are contaminated with a greatly reduced volume of challenge organism (200 microliters). The reduced volume leaves the hands dry and relatively unsoiled when product is applied, allowing typical product volumes to be tested and resulting in realistic product dry times".

We propose using ASTM E2755 (*Standard Test Method for Determining the Bacteria-Eliminating Effectiveness of Health Care Personnel Hand Rub Formulations Using Hands of Adults*) to conduct clinical simulation studies to demonstrate the efficacy of ethanol for use as a Healthcare Personnel Hand Rub.

Question #14 (HCPHR)

Does FDA agree that the proposed *in vivo* study using ASTM E2755 (Appendix H) is acceptable for efficacy evaluation of ethanol for a Healthcare Personnel Hand Rub indication?

Summary for Questions #15 and #16 (HCPHR)

The Healthcare Antiseptic TFM released May 1, 2015, proposes ethanol at concentrations of 60%-95% v/v for each of the three antiseptic uses (80 FR 25166 at 25171).

Rationale for Questions #15 and #16 (HCPHR)

GOJO has not evaluated ethanol by ASTM E 2755 using the success criteria proposed in the 2015 TFM and must therefore conduct a pilot study to identify the most appropriate ethanol concentration and application volume to evaluate in the pivotal study. The initial pilot test of approximately 3-5 test arms will be used to identify the minimum concentration of ethanol and the minimum application volume needed to meet the proposed efficacy requirement.

Table: Example Pilot E2755 Study

Test Product	Application Volume
Ethanol <60% v/v (e.g., 50%)	3 mL
Ethanol 60% v/v	3 mL
Ethanol 70% v/v	2 mL
Ethanol 70% v/v	3 mL

*Per Directions for use as Healthcare Personnel Hand Antiseptic

In the pivotal study, we propose to test a single concentration of ethanol (equal to or greater than 60% volume/volume) at one application volume, which would establish the minimum concentration of ethanol for a Health Care Personnel Hand Rub indication. We propose that by establishing the lower range of the effective dose for ethanol, it will not be necessary to test additional concentrations of ethanol to establish 95% volume/volume as the upper range of a GRAE.

Question #15 (HCPHR)

We proposed to conduct a pilot study(ies) (Appendix I) to evaluate multiple concentrations of ethanol at multiple application volumes and a pivotal study evaluating a single concentration of alcohol at one application volume as determined by the pilot study. Does FDA agree with the proposed approach?

Question #16 (HCPHR)

If we were to test a concentration of ethanol below 60% (e.g., 50%) and it met the efficacy proposed in the 2015 TFM, would FDA set the lower limit of efficacy for ethanol at that level?

Summary for Question #17 (HCPHR)

We are aware of only one FDA approved, alcohol-based product with a Health Care Personnel Hand Rub indication (Avagard, NDA 21-074). Based on the data provided in the NDA for this product, (see excerpt below), it is highly unlikely that Avagard would meet the proposed FDA success criteria using E1174.

Excerpt from Avagard NDA 21-074 Medical Review:

1. Efficacy: The following table is taken from volume 35, p. 8-1384 of the NDA. The values represent log reduction seen after the first and tenth washes. That is, the baseline log count minus the log count measured is shown.

Table 4 Log Transformed Bacterial Counts (CFU/Hand) After Wash 1 and Wash 10 (HPD-5a=Avagard)

Log Reduction	Statistic	HPD-5a N=24	Hibiclens N=24
Wash 1	Mean	2.1	2.6
	SD	0.55	0.45
	Range	[redacted]	
	95% C.I.	(1.9, 2.4)	(2.4, 2.8)
Wash 10	Mean	3.7	3.7
	SD	0.98	0.70
	Range	[redacted]	
	95% C.I.	(3.3, 4.2)	(3.4, 4.0)

Additionally, Avagard is a dual-active drug product (61% Ethyl Alcohol and 1% CHG w/w). Therefore, it should not be assumed it will perform similarly to ethyl alcohol alone.

Furthermore, no data is available for an FDA approved health care personnel hand rub using the E 2755 method, which we propose to use.

Rationale for Question #17 (HCPHR)

Because we are not aware of any FDA approved product that has been shown to meet the new requirements for active ingredients outlined in the 2015 TFM, GOJO proposes that no active control should be required for inclusion in the pivotal study. Instead, we propose that the data provided for ethanol at test conditions used in our proposed study be used to establish ethanol as the active control in future studies for products seeking a Health Care Personnel Hand Rub indication.

Question #17 (HCPHR)

In the proposed Study Synopsis (Appendix J), GOJO proposes that the *in vivo* E 2755 study be conducted without an active control. Does FDA agree with this proposal?

Summary for Question #18 (HCPHR)

A cross-over design is routinely used by FDA and is preferable in this scenario over a parallel design because of the efficiency gained in the analysis by subjugating the within subject error term and the among subject error term. As noted in the E9 guidance entitled **Statistical Principles for Clinical Trials**, under Section III **Trial Design Considerations**:

In the crossover design, each subject is randomized to a sequence of two or more treatments and hence acts as his own control for treatment comparisons. This simple maneuver is attractive primarily because it reduces the number of subjects and usually the number of assessments needed to achieve a specific power, sometimes to a marked extent.

Rationale for Question #18 (HCPHR)

In Appendix J, for *in vivo* assessment of ethanol for the indication of Health Care Personnel Handrub, the proposed design will be randomized cross-over designs; sequence and period will be added to models for evaluation.

Question #18 (HCPHR)

Does FDA agree with using a randomized cross-over design?

Rationale for Question #19 (General)

In the April 30, 2015 FDA Media Briefing related to release of the May 1, 2015 TFM for healthcare antiseptics it was stated "The FDA does not believe that these ingredients are ineffective or unsafe, but is requesting additional data to establish the safety of long-term, daily repeated exposure to these ingredients in the health care setting. The FDA recommends that health care personnel

continue to use these products consistent with infection control guidelines while additional data are gathered. Health care antiseptics are an important component of infection control strategies in hospitals, clinics, and other health care settings, and they remain a standard of care to prevent illness and the spread of infection."

However, contrary to FDA's stated intent, the reclassification of alcohol from Category I in the 1994 TFM to Category III in the 2015 TFM may be causing some confusion in the health care community and affecting acceptance and use of ABHRs (See Medscape article http://www.healthleadersmedia.com/page-2/QUA-315924/FDA-Seeks-More-Safety-Data-on-Hospital-Antiseptics-Proposes-Rule).

FDA has announced its intent to issue a final monograph for Healthcare Antiseptics in 2018. This schedule results in a potential multi-year scenario of confusion and changes in use patterns for ABHRs. In the interest of public safety FDA should consider completing the safety assessment of alcohol and reclassification to Category I as soon as possible.

Question #19 (General)

If FDA receives new efficacy data based upon approved protocols and agrees that these data are sufficient to make a GRAE determination for alcohol, would FDA change the category status from III to IS prior to issuance of the Final Monograph for Healthcare Antiseptics?

Summary for Question #20 (General)

FDA introduced a model for antiseptic hand hygiene products with three distinct user categories: Consumer Hand Wash, Healthcare Antiseptics and Consumer Hand Rubs. In the Dec 2013 TFM for Consumer Hand Wash FDA indicated that in order for an active ingredient to be considered GRAE, additional benefit from the use of consumer antiseptic hand or body washes compared to non-antibacterial soap would need to be shown in an outcome study.

In the 2015 Healthcare TFM FDA indicated for a variety of reasons, including ethical ones, that outcome studies would not needed to show GRAE status for a healthcare antiseptic active ingredient.

In the NRDC related Consent Decree filed November 2013, FDA indicated that a TFM for Consumer Hand Rub products would be introduced on June 30, 2016. In a June 5, 2015 Defendants Status Report under the Consent Decree for Nov. 21, 2014-May 21, 2015, FDA indicated "FDA anticipates that its work on both the

TFM for Consumer Antiseptic Hand Wash Products and TFM for Healthcare Antiseptic Products will also inform the development of the TFM for Consumer Antiseptic Hand Rub Products and FDA has begun drafting the TFM for those hand rub products."

Rationale for Question #20 (General)

Hand rubs can be used by consumers in situations where soap and water are not available. The statements by Dr. Tinetti during the October 20, 2005 NDAC Meeting[10] support the conclusion that the availability of ABHRs to consumers is important. The efficacy of ABHRs to kill germs is well established and therefore a GRAE determination for alcohol in healthcare settings should be sufficient for GRAE classification in the Consumer category for "leave-on" products.

Question #20 (General)

If FDA determines the data submitted are sufficient to determine GRAE for ethyl alcohol in healthcare indications, would FDA also consider the data sufficient for a GRAE determination for ethyl alcohol in the upcoming Proposed Rule for Consumer "Leave-On" products?

[10] NDAC Meeting of Thursday, October 20, 2005 Topical Antiseptic Review Team. http://www.fda.gov/ohrms/dockets/ac/05/transcripts/2005-4184T1.pdf

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Appendices

Appendix A: Society of Toxicology Meeting (March 2015) Poster

Note to the Reviewer

Please see back binder pocket for copy of poster or link to TERA website below

http://www.tera.org/ART/antiseptics.html

Appendix B: In Vitro Efficacy MIC/MBC Study Synopsis

Name of Sponsor: GOJO Industries, Inc. **Name/Active Ingredients:** Ethyl Alcohol	150545-202
Study Title	Determination of the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of one (1) Test Material (95% Ethanol)
Study Center	BioScience Laboratories Inc.
Study Rationale	The 1994 TFM requires that the finished product be challenged with 25 ATCC strains and 22 fresh clinically isolated strains of each of the 12 Gram-negative and 11 Gram-positive bacterial species and 2 yeast groups of species (*Candida albicans* and *Candida* spp., other than *albicans*).
Design	This study, a Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) evaluation of one (1) Test Material (95% Ethanol) will be performed based upon the Macrodilution Broth Method outlined in CLSI Document M07-A9, Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically, Ninth Edition, as well as NCCLS (currently known as CLSI) Document M26-A, Methods for Determining Bactericidal Activity of Antimicrobial Agents (September 1999). The Test Materials will each be evaluated using one thousand one-hundred and seventy five (1,100) different microorganism strains. Twenty-five (25) American Type Culture Collection [ATCC] strains and Twenty-five (25) Clinical Isolates of each of the twenty-two (22) microorganism species listed in the Tentative Final Monograph, Federal Register, 17 June 1994, vol. 59:116, p. 31444, will be evaluated. Specific Microorganisms: 1 *Acinetobacter baumannii* 2 *Bacteroides fragilis* 3 *Haemophilus influenza* 4 Enterobacter species 5 *Escherichia coli* 6 Klebsiella species 7 *Klebsiella pneumoniae* 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 pneumoniae* 21 Candida species 22 *Candida albicans*
Approximate Duration of Study	12 weeks
And Efficacy Evaluation	A suspension of each challenge strain will be prepared and exposed to each of 12 doubling dilutions of the Test Material prepared in the appropriate nutrient broth. Following incubation for 18 to 24 hours, the Minimum Inhibitory Concentration (MIC) of each test product will be determined visually and documented. Aliquots of the three highest dilutions of each test product 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 the each test product will be reported as the highest dilution (lowest product concentration) resulting in a ≥ 3.0 Log_{10} ($\geq 99.9\%$) reduction in the population of the challenge strain.
Data Analysis	A statistical analysis may be used.

