September 17, 2015

Alina W. Salvatore, R.Ph., M.S.
CDR, United States Public Health Service
Senior Regulatory Project Manager
FDA/Center for Drug Evaluation and Research Division of Nonprescription Drug Products
Office of Drug Evaluation IV
WO22 - Room 5416 10903
New Hampshire Avenue
Silver Spring, MD 20993

RE: Meeting Information Package for Type C Meeting with the FDA Center for Drug Evaluation and Research Division of Nonprescription Drug Products, Office of Drug Evaluation IV Concerning Isopropyl Alcohol

Dear Dr. Salvatore,

On behalf of the American Chemistry Council's Isopropanol Panel,[1] I am submitting the enclosed meeting information packet for the Type C meeting the Isopropanol Panel has scheduled with the U.S. Food and Drug Administration's (FDA) Center for Drug Evaluation and Research Division of Nonprescription Drug Products' Office of Drug Evaluation IV on October 20, 2015. The meeting was requested to discuss the data gaps identified for isopropyl alcohol (CAS Number: 67-63-0) for use in patient preoperative skin preparations, health care personnel hand rub, and surgical hand rubs as indicated in the May 1, 2015, proposed rule *Safety and Effectiveness of Health Care Antiseptics; Topical Antimicrobial Drug Products for Over-the-Counter Human Use; Proposed Amendment of the Tentative Final Monograph; Reopening of the Administrative Record* (Proposed Rule).[2]

Please do not hesitate to contact me at 202-249-6708 or angela_lynch@americanchemistry.com with questions about this information.

Sincerely,

Angela Lynch, MSPH, PhD
Isopropanol Panel Manager
American Chemistry Council
700 2nd St., NE
Washington, DC 20002

Enclosure: Meeting Information Package

[1]The American Chemistry Council (ACC) Isopropanol Panel including The Dow Chemical Company, ExxonMobil, and Shell International, represents manufacturers of isopropanol in the United States. The Panel is committed to health, safety, security and environmental issues relating to the production, transportation or use of isopropanol.
[2] 80 Fed. Reg. 25166 (May 1, 2015).

HEALTHCARE ANTISEPTIC

TENTATIVE FINAL MONOGRAPH

[Docket FDA-2015-N-0101]

Isopropyl Alcohol

Meeting Information Package

FDA Meeting October 20, 2015

IPA Meeting Information Package
Table of Contents

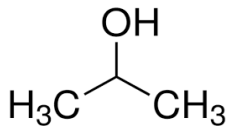
Table of Contents

Product Name

Isopropyl Alcohol 60-91.3%

Chemical Name and Structure

Isopropyl Alcohol, CAS Number 67-63-0



Proposed Indication

Healthcare Antiseptic for use as an active ingredient in the following:

1) patient preoperative skin preparations,
2) healthcare personnel hand rubs, and
3) surgical handrubs.

Dosage Form, Route and Dosing Regimen

Leave-on topical gel, liquid, foam or wipe applied to hands as needed or as a surgical pre-operative skin preparation.

Attendees

Name	Title	Affiliation
Angela Lynch, MSPH, PhD	Isopropanol Panel Manager Toxicologist	American Chemistry Council
Neeraja Erraguntla, PhD, DABT	Director, Chemical Products & Technology	American Chemistry Council
Komal Jain, JD	Isopropanol Panel Legal Counsel	American Chemistry Council
Brian Hughes, MPH, PhD, DABT	Toxicology Consultant	The Dow Chemical Company
Laura Keller	Regulatory Affairs and Advocacy Advisor	ExxonMobil
David Adenuga, PhD	Senior Toxicologist	ExxonMobil
Satinder Sarang, PhD, DABT	Senior Toxicologist	Shell International
Mike Ebers	Director of Regulatory Affairs	STERIS
Dan Klein	Senior Manager Microbiology	STERIS
John L. O'Donoghue, VMD, PhD, DABT	Consulting Toxicologist and Pathologist	STERIS

Background

Isopropyl alcohol (IPA) and products containing IPA have been the subject of a number of interagency, intergovernmental, and agency-specific activities over a period of more than 40 years. On September 3, 2014, the U.S. Food and Drug Administration's (FDA) Nonprescription Drugs Advisory Committee (Advisory Committee) convened to discuss the "Pre-market safety testing framework for over-the-counter healthcare antiseptic drugs," which included a discussion on the adequacy of safety and effectiveness data for IPA (FDA, 2014). FDA's May 1, 2015, proposed rule on *Safety and Effectiveness of Health Care Antiseptics; Topical Antimicrobial Drug Products for Over-the-Counter Human Use; Proposed Amendment of the Tentative Final Monograph; Reopening of the Administrative Record* (Proposed Rule) states that additional safety and effectiveness data may be necessary to support a determination of generally recognized as safe/generally recognized as effective (GRAS/GRAE) for IPA (FDA, 2015).