Appendix C: In Vitro Efficacy Time Kill Study Synopsis

Name of Sponsor: GOJO Industries, Inc. **Name/Active Ingredients:** 60%, 70%, and 95% Ethyl Alcohol	150546-201
Study Title	An In-Vitro evaluation of three concentrations of ethanol, 60%, 70%, & 95% for their antimicrobial properties using the standardized ASTM E2783 Time-Kill Method.
Study Center	BioScience Laboratories Inc.
Study Rationale	The 1994 TFM requires that the microorganisms tested in the time kill study are to be the 25 organisms identified in paragraph (a)(1)(ii) of the in-vitro section.
Design	This study will use an In-Vitro Time-Kill Method to evaluate the antimicrobial properties of three test materials when challenged with suspensions of the twenty-five (25) microorganism specifically called out in the 1994 FDA TFM. This time-kill evaluation will follow ASTM E2783-11, Standard Test Method for Assessment of Antimicrobial Activity for Water Miscible Compounds Using a Time-Kill Procedure. The percent and log10 reduction in the microbial population of each challenge strain will be determined following exposures to each test material for fifteen seconds. All exposures will be performed once and all agar-plating will be performed in triplicate. 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 E 1054-08(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.
Approximate Duration of Study	3 weeks
Efficacy Evaluation	A single replicate of the procedure will be performed for each individual evaluation. A dilution/aliquot of the each test material will be brought into contact with a known population of the test organisms for the specified period of time, at a specified temperature. The activity of each test material will be quenched at the specified sampling interval, 15 seconds with a validated neutralizing technique. The test material will be neutralized at the sampling time and the surviving microorganisms enumerated. The percent and $\log_{10}$ reduction, from an initial microbial population will then be calculated.
Data Analysis	A statistical analysis may be used.

Appendix D: *In Vitro* Time Kill Data

PURELL® Advanced Hand Sanitizer

Objective: Evaluate the antimicrobial effectiveness of the product *in vitro*.

Description of Test: Fifteen (15) second exposure kill evaluations were performed utilizing fifty-six (56) challenge microorganism strains. The challenge inoculum was introduced to the test product at time zero; a portion of the sample was removed and placed in neutralizing media at the appropriate time (15 seconds). Standard plate counting techniques were used to enumerate viable challenge microorganisms.

Independent Laboratory: BioScience Laboratories, Inc., Bozeman, MT, USA

Date: 19 October 2010

Results:

Bacteria	ATCC No.	Exposure (seconds)	Log_{10} Reduction
Acinetobacter baumannii	19606	15	6.4241
Bacteroides fragilis	25285	15	4.0588
Burkholderia cepacia	25416	15	6.0588
Burkholderia cepacia	25608	15	6.0149
Campylobacter jejuni	29428	15	7.8451
Citrobacter freundii	8090	15	6.4983
Clostridium difficile (vegetative cells)	9689	15	4.2418
Clostridium perfringens (vegetative cells)	13124	15	6.1959
Corynebacterium diphtheriae	11913	15	7.2292
Enterobacter aerogenes	13048	15	6.1189
Enterococcus faecalis	19433	15	6.266
Enterococcus faecalis	29212	15	6.4306
Enterococcus faecalis VRE	51299	15	6.5798
Enterococcus faecalis VRE	51575	15	6.4661
Enterococcus faecium	19434	15	5.9823
Enterococcus faecium (MDR, VRE)	51559	15	5.9956
Escherichia coli	11775	15	6.3345
Escherichia coli	25922	15	5.9777
Escherichia coli (O157:H7)	43888	15	5.9912

Escherichia coli (MDR, ESBL)	BAA-196	15	5.8692
Escherichia coli ESBL; Carbapenemase-Producing	BSLI #082710EcCP1*	15	5.7443
Haemophilus influenzae MDR	33930	15	6.5966
Klebsiella pneumonia Ozaenae	11296	15	5.8949
Klebsiella pneumonia pneumoniae	13883	15	5.7853
Klebsiella pneumoniae pneumoniae	27736	15	5.7443
Klebsiella pneumonia KPC 2 Positive; Carbapenemase Producing	BSLI#081710KPCI*	15	5.7672
Lactobacillus plantarum	14917	15	5.9494
Listeria monocytogenes	7644	15	6.618
Micrococcus luteus	7468	15	5.0719
Proteus hauseri	13315	15	6.1508
Proteus mirabilis	7002	15	6.1889
Pseudomonas aeruginosa	15442	15	6.1945
Pseudomonas aeruginosa	27853	15	5.9243
Salmonella enterica enterica serovar Enteritidis	13076	15	6.0512
Serratia marcescens	8100	15	6.243
Serratia marcescens	14756	15	6.3738
Shigella dysenteriae	13313	15	5.9165
Shigella sonnei	11060	15	5.9845
Staphylococcus aureus aureus	6538	15	6.3901
Staphylococcus aureus aureus	29213	15	6.1523
Staphylococcus aureus aureus (MRSA)	33591	15	6.1414
Staphylococcus aureus aureus (MRSA)	33592	15	6.2279
Staphylococcus aureus (MRSA) (VRSA)	062707 NARSAVRSal	15	6.444
Staphylococcus aureus (MRSA) (NARSA Strain NRS384;USA 300)	12085 NRSa384	15	6.1477
Staphylococcus epidermidis	12228	15	5.8633
Staphylococcus epidermidis MRSE	51625	15	5.8096
Staphylococcus haemolyticus	43252	15	5.6335
Staphylococcus hominis hominis	27845	15	5.4742
Staphylococcus saprophyticus	49453	15	6.0492
Streptococcus pneumoniae	6303	15	6.1614
Streptococcus pneumoniae	49619	15	7.1804
Streptococcus pyogenes	14289	15	6.5563

Streptococcus pyogenes	19615	15	6.752
Yeasts	**ATCC No.**	**Exposure (seconds)**	**Log_{10} Reduction**
Candida albicans	18804	15	6.2516
Candida albicans	66027	15	6.0374
Candida tropicalis	13803	15	6.4579

ESBL Extended Spectrum Beta-Lactamase Producer
MDR Multi-Drug Resistant
MRSA Methicillin Resistant *Staphylococcus aureus*
MRSE Methicillin Resistant *Stpaphyococcus epidemidis*
NARSA Network on the Antimicrobial Resistance in *Staphylococcus aureus*
VRE Vancomycin-Resistant *Enterococcus*
* Clinical Isolate

Appendix E: *In Vivo* Test Method ASTM E1115

Standard Test Method for Evaluation of Surgical Hand Scrub Formulations

Designation: E1115 – 10

Standard Test Method for Evaluation of Surgical Hand Scrub Formulations[1]

This standard is issued under the fixed designation E1115; 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 This test method is designed to measure the reduction of microbial flora on the skin. It is intended for determining both immediate and persistent (continuing antimicrobial effect) microbial reductions, after single or repetitive treatments, or both. It may also be used to measure cumulative antimicrobial activity after repetitive treatments.

1.2 A knowledge of microbiological techniques is required for these procedures.

1.3 Performance of this procedure requires the knowledge of regulations pertaining to the protection of human subjects (21 CFR, Parts 50 and 56)

1.4 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.4.1 In this test method, SI units are used for all applications, except for distance, in which case inches are used and SI units follow in parentheses.

1.5 *This standard does not purport to address all of the safety concerns, 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:*[2]

D1193 Specification for Reagent Water

E1054 Test Methods for Evaluation of Inactivators of Antimicrobial Agents

E2180 Test Method for Determining the Activity of Incorporated Antimicrobial Agent(s) In Polymeric or Hydrophobic Materials

2.2 *Other Documents:*

21 CFR Parts 50 and 56[3]

AATCC 147–2004 Antibacterial Assessment of Textile Materials: Parallel Streak Method[4]

JIS Z 2801 :2000, Antimicrobial Products—Test for Antimicrobial Activity and Efficacy[5]

USP 32 United States Pharmacopeia, Chapter 61 "Microbial Limits Test", 2009[6]

3. Terminology

3.1 *Definitions:*

3.1.1 *active ingredient*—a substance added to a formulation specifically for the inhibition or inactivation of microorganisms.

3.1.2 *cleansing wash*—a non-antimicrobial wash intended to remove gross soil or residues from the hands.

3.1.3 *cleansing wash formulation*—a liquid castile soap or other liquid soap with neutral pH which does not contain an antimicrobial.

3.1.4 *cumulative effect*—a progressive decrease in the number of microorganisms recovered following repeated applications.

3.1.5 *internal reference formulation*—a formulation with demonstrated performance characteristics within the laboratory.

3.1.6 *neutralization*—a process that results in quenching or inactivation of the antimicrobial activity of a formulation. This may be achieved through dilution of the formulation or through the use of chemical agents called neutralizers.

3.1.7 *persistence*—prolonged or extended antimicrobial activity that prevents or inhibits the proliferation or survival of microorganisms after treatment.

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[1] This test method is under the jurisdiction of ASTM Committee E35 on Pesticides and Alternative Control Agents and is the direct responsibility of Subcommittee E35.15 on Antimicrobial Agents.

Current edition approved April 1, 2010. Published August 2010. Originally approved in 1986. Last previous edition approved in 2002 as E1115 – 02. DOI: 10.1520/E1115-10.

[2] 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.

[3] Available from U.S. Government Printing Office, 732 N. Capitol St., Washington, DC 20401, U.S. Government Bookstore, http://bookstore.gpo.gov/baskets/cfr-listing.jsp.

[4] Technical Manual of the American Association of Textile Chemists and Colorists (AATCC), 2009, Vol 82, P.O. Box 12215, Research Triangle Park, NC 27709, http://www.aatcc.org.

[5] Available from Japanese Industrial Standards Committee, Divisional Council on Consumer Life, Japanese Standards Association (JSA), 4-1-24 Akasaka Minato-Ku, Tokyo, 107-8440, Japan, http://www.jsa.or.jp.

[6] Available from U.S. Pharmacopeia (USP), 12601 Twinbrook Pkwy., Rockville, MD 20852-1790, http://www.usp.org.

3.1.8 *sampling fluid*—a buffered solution that aids in recovery of microorganisms from the skin and neutralization of the active ingredient in test and internal reference formulations.

3.1.9 *test formulation*—a formulation containing an active ingredient(s).

4. Summary of Test Method

4.1 This test method is conducted on individuals selected from a group of subjects who have refrained from using any antimicrobials for at least one week prior to initiation of the test. Subjects are selected from this group on the basis of high initial bacterial count, $\geq 1 \times 10^5$ CFU/per hand as determined by baseline measurements of the bacteria on their hands using the recovery techniques in this method.