Purpose of Meeting

The purpose of this meeting is to discuss the data gaps identified by FDA and provide additional data and methodologies to fulfill those data requirements including data requests for:

- Human Pharmacokinetics and Animal ADME;
- Oral /Dermal Carcinogenicity;
- Hormonal Effects; and
- Efficacy Studies

Proposed Agenda

Agenda Item		Designated Speaker
I.	Introductions	5 minutes
II.	ACC IPA Panel Opening Remarks	5 minutes
III.	Discussion	40 minutes
IV.	Summarize agreements and action items	10 minutes
		Estimated Total Time: 60 minutes

Data Discussion for Human Pharmacokinetics and Animal ADME

The development of human and animal PBPK models for IPA and IPA derived acetone adds significant new information about IPA pharmacokinetics that should be considered by FDA and its Advisory Committee. In the FDA Briefing Document for a Meeting of the Nonprescription Drugs Advisory Committee, "Pre-market safety testing framework for over-the-counter healthcare antiseptic drugs," September 3, 2014, Appendix B7 includes references to Boatman et al. and Kapp et al. which are peer-reviewed manuscripts of studies conducted under the United States Environmental Protection Agency (EPA) TSCA Test Rule, referred to as "the Test Rule"(EPA, 1989;Boatman, 1998; Kapp, 1996; FDA, 2014). However, other peer-reviewed manuscripts on the pharmacokinetics of IPA and acetone (the primary metabolite of IPA) in humans and animals following IPA administration are not included in Appendix B7 of the Briefing Document.

Based on data from the Test Rule, Clewell et al. developed a physiologically based pharmacokinetic (PBPK) model for IPA and its major metabolite (acetone) (Clewell, 2001). The model provides a coherent description of IPA and acetone kinetics in the rat and human for exposures to IPA by several routes: intravenous, intraperitoneal, oral, inhalation, and dermal. The model is a validated framework for performing chemical-specific route-to-route extrapolation and cross-species dosimetry in support of risk assessments for IPA and acetone. As an example, others have extended the PBPK model to derive reference values (RfC and/or RfD) for humans (Gentry, 2002, Clewell, 2001), estimate internal dose metrics for mother and fetus exposed during pregnancy and during lactation (Gentry, 2003) and evaluate age- and gender-specific differences in tissue dosimetry for IPA and acetone (Clewell, 2004).

In addition, Gentry et al. used the PBPK model developed by Clewell et al. to derive a reference dose (RfD) and a reference concentration (RfC) for IPA (Gentry, 2002; Clewell, 2001). Adult PBPK models for rats and humans were extended by Gentry et al. to simulate exposure to IPA during pregnancy and used to estimate internal dose metrics in the mother and fetus during development (Gentry, 2002; Gentry, 2003). Endpoints for chronic, developmental, and reproductive toxicity from the Test Rule studies were used for the derivation of RfDs and RfCs. Recommended RfD and RfC values for IPA determined by Gentry et al. are 10 mg/kg/day and 40 ppm, respectively, based on decreased fetal body weights. All of the PBPK-derived RfD or RfC values for various endpoints were similar (within a factor of 3), regardless of route of exposure in the animal study (Gentry, 2002).

Clewell et al. further employed the human PBPK model developed to evaluate the potential impact of age- and gender-specific pharmacokinetics differences in tissue dosimetry for IPA and its circulating metabolite acetone (Clewell, 2004). Age-dependent metabolism of IPA was based on data for aldehyde dehydrogenase (ADH), while age-dependent metabolism of acetone was based on data for CYP2E1. Predictions of age-dependent dosimetry were conducted for three routes of exposure: oral, dermal, and inhalation. Artificial continuous exposure scenarios for dermal exposure (0.07 mg/l over 18.5% body surface area for males and 20% body surface area for females) and inhalation exposure (1 ppb

continuous) were selected to result in values of the dose metrics on the same order of magnitude as the oral exposure, so comparisons could readily be made of the impact of route of exposure on age-dependent behavior. Oral exposure was characterized as a constant daily intake, while inhalation and dermal exposures were characterized as a constant media concentration (in the air or in a water vehicle on a constant fraction of the total skin surface area). There were no gender-related differences in average daily dose for IPA or acetone. Maximum age-related differences in average daily dose were about a factor of 2 for IPA and highest in the age period birth to six-months when exposure was by inhalation and lowest by dermal exposure. Age-related differences in acetone dose were also highest in the birth to six month age group following inhalation. The acetone differences appear to be due to the maturity of the metabolic enzyme systems in infants (Sarangapani, 2003). Gentry et al. additionally studied IPA pharmacokinetic differences in offspring during pregnancy and lactation (Gentry, 2003). This work showed that changes in dosimetry during pregnancy predicted by modeling IPA were mainly attributable to the development of enzymatic pathways in the fetus or to changes in tissue composition in the mother and fetus during pregnancy. In general, blood concentrations were lower in the neonate during the lactation period than in the fetus during gestation.