4.2 The selected subjects perform a simulated surgical scrub under the supervision of an individual competent in aseptic technique. One hand of each subject is sampled immediately after the scrub (within 1 min), and the other hand, 6 h after scrubbing. Only one hand of a subject is sampled at a specified time. Optionally, another sampling time, 3 h for example, can be added between the immediate and 6 h sampling times. If this is desired, the panel size must be increased by 50 % to obtain the same number of data points at each designated sampling interval. Also, a sampling time randomization must be generated such that one-third of the hands are sampled at each sampling interval with only one hand of a subject being sampled at a sampling time interval.

NOTE 1—Data for submission to some regulatory bodies may require the addition of a positive and negative control in addition to the test product. For the negative control, 0.9 % saline can be used when testing alcohol products and the product vehicle can be used as the negative control when testing non-alcoholic products.

4.3 If demonstration of cumulative activity is desired, eleven additional scrubs are performed over a 5-day period, one additional time on Day 1, three times on Days 2, 3, and 4 and once on Day 5. The hands are sampled again after the last scheduled scrub.

5. Significance and Use

5.1 The procedure in this test method should be used to evaluate the activity of the test formulation in reducing the bacterial population of the hands immediately after a single use and to determine persistent activity (inhibition of growth) after 6 h. Optionally, measurements of persistent activity after a 3 h period and measurements of cumulative activity may be made after repetitive uses over a five day period.

6. Apparatus

6.1 *Colony Counter*—Use any of several types.

6.2 *Incubator*—Any incubator capable of maintaining a temperature of 30 ± 2°C.

6.3 *Sterilizer*—Any suitable steam sterilizer capable of producing the conditions of sterilization.

6.4 *Timer (stop-clock)*—one that displays minutes and seconds.

6.5 *Hand Washing Sink*—A sink of sufficient size to permit subjects to wash without touching hands to sink surface or other subjects.

6.5.1 *Water Faucet(s)*—To be located above the sink at a height that permits the hands to be held higher than the elbows during the washing procedure. (It is desirable for the height of the faucet(s) to be adjustable.)

6.6 *Tap Water Temperature Regulator and Temperature Monitor*—To monitor and regulate water temperature to 40 ± 2°C.

7. Reagents and Materials

7.1 *Petri Dishes*—100 by 15 mm. Required for performing Standard Plate Count.

NOTE 2—Pre-sterilized/disposable plastic petri dishes are available from most local laboratory supply houses.

7.2 *Bacteriological Pipets*—10.0 and 2.2 or 1.1-mL capacity.

NOTE 3—Pre-sterilized/disposable bacteriological pipets are available from most local laboratory supply houses.

7.3 *Water-Dilution Bottles*—Any sterilizable container having a 150 to 200-mL capacity and tight closures may be used.

NOTE 4—Dilution bottles of 160-mL capacity having a screw-cap closure are available from most local laboratory supply houses.

7.4 *Cleansing Wash Formulation*—A mild, non-antimicrobial soft soap such as the following or any other liquid soap with neutral pH which does not contain an antimicrobial:

Soft soap, 200 g/L

Linseed oil	50 parts by weight
Potassium hydroxide	9.5 parts
Ethanol	7 parts
Distilled or high purity water	as needed

7.4.1 Add linseed oil to a solution of potassium hydroxide in 15 parts water and heat up to approximately 70°C while constantly stirring. Add the ethanol and continue heating 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 is then brought up to 100 parts by addition of hot water. Take 200 g of the soft soap in 1 L of water. Dispense in to appropriate containers and sterilize in an autoclave.

7.5 *Gloves for Sampling*—Loose-fitting, unlined, powder-free latex gloves which possess no antimicrobial properties,[7] or equivalent.

NOTE 5—A zone of inhibition test such as AATCC 147–2004, Test Method E2180, or Japanese Standard JIS Z 2801 may be used to evaluate antimicrobial properties of gloves.

7.6 *Test Formulation*—Directions for use of active test formulation should be utilized if available. If not available, use directions provided in this test method (see 11.3).

[7] The sole source of supply of the apparatus (Ansell #579500, sterile, Encore Acclaim Latex Surgical Gloves) known to the committee at this time is PSS Medical, Inc. (Cat #105613). If you are aware of alternative suppliers, 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.

7.7 *Water*—Sterile deionized water or equivalent (Specification D1193, Type III).

7.8 *Sampling Fluid*[8]—Dissolve 0.4 g KH_2PO_4, 10.1 g Na_2HPO_4, 1.0 g isooctylphenoxypolyethoxyethanol (for example, Triton X-100), and appropriately validated neutralizers in 1 L distilled water. Adjust pH to 7.8 ± 1 with 0.1 *N* HCl or 01. *N* NaOH. Dispense to achieve a final volume of 75 ± 1 mL and sterilize.

7.9 *Dilution Fluid*—Sterile Butterfield's buffer[9] or other suitable diluent adjusted to pH 7.2 ± 0.1 with effective neutralizer for the test material. Adjust pH with 0.1 *N* HCl or 0.1 *N* NaOH. See Test Methods E1054.

7.10 *Agar*—Soybean-casein Digest agar (USP 32) or other solid media appropriately validated to support growth of the test organism with appropriate neutralizers if needed.

NOTE 6—Inadequate neutralization may result in false interpretation of the test data. The use of excess chemical neutralizers may exert a toxic effect on the recovery of bacterial cells. The goal, therefore, is to stop antimicrobial activity as early as possible in the sampling/plating process. If it can be demonstrated that antimicrobial activity is quenched or inactivated in the sampling fluid then, to reduce the chance of possible toxic effects, inactivators should not be added to the dilution fluid or plating media.

7.11 *Scrub Sponge and Nail Cleaner Stick*—Such as E-Z Scrub 160[10] or any equivalent may be used.

8. Subjects

8.1 Recruit a sufficient number (see X1.1) of healthy subjects who have no clinical evidence of dermatoses, open wounds, or other skin disorders. Exclude any individual receiving antibiotic therapy and any individual sensitive to natural rubber or latex or to a component of the formulation(s) being tested.

8.2 Instruct the subjects to avoid contact with antimicrobial products (other than the test formulation(s) as dispensed for each scrub) for the duration of the test and for at least one week prior to the test. This restriction includes antimicrobial-containing antiperspirants, deodorants, shampoos, lotions, dishwashing liquids and soaps, and also such materials as acids, bases, and solvents. Bathing in biocide treated pools, hot tubs, or spas should be avoided. Subjects are provided with a kit of non-antimicrobial personal care products for exclusive use during the test and rubber gloves to be worn when contact with antimicrobials agents cannot be avoided.

9. Procedure

9.1 After subjects have refrained from using antimicrobials for at least one week, perform wash with cleansing wash formulation (see 7.4) using methodology outlined in 10.1-10.4. Subjects are not to have washed their hands on this day 2 h prior to baseline determination. After washing, determine first estimate of baseline bacterial population by sampling hands and enumerating the bacteria in the sampling fluid. This is Day 1 of "Baseline Period." Repeat this baseline determination procedure on Days 3 and 7, Days 3 and 5, or Days 5 and 7 of "Baseline Period" to obtain three estimates of baseline population. After obtaining the first and second estimates of the baseline populations, select subjects who exhibited at each sampling time counts $\geq 1 \times 10^5$ per hand. The three estimates of the baseline population obtained for each of the selected subjects are averaged to obtain the mean baseline counts.

9.2 A basic random bacterial recovery sampling plan should be followed. The number of subjects and sampling times depend on the test formulation but must establish the onset and extent of the bacterial suppression and the duration of suppression below the baseline counts. Equal numbers of subjects should be assigned per sampling time, test formulation and hand. A typical balanced randomization plan for testing a block of six subjects follows with sampling at 0 h, 3 h (optional), and 6 h.

Subject No.	Post Scrub Sampling Time, h		
	0-h	3-h	6-h
1	left hand	right hand	
2	left hand		right hand
3	right hand	left hand	
4	right hand		left hand
5		left hand	right hand
6		right hand	left hand

If only 0 h and 6 h post scrub samples are collected the 0 h will be randomized to the right or left hand.

9.2.1 The number of subjects per block may vary but must be divisible by two and by the number of sampling times in order to assign equal number of left and right hands to each sampling time.

9.3 No sooner than 24 h and no longer than 96 h after completion of the baseline determination, subjects perform scrub with the test formulation. The starting interval should be same for all subjects participating in the study. According to the random sampling plan, determine the bacterial populations on the subjects' hands at the assigned sampling times after scrubbing. Determine bacterial population by sampling hands and enumerating the bacteria in the sampling fluid as specified in Sections 13 and 14.

9.4 If measurement of cumulative effect is desired, the hands are sampled one more time after performing 11 additional scrubs with the active test formulation over a 5 day period. Repeat the treatment procedure with the test formulation one additional time after the sampling on Day 1 and treat three additional times on Day 2, Day 3 and Day 4 with at least a 1-h interval between scrub treatments. On day 5 perform one scrub treatment prior to sampling.

9.5 In summary, measurement of immediate activity is made following a single scrub. Persistent activity may be measured by collecting samples after 3 and or 6 h of glove wear or other selected times after the immediate sampling. If measurement of cumulative activity is desired the subjects are to scrub a total of twelve times with the test formulation, twice on Day 1 and three times per day on Days 2, 3, and 4, and once on Day 5.

[8] Peterson, A. F., "The Microbiology of the Hands: Evaluating the Effects of Surgical Scrubs," *Developments in Industrial Microbiology*, Vol 14, 1973, pp. 125–130.

[9] Horowitz, W. (Ed.) , 2006 *Official Methods of Analysis of AOAC International,* 2006, 18th Ed., Revision 1, Ch 17, p. 4, Sec. 17.2.01 (m). AOAC, Washington, D.C.

[10] The sole source of supply of the apparatus known to the committee at this time is E-Z Scrub 160, Cat. No. 371603, manufactured by Becton Dickinson Div., Franklin Lakes, NJ 07417–1884. If you are aware of alternative suppliers, 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.

Collect the samples following single scrubs on Days 1 and 5. This mimics typical usage and permits determination of reduction immediately after a single use and after repeated uses.

9.6 The schedule for scrubbing and sampling is shown in the following table. Samples collected immediately after the first scrub are used to measure the immediate reduction; optionally samples collected after scrub 12 are used to measure cumulative activity.

Day	Scrubs	Sample
1	2 (1 before and 1 after sample)	1
2	3	0
3	3	0
4	3	0
5	1	1
Totals	12	2

10. Washing Technique for Baseline Determinations

10.1 Subjects clean under fingernails with nail stick and clip fingernails to ±2-mm free edge. Remove all jewelry from hands and arms.

10.2 Rinse hands including two thirds of forearm under running tap water for 30 s. Maintain hands higher than elbows during this procedure and steps outlined in 10.3-10.5. Adjust water temperature to 40 ± 2°C and the water flow rate to 4 L per minute.

NOTE 7—This may be accomplished by placing a 2000 mL glass beaker or flask under each spigot to be used for subjects' hand washing. Allow the water to flow into the beaker. Adjust the water flow at each spigot accordingly, so that the beaker fills within 30 s.