In regards to the request for animal ADME data by the dermal route of exposure, it is expected that the available IPA PBPK model provides a methodology for predicting the fate of IPA through existing dermal component of the PBPK model. *In lieu* of a MUsT assay in humans, the PBPK model can be adjusted to account for the higher exposure requirements of the MUsT assay to predict potential human fate. The development of human and animal PBPK models for IPA and IPA-derived acetone adds significant new information about IPA pharmacokinetics that should be considered along with the studies already evaluated by FDA and the Nonprescription Drugs Advisory Committee.

Human Pharmacokinetics and ADME Animal Studies Questions

i. Does the FDA agree that the published, peer-reviewed human PBPK models for IPA and acetone can be used in lieu of a MUsT study to address the data gaps that FDA perceives to exist for IPA? If not, what data cannot be provided by the PBPK models and how would the data from a MUsT study be used in the assessment of IPA?

ii. Does the FDA agree that the published, peer-reviewed animal PBPK models for IPA and acetone can be used to address the ADME data gaps that FDA perceives to exist for IPA? If not, what data cannot be provided by the PBPK models and how would the data from an additional animal pharmacokinetic study be used in the assessment of IPA?

Data Discussion for Oral and Dermal Carcinogenicity Studies

Regulatory Requirement

In Table 9 of the Proposed Rule, the FDA lists the criteria that must be fulfilled specifically with regard to the carcinogenicity endpoint (ICH S1A, S1B, and S1C) (FDA, 2015) These criteria include a minimum of one oral and one dermal study for topical products. According to FDA, these data will be used to identify potential carcinogenic risk associated with systemic and dermal exposure (dermal being the most appropriate route of exposure for topical products) with the active ingredient in the topical product, taking into consideration type of toxicity, level of exposure that produces these toxicities, and the highest level at which no adverse effects are expected to occur, or rather, the No Observed Adverse Effect level (NOAEL).

FDA Review of Available Data on Isopropyl Alcohol (IPA)

With regard to IPA, the FDA surveyed the available data and concluded that there is no data available on oral carcinogenicity while the data on dermal carcinogenicity is incomplete (FDA, 2015) This conclusion was based on an International Agency for Research on Cancer (IARC) review of the carcinogenicity of isopropyl alcohol in humans and rodents. In all the studies evaluated, an increased incidence of cancer of the paranasal sinus was noted in workers at factories where IPA was manufactured by the strong-acid process (IARC, 1999). Another case-control study of IPA exposure did not show any evidence for an excess risk of cancer and lifetime inhalation studies in both mice and rats and were negative for nasal neoplasms and/or neoplastic lesions (Burleigh-Flayer,1997). In this initial review, IARC concluded that there was *inadequate evidence* for the carcinogenicity of IPA in both humans and experimental animals. IARC's overall evaluation was that IPA was not classifiable as to its carcinogenicity to humans (Group 3). In 2012, IARC conducted a more extensive review of the available epidemiological data in workers at factories where IPA is manufactured by the strong-acid process (leading to the exposure to strong acid mists and/or the presence of diisopropyl sulfate) and other occupations where exposure to strong inorganic acids are likely to occur (manufacture of phosphate fertilizer, synthetic ethanol, nitric acid, etc.) (IARC, 2012a; IARC, 2012b). In both reviews, IARC concluded that there was *sufficient evidence* in humans for carcinogenicity of mists from strong inorganic acids as a result of multiple lines of evidence showing an increased risk for cancer of the respiratory tract (including laryngeal cancer, paranasal cancer and lung cancer). In both cases, the increased carcinogenic risk was significantly associated with acid mist irritation of the mucous epithelia of the respiratory tract and increased macroscopic and/or microscopic lesions of the nasal mucosa (such as squamous metaplasia and atypia). As a result, IARC indicated that the most plausible mode of action was likely due to localized cytotoxic damage subsequently leading to DNA damage as a result of exposure to low pH inorganic acid mists. IARC further concluded that there was no evidence that could support DNA damage through any other mechanism of carcinogenesis and no evidence for an increased risk of cancer in humans was reported for other exposures to IPA that did not involve concurrent exposure to strong acid mists. Both reviews provided conclusive evidence that the cancer risk was caused in response to the cytotoxic effects of inorganic acid

mists formed during the strong-acid process and not by the presence of IPA itself or one of its metabolites; a conclusion that is further corroborated by the absence of similar non-neoplastic lesions and respiratory tract tumors with lifetime exposures to significantly high levels of IPA by inhalation in both rats and mice (Burleigh-Flayer , 1997).

With regard to dermal carcinogenicity, the FDA reviewed a 1-year carcinogenicity study of mice dermally treated with IPA, three times a week. No skin tumors were observed. However, the FDA concluded that the administrative record for the safety of isopropyl alcohol is incomplete with respect to oral and dermal carcinogenicity (FDA, 2015).

Evaluation of FDA-Identified Data Gaps

In reviewing the Proposed Rule, it is not clear why oral and dermal carcinogenicity studies are cited as data gaps (considering that a well-conducted carcinogenicity study via the inhalation route of exposure is available) other than the fact that carcinogenicity is a required endpoint as indicated in the ICH guideline for carcinogenicity studies of pharmaceuticals (FDA, 2015; ICH, 1995). According to the ICH guidelines, the objective for requiring a carcinogenicity study for a pharmaceutical product (expected to be administered regularly over a substantial part of a patient's lifetime) is to identify the potential for tumorigenicity in animals with the view to assessing the relevance of this finding in humans. The guideline document, however, highlights that the original intent of requiring these studies was defined at a time when current technology capable of detecting genotoxicity potential and improved understanding of modes of action for tumorigenicity with non-genotoxic agents was not available. According to the S1A guideline, these additional data (including data on toxicokinetics and mechanistic information) are useful, not only in providing a means of interpreting study outcomes of a carcinogenicity study but also in determining whether there is a need to perform a carcinogenicity study for the particular agent in the first place. In other words, the need for a carcinogenicity study should not be considered a default requirement, but must be assessed in light of all other available toxicological data.

In Section 4.2 of the S1A guideline, the ICH lists four important criteria (among others) that could be considered to determine whether there is a "cause for concern" such as to warrant the need to conduct a carcinogenicity study. These include:

1. *Previous demonstration of carcinogenic potential in the product class that is considered relevant to humans* –

a) Genotoxicity – The *in vitro* genotoxicity potential of IPA was studied in bacteria (*Salmonella typhimurium* (TA 97, TA 98, TA 100, TA 1535, TA 1537) at concentrations up to 10000 µg/plate, with or without metabolic activation (Zeiger, 1992). The authors reported no evidence for an increase in revertant colony counts in the study. IARC, in its 1999 review of IPA, evaluated additional genotoxicity studies on IPA including *in vitro* bacterial reverse mutation tests (*S. typhimurium* and *E. coli* WP2 *uvr*A), mammalian gene mutation studies in CHO cells, sister chromatid exchange test in

Chinese Hamster V79 cells and *in vivo* micronucleus tests in the bone marrow of ICR mice (IARC, 1999). Results of all studies were determined to be negative, with or without metabolic activation.
As has been shown, IPA is rapidly metabolized to acetone, regardless of route of exposure or test species (mice, rabbits, rats and humans) (Martinez, 1986; Slauter, 1994). Hence, an evaluation of the genotoxic potential of IPA must include evaluation of the same endpoint for acetone since exposure to IPA translates to an indirect exposure to acetone. A thorough evaluation of the available genotoxicity data on acetone has been conducted through the EPA's Integrated Risk Information System (IRIS) program (USEPA, 2003a). The data evaluated include reverse mutation assays in bacteria; cell transformation tests in Syrian Hamster embryo cells; *in vivo* bone marrow micronucleus tests in hamsters; unscheduled DNA synthesis assay in cultured human epithelial cells; sister chromatid exchange tests in human and non-human cells; and *in vitro* chromosome aberration assays. In all cases, no evidence for genotoxicity of acetone was observed.

No evidence for genotoxicity has been reported for substances similar to either IPA or acetone. These include n-butanol, 2-butanol and its metabolite, methylethylketone (USEPA, 2003b; USEPA, 2011). Studies indicate that secondary alcohols (such as IPA) and their corresponding ketone metabolites, do not possess genotoxic properties such that may suggest the potential for carcinogenicity in rodents and/or humans.

b) *Carcinogenic potential in substances with similar structure and/or metabolites* – No oral carcinogenicity studies have been conducted for acetone. However, acetone has a long history of being utilized as a vehicle in dermal carcinogenicity studies with no evidence of increased incidence of tumors compared to concurrent no treatment groups (Van Duuren, 1978). In one other study, 0.2 ml acetone vehicle was applied to the shaved skin of male/female CF-1 mice, once per week for 2 years. The authors reported no carcinogenic effects, with no adverse effects on survival of all animals tested (Zakova, 1985). Ward et al.,evaluated 60 female mice using acetone for vehicle controls in skin painting studies. Mice were dermally exposed to 0.2 ml acetone, 1-2 times per week for up to 92 weeks. There was no evidence for a treatment-related increase in tumor incidence in any of the organs investigated (Ward, 1986).

No oral studies are available for methylethylketone; however, a mouse skin painting study investigating the potential carcinogenicity of organic sulfur compounds, using methylethylketone as a vehicle, is available (Horton, 1965). Similar to acetone, methylethylketone showed no potential to induce skin tumors with prolonged exposure.