10.3 Perform a cleansing wash of hands and forearms with cleansing wash formulation for 30 s using water as required to develop lather.

10.4 Rinse hands and forearms for 30 s under tap water thoroughly removing all lather.

10.5 Place gloves (see 7.5) used for sampling on right and left hands and secure gloves at wrist.

10.6 Sample hands for recoverable bacteria as described in Section 13.

11. Surgical Scrub Technique to Be Used Prior to Bacterial Sampling

11.1 Repeat 10.1 and 10.2.

11.2 Perform scrub with test formulation in accordance with directions furnished with the active test formulation(s) and/or the negative controls.

NOTE 8—If no instructions are provided with the active test formulation, use the 10-min scrub procedure in 11.3.

11.3 *Ten-Minute Scrub Procedure*:

11.3.1 Dispense prescribed amount of formulation into hands.

11.3.2 Set and start timer for 5 min (time required for the steps in 11.3.3-11.3.7).

11.3.3 With hands, distribute formulation over hands and lower two thirds of forearms.

11.3.4 If scrub brush is to be used, pick up with fingertips and pass under tap to wet, without rinsing formulation from hands.

11.3.5 Alternately scrub right hand and lower two thirds of forearm and left hand and lower two thirds of forearm.

11.3.6 Rinse both hands, the lower two thirds of both forearms, and the brush for 30 s.

11.3.7 Place brush in sterile dish within easy reach.

11.3.8 Repeat 11.3.1-11.3.6 so that each hand and forearm is washed twice. The second wash and rinse should be limited to the lower one third of the forearms and the hands.

11.3.9 Perform final rinse. Rinse each hand and forearm separately for 1 min per hand.

11.3.10 Immediately (within less than 30 sec), place gloves used for sampling on right and left hands and secure gloves at wrist.

11.4 Sample hands as described in Section 13 at assigned sample times.

12. Surgical Scrub Technique When Bacterial Samples Are Not Specified

12.1 Perform technique as described in Section 11, except omit 11.3.10. Subjects dry hands with clean paper towels after final rinse of hands.

13. Bacterial Recovery

13.1 At each specified sampling time, (for example, immediate, 3h, 6h) aseptically add 75 mL of sampling fluid with neutralizer (see 7.8) to the gloved hand to be sampled and secure the glove above the wrist.

13.2 Within one minute of donning gloves, uniformly massage all surfaces of the hand for 1 min ± 5 s, paying particular attention to the fingers and flipping the hand after 30 s to ensure both the palm and back of the hand are thoroughly massaged.

13.3 Aseptically retrieve a 3 to 5 mL sample of the fluid in the glove by pulling the glove away from the wrist, inserting a pipet into the finger region of the glove, and withdrawing the fluid.

13.4 Rinse hands under running tap water to remove residual sampling fluid.

13.5 The first dilution of sampling fluid is to be made in dilution fluid with appropriate neutralizer within 1 min and 10 s of completing the massage. The plating of the recovered sampling solution is completed within 30 min after sampling.

14. Enumeration of Bacteria in Sampling Fluid

14.1 Enumerate the bacteria in the sampling fluid by microbiological techniques such as surface inoculation technique (spread plating or spiral plating) or pour-plate technique.

14.2 Prepare sample dilutions in Dilution Fluid (see 7.9). Use Soybean-Casein Digest Agar (see 7.10). Plate in duplicate.

14.3 Incubate plated sample at 30 ± 2°C for 48 to 72 h before reading. Standard plate counting procedures are to be used.

14.4 Calculate for each hand sampled at each sampling time the average number of colony forming units (CFUs) recovered.

15. Determination of Reduction Obtained

15.1 For each post-treatment sampling time determine changes from baseline counts obtained with the test formulation.

15.2 To determine the activity of the test formulation, all counts of colony forming units per hand should be converted to

common (base10) logarithms. At each sampling time $\log_{10}$ reductions should be calculated.

16. Method of Statistical Analysis

16.1 Prior to initiating the statistical analyses, the subjects' first and second baseline count for each hand are to be examined to determine if they meet the qualification criterion ($>1.0 \times 10^5$ CFU/hand).

16.2 *Check for Significant Difference Between Right and Left Hand Bioburdens at Baseline*—The source data for the baseline analysis are the 3-day average $\log_{10}$ values for the right and left hands of each subject. Potential differences between right and left hand bioburdens at the baseline are examined using a two-factor, subject × hand, analysis of variance procedure.

16.3 *Activity*—The mean $\log_{10}$ reductions and the 95 % confidence intervals for the test formulation(s) after 1 or 5 days of usage are to be calculated for each sampling time. Log_{10} reductions for each subject are calculated as average baseline $\log_{10}$ of a hand minus $\log_{10}$ of the post-treatment count for that hand.

16.3.1 *Persistent Activity (Within-treatments)*—Analysis of variance techniques are to be performed to evaluate differences between sampling intervals in a given day. Log_{10} reduction values from baseline are used in this analysis.

16.3.2 *Cumulative Activity (Within-treatments)*—Analysis of variance techniques are to be performed to calculate differences between similar sampling times on different test days. (that is, comparing 6 h, Day 5 to 6 h, Day 1). Log reduction values from baseline are used in this analysis.

17. Internal Reference Standard

17.1 To measure the validity of the test method within a study an internal reference formulation and a negative control should be evaluated.

18. Precision and Bias

18.1 A precision and bias statement can not be made for this test method at this time.

19. Keywords

19.1 antimicrobial; efficacy; glove juice; handwash; healthcare; surgical scrub

APPENDIX

(Nonmandatory Information)

X1. SAMPLE SIZE CALCULATIONS

X1.1 Sample size calculations should be done to determine the number of subjects 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 (that is, variability in reductions from baseline), and the expected efficacy of the test product (that is, its expected reduction from baseline). This number of subjects (n) can be estimated from the following equation:

$$n > S^2\left[\frac{(Z_{\alpha/2} + Z_{\beta})^2}{D^2}\right]$$

where:

S^2 = estimate of variance (of reduction from baseline based on in-house data pool or published data),
$Z_{\alpha/2}$ = 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).

Appendix F: *In Vivo* Surgical Hand Rub Protocol Synopsis (Pilot)

Name of Sponsor: GOJO Industries, Inc.	150622-102
Name/Active Ingredients: Three or more ethanol concentrations	

Study Title	Pilot study to determine the antimicrobial efficacy of three or more ethanol concentrations using a modification of the standardized ASTM E1115-11 test method
Clinical Phase	Phase III
Study Center	BioScience Laboratories Inc.
Study Rationale	This study will evaluate the antimicrobial effect of three or more ethanol concentrations for use as a surgical hand rub in order to select the lead candidate for a pivotal surgical hand rub efficacy study. Additionally this data will be used to inform the sample size for the pivotal trial.
Trial Design	Twenty-four subjects per arm will be evaluated over the course of five consecutive hand sampling events. The first three samples will be collected for baseline, the fourth sample will be collected within 1 minute after test material application, and the fifth will be conducted 6 hours after test material application. Log_{10} microbial reductions from baseline will be calculated for the "immediate" and "6 hours" time points.
Treatment Duration	Single application of test product
Approximate Duration of Study	3 weeks
Study Objective	The purpose of this study is to evaluate the antimicrobial effectiveness of three or more test materials for use in a pivotal surgical hand rub efficacy study.
Diagnosis and Main Criteria for Inclusion	1. Subjects may be of either sex, at least 18 years of age, and of any race. Subjects must possess both hands. 2. Subjects must have an average baseline microbial population of $\geq 1.5 \times 10^5$ CFU/mL on the hands. 3. Subjects must be in good general health, as evidenced by the Subject Confidential Information and Acceptance Criteria. 4. Subjects must have read and signed a Study Description and Informed Consent Form, Subject Confidential Information and Acceptance Criteria, Authorization to Use and Disclose Protected

Name of Sponsor: GOJO Industries, Inc.	150622-102
Name/Active Ingredients: Three or more ethanol concentrations	
	Health Information Form and List of Restricted Products prior to participating in the study.
Main Criteria for Exclusion	1. Exposure of ungloved hands and/or forearms to antimicrobial agents, medicated soaps, medicated shampoos, medicated lotions, or hair mousses during the 7-day pre-test conditioning period and throughout the baseline/test periods. 2. Use of nail polish, artificial nails, nail or cuticle treatments during the 7-day pre-test conditioning period or during the baseline/test periods. 3. Use of biocide-treated pools or hot tubs, use of tanning beds, or sunbathing during the 7-day pre-test conditioning period and throughout the baseline/test periods. 4. Exposure of ungloved hands or forearms to strong detergents, solvents, or other irritants during the 7-day pre-test conditioning period and throughout the baseline/test periods. 5. Use of systemic or topical antimicrobials, antibiotic medications, hormone-containing contraceptives (e.g., oral, patch, injectables, or any device that delivers hormones or any other steroid), steroid medications, or any other product known to affect the normal microbial flora of the skin during the 7-day pre-test conditioning period and throughout the baseline/test periods. 6. Known allergies to latex (rubber), alcohols, detergents, or to common antibacterial agents found in soaps, lotions, or ointments, particularly Ethanol. 7. A medical diagnosis of a physical condition, such as a current or recent severe illness, asthma, diabetes, hepatitis, an organ transplant, any immunocompromised conditions such as AIDS (or HIV positive). 8. Pregnancy, plans to become pregnant within the pre-test and baseline/test periods of the study, or nursing a child. 9. Any active skin rashes, breaks in the skin, or excessive skin dryness of hands or forearms. Dermatoses, cuts, lesions, hangnails, or other skin disorders on the hands and forearms. 10. A currently active skin disease or inflammatory skin condition, including contact dermatitis anywhere on the body. 11. Washing the hands or applying lotion within the 2-hour period prior to testing. 12. Participation in a clinical study in the past 7 days or current participation in another clinical study.

Name of Sponsor: GOJO Industries, Inc.	150622-102
Name/Active Ingredients: Three or more ethanol concentrations	
	13. Any medical condition or use of any medications that, in the opinion of the Study Director, should preclude participation. 14. Unwillingness to fulfill the performance requirements of the study.
Approximate Number of Subjects	24 subjects per test material arm
Approximate Number of Study Centers	1
Study Materials and Administration	Three or more ethanol concentrations applied with 3 applications of 2ml using standard application instructions for a surgical hand rub.
Efficacy Evaluation	Two primary endpoints: 1) Lower limit of the 95% confidence interval for the responder rate is equal to or greater than 70%, where a successful response is set at a 2.0 $\log_{10}$ reduction within 1 minute ("immediately") after a single rub. 2) Mean $\log_{10}$ recovery does not exceed baseline $\log_{10}$ recover at 6 hours sampling period. Additionally there will be use of an appropriate neutralizer in all recovery media (i.e., sampling solution, dilution fluid, and plating media) and a demonstration of neutralizer validation. The purpose of neutralizer validation is to show that the neutralizer used is effective against the test and control materials, and that it is not toxic to the test microorganisms. If a test product can be neutralized through dilution, this should be demonstrated in the neutralizer validation study.

Appendix G: *In Vivo* Surgical Hand Rub Study Synopsis (Pivotal)

Name of Sponsor: GOJO Industries, Inc.	150548-102
Name/Active Ingredients: Ethanol (Concentration TBD based on Pilot) and Saline (Negative Control)	
Study Title	Pivotal study to determine the antimicrobial efficacy of one test material with a negative control using a modification of the standardized ASTM E1115-11 test method
Clinical Phase	Phase III
Study Center	BioScience Laboratories Inc.
Study Rationale	This study is being conducted to support ethanol as an effective antimicrobial active for a surgical hand rub indication.
Trial Design	One-hundred and fifty two subjects, seventy-six per test and control material, will be evaluated over the course of five consecutive hand sampling events, the first three samples will be conducted for baseline, the fourth will be conducted within 1 minute post-test material or negative control application and the fifth will be conducted 6 hours post-test material or negative control application. Log_{10} microbial reductions from baseline will be calculated for the "immediate" and "6 hours" time points.
Treatment Duration	Single application of test or control article
Approximate Duration of Study	30 weeks
Study Objective	The purpose of this study is to determine the antimicrobial effectiveness of one ethanol test material for use as a surgical hand rub.
Diagnosis and Main Criteria for Inclusion	1. Subjects may be of either sex, at least 18 years of age, and of any race. Subjects must possess both hands. 2. Subjects must have an average baseline microbial population of $\geq 1.5 \times 10^5$ CFU/mL on the hands. 3. Subjects must be in good general health, as evidenced by the Subject Confidential Information and Acceptance Criteria. 4. Subjects must have read and signed a Study Description and Informed Consent Form, Subject Confidential Information and Acceptance Criteria, Authorization to Use and Disclose Protected Health Information Form and List of Restricted Products prior to

Name of Sponsor: GOJO Industries, Inc.	150548-102
Name/Active Ingredients: Ethanol (Concentration TBD based on Pilot) and Saline (Negative Control)	

	participating in the study.
Main Criteria for Exclusion	1. Exposure of ungloved hands and/or forearms to antimicrobial agents, medicated soaps, medicated shampoos, medicated lotions, or hair mousses during the 7-day pre-test conditioning period and throughout the baseline/test periods. 2. Use of nail polish, artificial nails, nail or cuticle treatments during the 7-day pre-test conditioning period or during the baseline/test periods. 3. Use of biocide-treated pools or hot tubs, use of tanning beds, or sunbathing during the 7-day pre-test conditioning period and throughout the baseline/test periods. 4. Exposure of ungloved hands or forearms to strong detergents, solvents, or other irritants during the 7-day pre-test conditioning period and throughout the baseline/test periods. 5. Use of systemic or topical antimicrobials, antibiotic medications, hormone-containing contraceptives (e.g., oral, patch, injectables, or any device that delivers hormones or any other steroid), steroid medications, or any other product known to affect the normal microbial flora of the skin during the 7-day pre-test conditioning period and throughout the baseline/test periods. 6. Known allergies to latex (rubber), alcohols, detergents, or to common antibacterial agents found in soaps, lotions, or ointments, particularly Ethanol. 7. A medical diagnosis of a physical condition, such as a current or recent severe illness, asthma, diabetes, hepatitis, an organ transplant, any immunocompromised conditions such as AIDS (or HIV positive). 8. Pregnancy, plans to become pregnant within the pre-test and baseline/test periods of the study, or nursing a child. 9. Any active skin rashes, breaks in the skin, or excessive skin dryness of hands or forearms. Dermatoses, cuts, lesions, hangnails, or other skin disorders on the hands and forearms. 10. A currently active skin disease or inflammatory skin condition, including contact dermatitis anywhere on the body. 11. Washing the hands or applying lotion within the 2-hour period prior to testing. 12. Participation in a clinical study in the past 7 days or current participation in another clinical study.

<table>
<tr><td colspan="2">Name of Sponsor: GOJO Industries, Inc.</td><td rowspan="2">150548-102</td></tr>
<tr><td colspan="2">Name/Active Ingredients:
Ethanol (Concentration TBD based on Pilot) and Saline (Negative Control)</td></tr>
<tr><td></td><td colspan="2">13. Any medical condition or use of any medications that, in the opinion of the Study Director, should preclude participation.
14. Unwillingness to fulfill the performance requirements of the study.</td></tr>
<tr><td>Approximate Number of Subjects</td><td colspan="2">Sample Size Calculation:
The sample size calculation has the formula:
$$n \geq \frac{x\, s^2 \left(z_{\alpha/2} + z_{\beta}\right)^2}{d^2}$$
where:
n = number of subjects per product
s= 1.2
x = number of products evaluated- 2 products
$z_{\alpha/2}$ =0.05 level of significance = 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
In this case where two products were evaluated:
$$n \geq \frac{2\, s^2 (1.96 + 0.842)^2}{0.5^2}$$
$$n \geq \frac{2\,(1.2)(1.96 + 0.842)^2}{0.5^2} = 75.37$$ subjects per the 2 test products
There must be 152 subjects minimum (76 subjects per study arm), to execute this study according to the new requirements.</td></tr>
</table>

Name of Sponsor: GOJO Industries, Inc.	150548-102
Name/Active Ingredients: Ethanol (Concentration TBD based on Pilot) and Saline (Negative Control)	
	Note that this sample size is for estimate purposes only. An updated calculation will be performed using data from the pilot evaluation.
Approximate Number of Study Centers	1
Study Materials and Administration	Ethanol in water (concentration TBD based on pilot data) Negative Control (saline) Applied with 3 applications of 2ml using standard surgical hand rub application instructions
Efficacy Evaluation	Three primary endpoints: 1. Lower limit of the 95% confidence interval for the responder rate is equal to or greater than 70%, where a successful response is set at a 2.0 $\log_{10}$ reduction within 1 minute ("immediately") after a single rub. 2. Mean $\log_{10}$ recovery does not exceed baseline $\log_{10}$ recover at 6 hours sampling period. 3. Test product mean $\log_{10}$ reduction is superior to vehicle mean $\log_{10}$ reduction from baseline at "immediate" sampling time point using a two-sided statistical test for superiority and a 95 percent confidence interval. Additionally, use of an appropriate neutralizer in all recovery media (i.e., sampling solution, dilution fluid, and plating media) and a demonstration of neutralizer validation. The purpose of neutralizer validation is to show that the neutralizer used is effective against the test and control materials, and that it is not toxic to the test microorganisms. If a test product can be neutralized through dilution, this should be demonstrated in the neutralizer validation study. An analysis of the portion of subjects who meet the log reduction criteria based on a two-sided statistical test for superiority to the vehicle and a 95 percent confidence interval approach.

Appendix H: *In Vivo* Test Method ASTM E2755-10

Standard Test Method for Determining the Bacteria-Eliminating Effectiveness of Health Care Personnel Hand Rub Formulations Using Hands of Adults

Note to the Reviewer

This test method was recently approved by ASTM and ASTM E2755-15 will be released within the next month

Designation: E2755 – 10

Standard Test Method for Determining the Bacteria-Eliminating Effectiveness of Hand Sanitizer Formulations Using Hands of Adults[1]

This standard is issued under the fixed designation E2755; 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 This test method is designed to determine the activity of hand sanitizers (also known as hand rubs, hygienic hand rubs, or hand antiseptics) against transient bacterial flora on the hands.

1.2 Performance of this procedure requires the knowledge of regulations pertaining to the protection of human subjects (see 21 CFR Parts 50 and 56).

1.3 This test method should be performed by persons with training in microbiology, in facilities designed and equipped for work with potentially infectious agents at biosafety level 2.[2]

1.4 *Units*—The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.5 *This standard does not purport to address all of the safety concerns, 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.* For more specific precautionary statements, see 8.2.

2. Referenced Documents

2.1 *ASTM Standards:*[3]

E1054 Test Methods for Evaluation of Inactivators of Antimicrobial Agents

E1174 Test Method for Evaluation of the Effectiveness of Health Care Personnel Handwash Formulations

E2276 Test Method for Determining the Bacteria-Eliminating Effectiveness of Hygienic Handwash and Handrub Agents Using the Fingerpads of Adults

2.2 *Other Standards:*

AATCC Test Method 147 2004 Antibacterial Activity Assessment of Textile Materials: Parallel Streak Method[4]

21 CFR Parts 50 and 56 Protection of Human Subjects; Institutional Review Boards[5]

3. Terminology

3.1 *Definitions:*

3.1.1 *antiseptic*, *n*—a material for use on living tissue that either destroys microorganisms or suppresses their growth.

3.1.2 *hand sanitizer*, *n*—an antimicrobial gel, foam, liquid, spray, or wipe, used on hands that are not visibly soiled to reduce the number of transient microorganisms, which is applied by rubbing, and does not require a post-treatment water rinse. Such agents may also be referred to as hand rubs, hygienic hand rubs, or hand antiseptics.

3.1.3 *healthcare personnel handwash*, *n*—a cleanser or waterless agent intended to reduce transient bacteria on the hands.

3.1.4 *neutralization*, *n*—the process for inactivating or quenching the activity of a microbicide, often achieved through physical (for example, filtration or dilution) or chemical means, or both.

3.1.5 *resident microorganisms*, *n*—microorganisms that survive and multiply on the skin, forming a stable population.

3.1.6 *test bacteria*, *n*—an applied inoculum of bacteria that has characteristics which allow it to be readily identified. Test bacteria are used to simulate a transient topical microbial contaminant. This may also be referred to as a test organism, marker organism, simulant, or contaminant.

3.1.7 *test material*, *n*—a product or formulation which incorporates an antimicrobial ingredient(s).

3.1.8 *transient microorganisms*, *n*—microorganisms that contaminate the skin but do not form a stable population.

4. Summary of Test Method

4.1 This test method uses adult subjects who have provided a written informed consent and whose hands have been

[1] This test method is under the jurisdiction of ASTM Committee E35 on Pesticides and Alternative Control Agents and is the direct responsibility of Subcommittee E35.15 on Antimicrobial Agents.

Current edition approved June 1, 2010. Published June 2010. DOI: 10.1520/E2755–10.

[2] *CDC-NIH, Biosafety in Microbiological and Biomedical Laboratories*, 5th ed., U.S. Department of Health and Human Services, U.S. Government Printing Office, Washington, DC, 2007.

[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.

[4] Available from American Association of Textile Chemists and Colorists (AATCC), P.O. Box 12215, Research Triangle Park, NC 27709, http://www.aatcc.org.

[5] Available from U.S. Government Printing Office Superintendent of Documents, 732 N. Capitol St., NW, Mail Stop: SDE, Washington, DC 20401, http://www.access.gpo.gov.

determined to be free from any apparent damage at the time of participation in the study. Subjects are to refrain from use of any antimicrobials for at least one week prior to the initiation of the test procedure (see Section 11).