No carcinogenicity studies were found for n-butanol and 2-butanol through any routes of exposure. But, it is generally accepted that these substances are not likely to pose a carcinogenic risk based on a lack of structural alerts for carcinogenicity, lack of carcinogenicity in studies with their respective metabolites and the lack of genotoxicity potential in both *in vivo* and *in vitro* studies. Two carcinogenicity studies of IPA, through the inhalation route of exposure, are described in the Proposed Rule. Both studies involved exposures up to 5000 ppm for 24 months and 18 months in rats and mice, respectively. Overall, no treatment-related increase in the incidence of tumors with

human relevance was noted (Burleigh-Flayer , 1997). Although this study was not conducted via the oral and/or dermal routes of exposure, it provides additional evidence that this class of substances do not pose a carcinogenic risk to humans.

2. *Structure-activity relationship suggesting carcinogenic risk* – Utilizing the rich database on carcinogenic compounds available, Benigni et al. developed a QSAR-based approach capable of identifying structural alerts to predict for both potential genotoxic and non-genotoxic carcinogens (Benigni, 2013). The predictive model for non-genotoxic carcinogens took into consideration common non-genotoxic carcinogenic modes of action such as peroxisome proliferation, AhR agonists/antagonists, induction of oxidative stress and hormonal imbalance. Of 56 functional groups identified as structural alerts, no alcohols and/or ketones were identified. Therefore, IPA and acetone do not possess structural alerts for a non-genotoxic mode of action for carcinogenicity, a conclusion that is corroborated by the available carcinogenicity evidence.

3. *Evidence of pre-neoplastic lesions in repeated dose toxicity studies* – Several repeat dose toxicity studies of IPA are available via the inhalation and oral routes of exposure. In one study, rats were exposed to up to 5000 ppm IPA by inhalation, 6 hours/day, and 5 days/week for 13 weeks (Burleigh-Flayer,1994). The authors reported that the only treatment-related tissue lesions in the rats were male rat-specific hyaline droplets in the kidneys. These effects are well defined male-rat specific effects of exposure to organic substances that have no relevance to humans (USEPA, 1991). Lehman et al. administered 600 and 2300 mg/kg (males) or 1000 and 3900 mg/kg (females) IPA to rats in drinking water continuously for 27 weeks. The authors reported body weight decreases in female rats throughout the duration of the study. However, no gross or macroscopic lesions were noted (Lehman , 1944). In a similar study, dogs administered 1300 mg/kg IPA in drinking water for 6 months showed transient evidence of acute CNS depression (common with volatile organic compounds) but no pathologic changes in tissues evaluated (Lehman, 1945). The same authors exposed parents and two successive generations of rats to 1300, 1400 0r 1500 mg/kg IPA continuously in drinking water. The authors reported transient growth retardation in the first generation offspring. No other pathologic effects were reported in any of the treated animals. Rats were administered 1, 2, 3 or 5% IPA in drinking water, continuously for 12 weeks (Pilegaard, 1993). The authors reported a dose-dependent increase in relative liver, kidney and adrenal weights. However, no histological lesions were observed in the rats apart from the male rat-specific hyaline droplet formation in the proximal tubules of the kidneys; lesions that are not relevant to humans.

Oral and inhalation repeat dose studies for acetone are summarized in the existing EPA IRIS toxicological assessment (USEPA, 2003a). Similar to IPA, tissue lesions appeared to be restricted to male rat-specific kidney effects and occasional increases in tissues weights (particularly for the liver). Overall, there was no evidence for pre-neoplastic lesions in repeated dose toxicity studies for IPA and acetone through the oral and inhalation routes of exposure.

4. *Long-term tissue retention of parent compound or metabolites resulting in local tissue reactions or other pathophysiological responses* – Although the ICH guideline document does not indicate what

length of time is considered "long-term", there is sufficient evidence to indicate that IPA and its metabolite, acetone, are rapidly eliminated following exposure and show minimal evidence of tissue accumulation. In a study of IPA intoxication in a woman, elimination of IPA and acetone were reported to follow a 1st-order kinetic model, with half-lives of 6.4 hours and 22.4 hours for IPA and acetone, respectively (Natowicz, 1985). Serum levels of IPA were below detection limit within 40 hours of admission. Similar half-life values (1-3 hours for IPA and 17-27 hours for acetone) were obtained from a patient who ingested IPA, with serum levels of IPA below detection levels between 25-40 hours after admission to hospital (Jones, 2000). These values are similar to those obtained in rats and dogs where elimination half-lives have been determined to be 2 and 4 hours, respectively (half-life for acetone was 5 and 11 hours for rats and dogs respectively) (Clayton, 1981).