4.2 Subjects' hands are artificially contaminated with 0.2 mL of a high-titer suspension of the test bacteria which is distributed over all surfaces of the hands and fingers to produce a minimum baseline recovery level of 10^8 cfu/hand. Because *Serratia-marcescens* is relatively sensitive to drying, the high titer suspension is prepared by growing in broth with vigorous aeration, followed by a 10-fold concentration with centrifugation. *Staphylococcus-aureus* is more resistant to drying and is therefore not concentrated after growth with vigorous aeration in broth.

4.3 Test material effectiveness is measured by comparing the number of test bacteria recovered from contaminated hands after use of the test material to the number recovered from contaminated hands not exposed to the test material. Activity of the test material is measured following a single application and may also be measured after multiple consecutive contamination/application cycles in a single day.

5. Significance and Use

5.1 Hand hygiene is considered one of the most important measures for preventing the spread of infectious microorganisms. Hand sanitizers reduce the microbial load on the hands without the use of soap and water, and are thus an important tool in the practice of good hand hygiene. Hand sanitizers are recommended for use on hands that are not visibly soiled. They are formulated to be applied full strength to dry hands, "rubbed in" until dry, and are not rinsed off.

5.2 This test method is specifically designed to evaluate the bacteria-eliminating activity of hand sanitizers from experimentally-contaminated hands. It is intended to be an alternative to Test Method E1174, which was designed primarily to evaluate antimicrobial handwashing agents that are lathered with the aid of water and then rinsed off. When using Test Method E1174 to evaluate hand sanitizers, inadequate drying of the hands after contamination dilutes the test product and can compromise activity, leading to an underestimation of effectiveness. By applying a higher titer test bacteria suspension in a smaller volume, soil load on the hands is minimized and hands are completely dry prior to application of the test material. These modifications result in a better approximation of the in-use conditions for hand sanitizers and thus provide a more reliable indication of their performance in the field.

5.3 This test method can be used to test any form of hand sanitizer, including gels, rubs, sprays, foams, and wipes according to label directions at typical "in-use" doses.

5.4 Susceptibility to biocides can vary among different species of bacteria and major differences have been noted between gram-negative and gram-positive organisms. This test method provides the option to use either a gram-negative bacterium (*Serratia marcescens*) or a gram-positive bacterium (*Staphylococcus aureus*) as the test organism. *S. marcescens* is used as a test organism in both Test Method E1174 and Test Method E2276. *S. aureus* is a highly relevant pathogen in healthcare, institutional, and community settings. Moreover, hands are an important vehicle in the transfer of *S. aureus* between people and the environment, and in the transfer between individuals.

5.5 This test method may be used as an alternative to Test Method E2276, which limits the test bacteria to the fingerpads and does not incorporate actual use conditions such as friction during hand decontamination.

5.6 The investigator should be aware of potential health risks associated with the use of these organisms and precautions similar to those referenced in Section 8 should be taken.

6. Apparatus

6.1 *Centrifuge*—For the sedimentation of *S. marcescens* for concentration.

6.2 *Centrifuge Tubes*—Sterile, for sedimentation of *S. marcescens* for concentration.

6.3 *Colony Counter*—Any of several types may be used; for example, Quebec colony counters and similar devices. Automated, computerized plater/counter systems may also be used.

6.4 *Gloves*—Sterile, loose-fitting, unlined, powder-free gloves possessing no antimicrobial properties. Perform a zone of inhibition test, such as AATCC Test Method 147, to evaluate the antibacterial activity.

6.5 *Handwashing Sink*—Sufficient in size to permit handwashing without the touching of hands to sink surface or other subjects.

6.5.1 *Water Faucet(s)*—Located above the sink at a height to permit hands to be held higher than the elbow during the washing procedure.

6.5.2 *Tap Water Temperature Regulator and Temperature Monitor*—To set and maintain the tap water temperature at 40 ± 2°C.

6.6 *Incubator*—Capable of maintaining temperatures of 35 ± 2°C and 25 ± 2°C. The latter temperature ensures adequate pigment production for *S. marcescens* on solid media.

6.7 *Miscellaneous Labware*—Continuously adjustable pipetters (1-mL and 0.2-mL capacity) and sterile pipette tips, sterile serological pipettes (5.0-mL capacity), sterile culture tubes, sterile disposable Petri dishes, sterile syringes, Erlenmeyer flasks, and beakers.

6.8 *Sampling Containers*—Sterile or sterilizable containers having tight closures and sufficient capacity to hold 75 mL sampling solution (see 7.7).

6.9 *Shaking Incubator*—Rotary platform shaking incubator capable of maintaining 35 ± 2°C and capable of shaking at 250 r/min. Alternatively, use an incubator capable of maintaining 35 ± 2°C and able to accommodate a portable rotary shaker, capable of shaking at 250 r/min.

6.10 *Sterilizer*—Any steam sterilizer capable of processing culture media and reagents.

6.11 *Timer (Stop-Clock)*—Type that can be read for minutes and seconds.

6.12 *Tourniquets*—Children's size or any style capable of securing gloves to the wrist.

6.13 *Vortex Mixer*—Any vortex that will ensure proper mixing of culture tubes.

7. Reagents and Materials

7.1 *Antibiotic Ointment*—A topical, triple-antibiotic ointment for application to the hands after the final decontamination.

7.2 *Cleansing Wash*—A mild, proven non-antimicrobial liquid soap. May be purchased commercially or prepared according to the instructions provided in Test Method E1174.

7.3 *Chlorhexidine Skin Cleanser*—Antiseptic skin cleanser containing 4 % chlorhexidine gluconate (w/v) for hand decontamination.

7.4 *Culture Media*:

7.4.1 *Broth*—Soybean-casein digest broth (tryptic soy broth) is recommended.

7.4.2 *Agar Plating Media*:

7.4.2.1 *S. aureus Plating Medium*—HardyCHROM (trademark) *Staph aureus*[6] is recommended. Other indicator media for *S. aureus* or MRSA may be appropriate but should be validated prior to use.

NOTE 1—*S. aureus* forms smooth, deep pink to fuchsia-colored colonies. The growth of most other organisms, including *Staphylococcus epidermidis* are partially to completely inhibited.

7.4.2.2 *S. marcescens Plating Medium*—Soybean-casein digest agar (tryptic soy agar) is recommended.

7.5 *Dilution Fluid*—Sterile Butterfield's buffered phosphate diluent[7] (or other suitable diluent) adjusted to pH 7.2 ± 0.1 and containing an effective inactivator for the test material, if necessary.

NOTE 2—Inactivator is only required if neutralization of the test material cannot be achieved upon dilution into the sampling solution (see 7.7).

7.6 *Ethanol Solution*—70 % ethanol in water (v/v) for hand decontamination.

7.7 *Sampling Solution*—Dissolve 0.4 g KH_2PO_4, 10.1 g Na_2HPO_4, 1.0 g isooctylphenoxypolyethoxyethanol (for example, Triton X-100, Igepal CA-630, or Protachem OP-9), and appropriately validated neutralizers, if necessary, in distilled water. Adjust pH to 7.8 ± 0.1 with 0.1 N HCl or 0.1 N NaOH and bring volume to 1 L with distilled water. Sterilize in an autoclave and aseptically dispense 75–mL portions into sterile sampling containers (see 6.8).[8]

NOTE 3—A neutralizer validation should be conducted according to Test Methods E1054 prior to the study. Test Methods E1054 provides a list of neutralizers appropriate for commonly used antimicrobial agents. In some cases (for example, some alcohol-based hand sanitizers) neutralization is achieved by dilution alone.

7.8 *Test Material*—Use directions provided with the test material. If directions are not provided, use the directions given in this method.

8. Test Bacteria

8.1 *Serratia marcescens* (ATCC 14756). This strain forms a stable red pigmentation at 25°C.

8.2 *Staphylococcus aureus* (ATCC 6538 (methicillin-sensitive) or ATCC 33591 (methicillin-resistant)) is an alternative test bacteria. *S. aureus* is differentiated from resident microorganisms (including *Staphylococcus epidermidis*) with chromogenic indicator medium (see 7.4.2.1). (**Warning**—Application of microorganisms to the skin may involve a health risk. Determine the antibiotic sensitivity profile of the test bacteria prior to applying to the skin. After the test has been completed, decontaminate the subject's hands and follow proper procedures to reduce infection risk (12.1-12.4). If an infection occurs, provide the antibiotic sensitivity profile to the attending clinician.)

9. Preparation of Test Bacteria Suspension

9.1 *Method 1 (for S. marcescens)*:

9.1.1 A homogeneous bacterial suspension is used to inoculate the subjects' hands. Prepare a stock culture of *S. marcescens* (ATCC 14756) by inoculating approximately 5 mL of soybean-casein digest broth (see 7.4.1) from a cryogenic stock or lyophilized vial or pellet and incubate for 25 ± 1 h at 35 ± 2°C. Inoculate the appropriate volume of soybean-casein digest broth with 1 mL of the stock culture of *S. marcescens*/125 mL of broth to yield the volume necessary to complete the study (that is, 2 mL per hand contamination (see 11.3) per test subject). The volume of the broth culture should not exceed about one fourth of the capacity of the Erlenmeyer flask to ensure adequate aeration. Incubate for 25 ± 1 h at 35 ± 2°C with shaking at 250 r/min to yield a titer of approximately 1.0×10^{10} cfu/mL.

NOTE 4—The frozen or lyophilized stock should be at least two but no more than four 24-h soybean-casein digest broth (see 7.4.1) transfers from the original ATCC culture.

9.1.2 Transfer the culture to appropriate sized sterile centrifuge tubes or bottles and centrifuge at conditions appropriate to sediment the culture completely (recommended conditions are 7000 G for 10 min). Decant the supernatant and resuspend the pellet to one-tenth the original volume with soybean-casein digest broth (see 7.4.1) to yield a homogeneous suspension containing between 5.0×10^{10} and 1.0×10^{11} cfu/mL.

9.2 *Method 2 (for S. aureus)*:

9.2.1 Use a homogeneous bacterial suspension to inoculate the subjects' hands. Prepare a stock culture of *S. aureus* (AATCC 6538 or ATCC 33591) by inoculating approximately 5 mL of soybean-casein digest broth (see 7.4.1) from a frozen stock or lyophilized vial and incubate for 25 ± 1 h at 35 ± 2°C (see Note 4). Inoculate the appropriate volume of soybean-casein digest broth with 1 mL of stock culture of *S. aureus*/125 mL of broth to yield the volume necessary to complete the study (that is, 0.2 mL per hand contamination (see 11.3) per test subject). The volume of the broth culture should not exceed about one fourth of the capacity of the Erlenmeyer flask to ensure adequate aeration. Incubate for 25 ± 1 h at 35 ± 2°C with shaking at 250 r/min to yield a titer of approximately 1.0×10^{10} cfu/ml.

[6] Available from Hardy Diagnostics, Santa Maria, CA.

[7] Horowitz, W., (Ed.), *Official Methods of Analysis of the AOAC International*, 18th Ed., Sec. 6.3.03 A.(f), Chapter 6, p. 10. AOAC International, Gaithersburg, MD, 2000.

[8] Peterson, A. F., "The Microbiology of the Hands: Evaluating the Effects of the Surgical Scrubs," *Developments in Industrial Microbiology*, Vol. 14, 1973, pp. 125–130.

9.3 Swirl or shake suspension before the withdrawal of each aliquot. Assay the suspension for the number of organisms present at the beginning and at the end of the use period. Do not use a suspension for more than 8 h. The suspension should not vary more than ± 0.5 $\log_{10}$ cfu/mL over an 8-h period.

10. Subjects

10.1 Recruit a sufficient number of healthy adult human subjects who have no clinical evidence of dermatosis, cuts, lesions, hangnails, or other skin disorders on the hands or forearms. A minimum of eight subjects should be used for each test material. The total number of subjects used will depend on the number of test materials, the purpose of the study, and the regulatory requirements governing the study.

10.2 It is the responsibility of the user of this test method to obtain the necessary approval from an Institutional Review Board (IRB) or Independent Ethics Commission (IEC) for the use of adult human subjects for testing and to obtain informed and written consent from those selected for the study before starting the tests.

10.3 Instruct subjects to avoid contact with antimicrobial products for the duration of the test and for at least one week prior to the test. This restriction includes antimicrobial-containing antiperspirants, deodorants, shampoos, lotions, and soaps. Bathing in biocide-treated pools, hot tubs, or spas should be avoided. Harsh chemicals such as acids, bases, and solvents should also be avoided. Subjects may not use topical or systemic antimicrobials, antibiotics, or steroids other than for contraception or post-menopausal indications, and must agree to abstain from these materials until the completion of the study. Provide subjects with a kit of non-antimicrobial personal care products for exclusive use during the test and include rubber gloves to be worn when contact with antimicrobial or harsh chemicals cannot be avoided.

11. Procedure

11.1 *Admission to Testing*—Instruct each subject to return to the laboratory for testing after they having refrained from using antimicrobials for at least seven days. Question the subject to confirm adherence to the study requirements (see 10.3). Inspect the subject's hands and forearms to confirm the absence of clinical signs of skin disorders as described in 10.1. Admit the subject into the test if each of the above criteria is met. Instruct the subject to remove all jewelry from their hands and arms and to clip their fingernails to a uniform length (free edge ≤ 1 mm).

11.2 *Cleansing Wash*—Instruct the subject to perform a 30-s cleansing wash (see 7.2). This procedure removes oil and dirt from the hands and forearms. For this and all other hand washes and rinses, adjust the water temperature to 40 ± 2°C and the water flow rate to 4 L per minute. To adjust the flow rate, place a 2000-mL glass beaker or flask under each water faucet and allow the water to flow into the beaker. Adjust the water flow at each faucet accordingly, so that the beaker fills within 30 s.

11.2.1 Have subject thoroughly wet their hands and forearms under tap water.

11.2.2 Dispense 5 mL of the cleansing wash (see 7.2) into the subject's cupped hands and instruct subject to spread over hands and lower third of forearms.

11.2.3 Instruct subject to wash all surfaces of the hands and the lower third of the forearm in a vigorous manner for 30 ± 5 s. If the lather becomes too dry, add a small amount of water to maintain lather.

11.2.4 Instruct subject to rinse thoroughly from fingertips to elbows under tap water for 30 ± 5 s. Have the subject exercise caution to avoid contact with the sink and fixtures, eliminating the chance of recontamination from the sink surfaces. Also instruct subject to avoid rubbing hands and forearms during the rinsing process.

11.2.5 Hand subject a clean, dry paper towel and instruct them to lightly pat their hands and forearms dry.

11.3 *Hand Contamination*—Use a liquid suspension of the test bacteria prepared as directed (see 9.1 or 9.2).

11.3.1 Dispense a 0.2-mL aliquot of the test bacteria suspension into the subject's cupped hands. Instruct the subject to evenly distribute the inoculum over all surfaces of both hands and fingers, not reaching above the wrist, for 30 ± 5 s, making sure that the hands are dry.

NOTE 5—Subjects should not touch their clothing, face, or other objects with their hands during the test period. This prevents contamination of the subject and the environment with the test bacteria.

11.4 *Contamination, Product Application, and Recovery Schedule*—The subject's hands are contaminated with the test bacteria prior to the baseline recovery (see 11.5) and prior to each test material application (see 11.6). The test material is evaluated after a single contamination/application cycle and may be evaluated after multiple cycles. Table 1 illustrates a typical design. Perform a total of twelve hand contaminations. Sample the hands for baseline recovery (see 11.5) immediately after the first hand contamination, and apply test material (see 11.6) after each of the following eleven hand contaminations. To determine the test product effectiveness, recover bacteria from the hands (see 11.7) after test material applications 1 and 11.

TABLE 1 Hand Contamination, Product Application and Recovery Schedule

Name	Contamination	Type of Application	Recovery
Cleansing Wash	No	Cleansing Wash	No
Baseline	Yes	None	Plate Recovered Sampling Solution with Neutralizer
Cleansing Wash	No	Cleansing Wash	No
Test Application 1	Yes	Test Material	Plate Recovered Sampling Solution with Neutralizer
Cleansing Wash	No	Cleansing Wash	No
Test Applications 2–10	Yes	Test Material	No
Test Application 11	Yes	Test Material	Plate Recovered Sampling Solution with Neutralizer

NOTE 6—It is strongly recommended that ATCC 6538 be chosen when multiple contamination/application cycles are to be performed using *S. aureus* as the test bacteria.

NOTE 7—When evaluating the test material after multiple cycles, alternative contamination, sampling, and recovery schedules may be followed such as that described in the Tentative Final Monograph for Health-Care Antiseptic Drug Products.[9] Alternative schedules may also be followed as long as the same schedule is followed for all test products in the study.

11.5 *Baseline Recovery*—Recover the test bacteria surviving on the hands after the initial hand contamination (see 11.3) following the procedures outlined in 11.7 and enumerate according to Section 13. This represents the baseline recovery, which is typically between 8.5 $\log_{10}$ and 9.0 $\log_{10}$cfu/hand and may not be less than 8.0 $\log_{10}$ cfu/hand.

11.6 *Test Material Application*—Conduct the test in accordance with the use directions for the test material. If test material directions are not available, use the appropriate test material application procedure described as follows.

11.6.1 *Liquid, Gel and Spray Hand Sanitizers*:

11.6.1.1 Dispense 1.5 mL of test material into the subject's cupped hands from an appropriate dispenser or syringe within 10 s of completing the contamination step in 11.3.1.

11.6.1.2 Within 10 s, instruct the subject to distribute test material over all surfaces of the hands, fingers and lower third of forearms, paying attention to the nails, and continue rubbing until the product is dry. Subject should exercise caution to retain the test material in the hands.

11.6.1.3 Have subject hold hands upright and motionless prior to bacterial recovery (see 11.7).

11.6.2 *Foaming Formulations*:

11.6.2.1 Dispense approximately 1.5 g of test material from an appropriate foaming dispenser into the subject's cupped hands within 10 s of completing the contamination step in 11.3.1.

11.6.2.2 Within 10 s, instruct the subject to distribute test material over all surfaces of the hands, fingers and lower third of forearms, paying attention to the nails, and continue rubbing until the product is dry. Caution should be exercised to retain the test material in the hands.

11.6.2.3 Have subject hold hands upright and motionless prior to bacterial recovery (see 11.7).

11.6.3 *Hand Sanitizing Wipes (Towelettes)*:

11.6.3.1 Subject should remove a single towelette, or be handed a single towelette from its package, taking care not to touch the package material, and clean their fingernails for approximately 10 s, paying attention to the underside, and the cuticles.

11.6.3.2 Have the subject wipe the towelette broadly over the front and back surfaces of both hands and the lower third of the forearms until wet (approximately 5 s).

11.6.3.3 Next, the subject should scrub the fingers and thumbs of each hand, wrapping the towelette around each digit to wet entire surface completely (approximately 15 s).

11.6.3.4 Subject should turn the towelette over and scrub the palms of their hands up to the wrist, then scrub the back of their hands up to the wrist (approximately 10 s). Subject continues wiping all surfaces of both hands and lower third of the forearms until all liquid has evaporated.

11.6.3.5 Have subject hold hands upright and motionless prior to bacterial recovery (see 11.7).

11.7 *Bacterial Recovery*:

11.7.1 Within one minute after specified test material applications (Table 1), place gloves (see 6.4) on the subject's hands. Add 75 mL of sampling solution (see 7.7) with neutralizer to each glove and secure gloves above the wrist with a tourniquet.

NOTE 8—When the hand sampling schedule described in Note 7 is used, neutralizer is only included in the sampling solution after product application 10.

11.7.2 Within one minute of donning gloves, thoroughly and uniformly massage all surfaces of the subject's hands and fingers for 1 min ± 5 s.

11.7.3 Within one minute of completing the massage, aseptically retrieve a 5-mL sample of the sampling solution from the glove by pulling the glove away from the wrist, inserting a pipette into the finger region of the glove, and withdrawing the fluid.

11.7.4 Within 10 s, prepare the first dilution (see 13.1.2) in dilution fluid with an appropriate neutralizer, if required. Complete the plating of the recovered sampling solution within 30 min after sampling.

12. Hand Decontamination

12.1 Upon completion of testing, have subject rinse their hands and forearms for 1 min with 70 % ethanol (see 7.6) and air-dry.

12.2 Supervise subject performing a 4-min wash with a 4 % chlorhexidine gluconate handwash (see 7.3). Have the subject use a scrub brush during the first minute of the wash.

12.3 Apply a topical, antibiotic ointment (see 7.1) to the subject's hands and forearms.

12.4 When *S. aureus* is the test bacteria, subject should return to the laboratory approximately 24 h following testing. Inspect hands and forearms for any signs of infection at that time.

13. Enumeration of Bacteria

13.1 *S. marcescens*:

13.1.1 Enumerate the *S. marcescens* in the recovered sampling solution (see 11.7.3) using standard microbiological techniques, such as spread plating or spiral plating. The pour plate technique is not recommended because subsurface *S. marcescens* colonies may not exhibit the red pigment.

13.1.2 Prepare dilutions of the recovered sampling solution (see 11.7.3) in dilution fluid (see 7.5). Use soybean-casein digest agar (see 7.4.2.2) with suitable neutralizer, if necessary, as the recovery medium.

13.1.3 Incubate prepared plates 48 ± 4 h at 25 ± 2°C. Count only the red pigmented *S. marcescens* using an appropriate colony counter (see 6.3).

13.2 *S. aureus*:

[9] *Tentative Final Monograph for Health-Care Antiseptic Drug Products*; Proposed Rule, 1994 Federal Register, Vol. 59, No. 116.

13.2.1 Enumerate the *S. aureus* in the recovered sampling solution (see 11.7.3) using standard microbiological techniques, such as spread plating or spiral plating. The pour plate technique is not recommended.