In conclusion, the overall weight of the evidence provides convincing evidence that IPA is not likely to cause carcinogenicity in rodents and/or humans. IPA and acetone are not genotoxicants. IPA and acetone are rapidly absorbed into the body via multiple exposure routes and are rapidly eliminated such that it is not likely that there is sufficient residence time in the body would to lead to local tissue reactions that may predispose the organism to potential neoplastic lesions with chronic exposure. To corroborate this, several inhalation and oral repeat dose toxicity studies (some as long as 6 months or more) are available showing minimal to no tissue-specific pathological lesions outside of the male rat-specific kidney effects that occur via a well-defined mode of action that is not relevant to humans. Acetone has been utilized as a vehicle for dermal carcinogenicity studies for years and has not been shown to induce an increase in tumor incidence compared to non-exposed controls. Although preliminary in nature, structure activity relationship models designed to predict the potential for non-genotoxic carcinogenicity based on an array of known non-genotoxic modes of action, did not identify either IPA and/or acetone as potential structural alerts.

Evaluation of the Oral Carcinogenicity Data Gap

Although the FDA has identified the oral carcinogenicity study as a data-gap in the IPA toxicity database according to the ICH guidelines, it should be noted that the ICH guideline specifies that the "*route of exposure in animals should be the same as the intended clinical route when feasible*" (FDA, 2015). As noted by the FDA, IPA is primarily used in the clinical setting for dermal use (patient pre-operative skin preparations, healthcare personnel hand rub and surgical hand rubs). Considering this to be the case, it would appear that the most logical routes of exposure for a potential carcinogenicity study should be strictly related to the most appropriate routes of human exposure. Based on the indicated clinical use and the high vapor pressure of IPA, the most appropriate routes of human exposure would be dermal (skin use in the clinical setting) and inhalation (vapor inhalation due to rapid volatilization during use). IPA is not expected to be used in the clinical setting for oral ingestion, and exposure through this route is not anticipated except in cases of accidental ingestion. For these reasons and based on the ICH guidelines, the requirement for an oral carcinogenicity study does not appear to be supported.

Evaluation of the Dermal Carcinogenicity Data Gap

According to the ICH guidelines, carcinogenicity studies should only be conducted by a single route of exposure if similar metabolism and systemic exposure can be demonstrated by differing routes of administration (ICH, 1995). As has been consistently reported, metabolism of IPA to acetone is consistent across species and across different routes of exposures (including oral, intravenous, inhalation and dermal) (Martinez , 1986; Slauter, 1994). Compared to oral/inhalation exposures, absorption through the dermal route of exposure is poor. In a study to evaluate the dermal uptake of IPA in rats, Boatman et al. determined that total absorbed dose accounted for approximately <7% of administered dose, with 84-86% of dose recovered at the application site after occlusion for four hours (Boatman, 1998). According to the authors, human exposure through the absorption route under typical use conditions would be expected to be considerably less than was predicted in the rat study (accounting for loss through volatilization), given that the rat study was conducted under conditions of complete occlusion. On this basis, it is assumed that the systemic dose from the 2-year inhalation carcinogenicity study would be approximately similar to that expected in a 2-year dermal carcinogenicity study. Considering the lack of systemic effects reported in the 2-year inhalation toxicity study, it is doubtful that requiring a 2-year dermal carcinogenicity would provide any additional information than is already available.

Conclusion

According to the ICH guidelines on the need for carcinogenicity studies of pharmaceuticals, the requirements for carcinogenicity studies require a careful evaluation of the available evidence to ensure that the carcinogenicity study being required actually provides meaningful information on the potential for hazard with human exposure. In many cases, this information can be provided by evaluation of other available data such as genotoxicity, presence of structural alerts for carcinogenicity and evidence for pre-neoplastic lesions with repeat dose studies. With regard to the oral carcinogenicity data gap identified for IPA by the FDA, this route of exposure is not expected for humans and is not in line with the ICH guidelines which specify that the route of exposure in the proposed carcinogenicity study be the same as that of the intended clinical route when feasible. With regard to the dermal carcinogenicity requirement, dermal absorption of IPA is considerably low such that systemic dose is not anticipated to be significantly higher than what would be expected in the 2-year carcinogenicity study of IPA (maximum concentration – 5000 ppm/13200 mg/m^3). Neither IPA nor acetone (used as a common vehicle for dermal carcinogenicity studies) is genotoxic, do not possess structural alerts for non-genotoxic carcinogenicity modes of action and have not been reported to cause local skin effects that might suggest the potential for skin tumors. It is thus doubtful what new information requiring a 2-year dermal carcinogenicity study will provide with regard to IPA. Overall, evaluation of the available data on IPA/acetone and the ICH guidelines suggest that requiring both oral and dermal carcinogenicity studies on IPA is not scientifically justified.

Oral/Dermal Carcinogenicity Questions

Does the FDA agree that the IPA inhalation carcinogenicity study and negative genotoxicity studies for IPA provide sufficient information to address the Agency's perceived data gaps regarding oral/dermal carcinogenicity?

If not, by what mechanism does FDA propose IPA may cause cancer since it is not genotoxic in vivo or in vitro and there is no proposed non-genotoxic mechanism that might result in carcinogenicity from exposure to IPA or its primary metabolite acetone?

Any information the Agency could provide in this matter could be used to design a targeted response to address the Agency's concern about IPA.

Discuss Data Request on Hormonal Effects

In the Proposed Rule, the FDA notes that additional testing will be required for the purpose of assessing IPA's potential for hormonal/endocrine effects (FDA, 2015). Further research into the hormonal effects of IPA has been justified citing the study of Gorkal et al. that indicated dopamine, noradrenaline, and serotonin were either decreased or increase depending on the area of the brain studied (Gorkal, 1989) However, this is a non-guideline, high dose study, through a novel route of exposure, unrepeated, and without an apical endpoint associated with the observations. The authors note that methanol and ethanol can alter monoamine levels in the brain although the pattern of response is different. Therefore, the changes in neurotransmitters are indicative of a "class effect" notably of the narcotic action observed for small molecular weight alcohols and not a function of hormonal/endocrine imbalance *per se*.

A review of the literature on the adverse effects of IPA including neurotoxicity concludes that there is a significant narcotic effect upon exposure to high levels for extended periods of time. These effects are consistent with other short-chained alcohols and are reversible. However, there is no evidence from any of these studies that perturbations in hormone levels are responsible for these effects (Kapp. 1996). Furthermore, evidence of neurologic damage or endocrine related effects have not been observed in a variety of studies. For instance, a 13-week inhalation study with Fischer 344 rats exposed to 0, 100, 500, 1500, or 5000 ppm IPA included a functional observation battery and detailed histopathology of the brain, spinal cord, and peripheral nerves. The histological examinations were conducted on a number of endocrine organs including: adrenal glands, testes, ovaries, pancreas, uterus, pituitary, thyroid/parathyroid, mammary gland, seminal vesicles, epididymis, and prostate. The neuroanatomic pathology evaluation did not reveal any exposure-related lesions in the central or peripheral nervous system of IPA exposed rats. No adverse effects were observed via histopathology in any of the endocrine organs (Burleigh-Flayer, 1997).

A developmental neurotoxicity study was conducted in Sprague-Dawley rats exposed to IPA from gestational day (GD) 6 through post-natal day (PD) 21. IPA was administered via oral gavage at 0, 200, 700, or 1200 mg/kg/day. On PND 22, some of the pups were sacrificed for histopathological examination of the central and peripheral nervous systems. Other neurodevelopmental indices (motor activity, auditory startle, etc.) were performed on the remaining pups up to PND 64. There were no biologically significant findings in the behavior tests and no changes in organ weights or pathology. Therefore, IPA was not associated with any developmental neurotoxicity up to 1200 mg/kg/day (Bates, 1994).

The hormonal effects section in Appendix B7 of the Briefing Document and the Proposed Rule fail to acknowledge the lack of apical endpoints associated with IPA as assessed in a number of reproductive (Bevan, 1995; Beyer, 1992;Clode, 1986), developmental (Tyl,1994), and oncogenicity (Burleigh-Flayer,. 1997) studies through the oral and inhalation routes of exposure in rats, rabbits, and mice. These studies, which include one and two generation reproduction studies, have been conducted to measure the potential effects of IPA on gonadal function, estrous cycles, mating behavior, conception,

parturition, lactation, weaning, and the growth and development of offspring. The results indicate that IPA is not a reproductive or developmental toxicant. A recent review of these studies was conducted by Faber et al. and concluded, “Based on all the available evidence, we conclude that IPA exposure does not affect male mating ability or fertility at dose levels up to 1000 mg/kg/day”, which is the limit dose for a guideline study (Faber, 2008). Again, reviews of the apical endpoints regarding reproduction and development and the aforementioned lack of any histopathologic findings in endocrine tissues do not justify additional testing of IPA for hormonal effects.

It should be noted that in animal studies following inhalation, dermal and oral exposures, IPA is quickly metabolized to its primary metabolite, acetone, with the majority excreted via exhalation (Slauter, 1991; Boatman,. 1995). Acetone was included in the EPA’s Endocrine Disruptor Screening Program Tier 1 assay battery. This battery includes tests designed to evaluate the potential of chemicals to interact with estrogen, androgen, and thyroid signaling pathways including receptor agonism and antagonism, altered steroidogenesis, and hypothalamic-pituitary-gonadal/thyroid axes. The EPA recently issued a report on acetone regarding the weight of evidence conclusions considering the Tier 1 screening assays and other scientifically relevant information. The report states the following regarding acetone, "Based on the weight of evidence considerations…there was no evidence of potential interactions with estrogen, androgen or thyroid pathways" (US EPA, 2015).

The weight of evidence for IPA and its metabolite, acetone, regarding any adverse effect on any apical endpoints of reproductive, developmental, and neurotoxicity as well as the endocrine disruption screening data does not justify the performance of additional endocrine/hormonal studies.