13.2.2 Prepare dilutions of the recovered sampling solution (see 11.7.3) in dilution fluid (see 7.5). Use an appropriate indicator medium (see 7.4.2.1) with suitable neutralizer, if necessary, as the recovery medium.

13.2.3 Incubate prepared plates 24 ± 4 h at 35 ± 2°C. Count *S. aureus* colonies using an appropriate colony counter (see 6.3) based on manufacturer's instructions for the indicator medium (see 7.4.2.1).

14. Determination of Reduction

14.1 Convert plate counts (cfu/hand) to $\log_{10}$. Average the left and right hand values for each sampling interval.

14.2 Determine $\log_{10}$ reductions at each recovery interval/wash using the following formula:

$$\text{Log}_{10}\text{ Reduction at Sampling Interval} = \text{Log}_{10}\text{ Baseline Recovery} - \text{Log}_{10}\text{ Sampling Interval}$$

15. Comparison of Test Material

15.1 When comparing different test materials, assign an equivalent number of test subjects to each test material on a random basis. Use equivalent test parameters for all of the test materials (product application procedures for commercial products may be different) and evaluate all test materials concurrently.

16. Precision and Bias

16.1 A precision and bias statement cannot be made for this method at this time.

17. Keywords

17.1 alcohol-based hand rub; antimicrobial; contaminant; efficacy; hand antiseptic; hand sanitizer; healthcare personnel handwash; *Serratia marcescens*; *Staphylococcus aureus*

Appendix I: *In Vivo* HCP Hand Rub Study Synopsis (Pilot)

Name of Sponsor: GOJO Industries, Inc. **Name/Active Ingredients:** 3-5 ethanol concentrations and/or application volumes	150607-101
Study Title	Pilot Study to determine the best dose/concentration for use in the pivotal study using the standardized ASTM E2755-10 test method with a cross-over design
Clinical Phase	Phase III
Study Center	BioScience Laboratories Inc.
Study Rationale	This study is being conducted to evaluate the antimicrobial effect of three or more ethanol concentrations and / or application doses for use as a health care personnel hand rub in order to select the lead candidate for a pivotal efficacy study, and inform the sample size calculation for the pivotal trial.
Trial Design	At least thirty (30) subjects will be evaluated in order to complete twenty-four (24) subjects using a cross-over sampling design. Baseline values and microbial log10 reductions for each test configuration will be calculated for each subject. The indicator microorganism will be *Serratia marcescens* (ATCC #14756). The testing methods are based on ASTM E 2755-10 *Determining the Bacteria-Eliminating Effectiveness of Hand Sanitizer Formulations Using Hands of Adults*. Mean $\log_{10}$ reductions of the indicator microorganism will be used to determine the antimicrobial effectiveness of each ethanol concentration and/or doses.
Treatment Duration	Single application
Approximate Duration of Study	1 week
Study Objective	The purpose of this study is to determine the best dose and / or concentration of ethanol for use in the pivotal study.
Diagnosis and Main Criteria for Inclusion	1. Subjects may be of either sex, at least 18 years of age, and of any race. 2. Subjects must possess both hands. 3. Subjects must be in good general health, as evidenced by the Subject Confidential Information and Acceptance Criteria. 4. Subjects must have read and signed an Informed Consent Form, Subject Confidential Information and Acceptance Criteria, Authorization to Use and Disclose Protected Health Information Form and List of Restricted Products.
Main Criteria for Exclusion	1. Exposure of ungloved hands or forearms to antimicrobial agents, medicated soaps, medicated shampoos, hair mousses, or medicated lotions, use of biocide-treated pools or hot tubs, or use of UV tanning beds or sunbathing during the 7-day pre-test

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	conditioning period or on the single test day. 2. 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. 3. Use of systemic or topical antibiotic medications, or steroids, other than for contraception or post-menopausal indications, during the 7-day pre-test conditioning period or on the single test day. 4. Application 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. 5. Known allergies to latex (rubber), alcohols, to common antibacterial agents found in soaps or lotions, particularly chlorhexidine gluconate, or to topical antibiotic ointments (e.g., Neosporin® or Polysporin® [neomycin/bacitracin/polymyxin B]). 6. A medical diagnosis of a physical condition, such as a current or recent severe illness, asthma, diabetes, hepatitis, an organ transplant, mitral valve prolapse, congenital heart disease, internal prostheses, or any immunocompromised conditions such as AIDS (or HIV positive). 7. Pregnancy, plans to become pregnant within the pre-test and test periods of the study, or nursing a child. 8. 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. 9. A currently active skin disease or inflammatory skin condition, such as contact dermatitis, anywhere on the body, that in the opinion of the Principal Investigator or consulting physician should preclude participation. 10. Participation in a clinical study in the past 7 days or current participation in another clinical study. 11. Any medical condition or use of any medications that, in the opinion of the Study Director, should preclude participation. 12. Unwillingness to fulfill the performance requirements of the study.
Approximate Number of Subjects	24
Approximate Number of Study Centers	1
Study Drug(s) and Administration	3-5 ethanol concentration and/or application volumes
Efficacy Evaluation	Primary endpoint: 1. Lower limit of the 95% confidence interval for the

Name of Sponsor: GOJO Industries, Inc.	150607-101
Name/Active Ingredients: 3-5 ethanol concentrations and/or application volumes	
	responder rate is equal to or greater than 70%, where a successful response is set at a 2.5 $\log_{10}$ reduction within 5 minutes after a single application. Additionally, use of an appropriate neutralizer in all recovery media (i.e., sampling solution, dilution fluid, and plating media) and a demonstration of neutralizer validation. The purpose of neutralizer validation is to show that the neutralizer used is effective against the test and control materials, and that it is not toxic to the test microorganisms. If a test product can be neutralized through dilution, this should be demonstrated in the neutralizer validation study.

Appendix J: *In Vivo* HCP Hand Rub Study Synopsis (Pivotal)

Name of Sponsor: GOJO Industries, Inc.	150606-101
Name/Active Ingredients: One Ethanol Concentration (TBD based on pilot) and a Negative Control (Saline)	
Study Title	Pivotal Study to Determine the antimicrobial efficacy of one ethanol concentration (TBD based on pilot data) compared to a negative (saline) control using the standardized ASTM E2755-10 test method with a cross-over design.
Clinical Phase	Phase III
Study Center	BioScience Laboratories Inc.
Study Rationale	This study is being conducted to support ethanol as an effective antimicrobial active for the health care personnel hand rub indication.
Trial Design	A minimum of sixty three subjects will be evaluated. Sampling for baseline log recovery and microbial log reductions after a single application will occur for the test and control materials. The indicator microorganism will be *Serratia marcescens* (ATCC #14756). The testing methods are based on ASTM E 2755-10 *Determining the Bacteria-Eliminating Effectiveness of Hand Sanitizer Formulations Using Hands of Adults*. Mean $\log_{10}$ reductions of the indicator microorganism will be used to determine the antimicrobial effectiveness of the test product when compared to the negative (saline) control.
Treatment Duration	Single application
Approximate Duration of Study	2 weeks
Study Objective	The purpose of this study is to determine the antimicrobial effectiveness of ethanol (concentration TBD) as compared to a negative (saline) control when applied once to the hands of volunteers.
Diagnosis and Main Criteria for Inclusion	1. Subjects may be of either sex, at least 18 years of age, and of any race. 2. Subjects must possess both hands. 3. Subjects must be in good general health, as evidenced by the Subject Confidential Information and Acceptance Criteria. 4. Subjects must have read and signed an Informed Consent Form,

Name of Sponsor: GOJO Industries, Inc.	150606-101
Name/Active Ingredients: One Ethanol Concentration (TBD based on pilot) and a Negative Control (Saline)	
	Subject Confidential Information and Acceptance Criteria, Authorization to Use and Disclose Protected Health Information Form and List of Restricted Products.
Main Criteria for Exclusion	1. Exposure of ungloved hands or forearms to antimicrobial agents, medicated soaps, medicated shampoos, hair mousses, or medicated lotions, 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. 2. 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. 3. Use of systemic or topical antibiotic medications, or steroids, other than for contraception or post-menopausal indications, during the 7-day pre-test conditioning period or on the single test day. 4. Application 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. 5. Known allergies to latex (rubber), alcohols, to common antibacterial agents found in soaps or lotions, particularly chlorhexidine gluconate, or to topical antibiotic ointments (e.g., Neosporin® or Polysporin® [neomycin/bacitracin/polymyxin B]). 6. A medical diagnosis of a physical condition, such as a current or recent severe illness, asthma, diabetes, hepatitis, an organ transplant, mitral valve prolapse, congenital heart disease, internal prostheses, or any immunocompromised conditions such as AIDS (or HIV positive). 7. Pregnancy, plans to become pregnant within the pre-test and test periods of the study, or nursing a child. 8. 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. 9. A currently active skin disease or inflammatory skin condition, such as contact dermatitis, anywhere on the body, that in the opinion of the Principal Investigator or consulting physician should preclude participation. 10. Participation in a clinical study in the past 7 days or current participation in another clinical study.

Name of Sponsor: GOJO Industries, Inc.	150606-101
Name/Active Ingredients: One Ethanol Concentration (TBD based on pilot) and a Negative Control (Saline)	
	11. Any medical condition or use of any medications that, in the opinion of the Study Director, should preclude participation. 12. Unwillingness to fulfill the performance requirements of the study.
Approximate Number of Subjects	The sample size calculation has the formula: $n \geq \frac{x\, s^2 \left(z_{\alpha/2} + z_{\beta}\right)^2}{d^2}$ where: n = number of subjects per test material s= 1 x = number of products evaluated- 2 test materials $z_{\alpha/2}$ =0.05 level of significance = 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 2 materials will be evaluated using a crossover design: $n \geq \frac{(2)\,(1)(1.96 + 0.842)^2}{0.5^2} = 63$ subjects There must be 63 subjects minimum to execute this study according to the new requirements. Note that this sample size is for estimate purposes only. An updated

Name of Sponsor: GOJO Industries, Inc.	150606-101
Name/Active Ingredients: One Ethanol Concentration (TBD based on pilot) and a Negative Control (Saline)	
	calculation will be performed using data from the pilot evaluation.
Approximate Number of Study Centers	1
Study Drug(s) and Administration	One ethanol concentration (TBD based on pilot data) with a negative control (saline) Dose (TBD based on pilot data)
Efficacy Evaluation	2 Primary Endpoints: 1. Lower limit of the 95% confidence interval for the responder rate is equal to or greater than 70%, where a successful response is set at a 2.5 $\log_{10}$ reduction within 5 minutes after a single application. 2. Test product mean $\log_{10}$ reduction is superior to vehicle mean $\log_{10}$ reduction from baseline using a two-sided statistical test for superiority and a 95 percent confidence interval. Use of an appropriate neutralizer in all recovery media (i.e., sampling solution, dilution fluid, and plating media) and a demonstration of neutralizer validation. The purpose of neutralizer validation is to show that the neutralizer used is effective against the test and control materials, and that it is not toxic to the test microorganisms. If a test product can be neutralized through dilution, this should be demonstrated in the neutralizer validation study.