Hormonal Effects Question

i. Does the FDA agree that there is sufficient data presented in this Meeting Information Packet (i.e., developmental neurotoxicity and reproductive/developmental studies with IPA and the EPA's report of no evidence of endocrine disruption with acetone) to conclude that the data gap for possible hormonal effects from IPA exposure has been addressed and that no further testing is required?

Discuss Data Request for Efficacy Studies

Previous efficacy data and comments submitted to the FDA in response to the 1994 Healthcare Antiseptic Tentative Final Monograph (TFM) proposed support of 60% v/v Isopropyl alcohol as a healthcare personnel handrub, which is within the range identified for isopropyl alcohol (50-91.3%v/v) as an antimicrobial active ingredient in the *Notice of Proposed Rulemaking for Alcohol Drug Products for Topical Antimicrobial OTC Human Use* (FDA, 1982). Therefore, IPA has been recognized as eligible for inclusion in the OTC Drug Review throughout the range of 50-91.3%. To support Isopropyl alcohol as a GRAE active ingredient, it is proposed that efficacy testing be conducted at a minimum 60% concentration of Isopropyl alcohol. This is proposed to support isopropyl alcohol as an active with a minimum concentration of 60% v/v activity as a healthcare personnel handrub.

In the Proposed Rule, FDA indicates 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" (FDA, 2015). To address this requirement, FDA has requested *in vitro* efficacy studies to demonstrate the spectrum of antiseptic activity as follows:

> *MIC or MBC testing of 25 representative clinical isolates and 25 reference (e.g., American Type Culture Collection) strains of each of the microorganisms listed in the 1994 TFM (FDA, 2015).*

To support this data requirement, it is proposed that data from the existing published literature be consolidated and submitted in a summary report to include available published data on the ATCC strains and clinical isolates for the organisms referenced in the 1994 TFM. To the extent the published literature may not contain the full battery of 25 ATCC and 25 clinical isolate strains of each organism, MIC or MBC data will be generated on Isopropyl alcohol in a subsequent test report to demonstrate Isopropyl alcohol's broad spectrum antiseptic activity. No additional resistance testing will be completed based on the confirmed lack of resistance of Isopropyl alcohol as published Proposed Rule. A copy of the proposed test outline is included as Appendix A-1.

Under the Proposed Rule, FDA identified the following proposed data requirement to evaluate the kinetics of antiseptic activity (FDA, 2015):

> *Time-kill testing of each of the microorganisms listed in the 1994 TFM 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.*

To address this testing consideration, it is proposed that testing be conducted in accordance with ASTM E2783-11, "*Standard Test Method for Assessment of Antimicrobial Activity for Water Miscible Compounds Using a Time-Kill Procedure*" (Appendix C). Testing will be completed at the minimum proposed concentration of Isopropyl alcohol (60% v/v). Testing at this concentration will verify the kinetics of the antiseptic activity at the lowest proposed concentration for

Isopropyl alcohol. It is proposed that by establishing the lower range of the proposed concentrations, it will not be necessary to conduct testing at the upper end of the proposed range for Isopropyl alcohol. Testing will be completed against the single ATCC strain of each organism referenced in the 1994 TFM. In addition, testing will also be conducted on select clinical strains identified as relevant to the healthcare clinical environment. Testing will be conducted at 15 and 30 seconds to simulate contact times relevant to the use pattern of healthcare personnel handrubs. Appropriate neutralization will be utilized. A copy of the proposed test outline is included as Appendix A-2.

Under the Proposed Rule, FDA specifies 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" (FDA, 2015) . To address this testing consideration, it is proposed that testing of Isopropyl alcohol as a GRAE active ingredient in a healthcare personnel handrub product be tested under the following conditions:

Testing conducted using a modified ASTM E2755-15 "*Standard Test Method for Determining the Bacteria-Eliminating Effectiveness of Healthcare Personnel Handrub Formulations Using Hands of Adults"* using a single application (Appendix C). A single concentration of Isopropyl alcohol will be tested (60% v/v) to establish the lower end of the concentration range acceptable for a Health Care Personnel Handrub indication. Testing will include a specified volume of product and contact time to be utilized in a single application of the test product to simulate use conditions. The test protocol will include a vehicle control (saline) and a positive control product. The positive control utilized to confirm the conduct of the study will be an NDA approved alcohol based handrub product. Testing application conditions will be established through conduct of a pilot study.A copy of the proposed test outlines are included as Appendices B-1, B-2

Efficacy Studies Questions

i. Does the FDA agree with the proposed *in vitro* testing proposals to support GRAE status of IPA?

ii. Does FDA require that an NDA approved IPA product be used as a positive control in clinical simulation use testing or would an ethanol based NDA approved product such as 3M™ Avagard™ be acceptable as a positive control?

iii. Does FDA agree that testing at the low end of the concentration range of 60-91.3% is an acceptable approach to supporting the overall range of IPA concentrations?

iv. Does FDA agree that testing to the ASTM E2755-15 method is acceptable for testing clinical efficacy of an IPA handrub?

References

Bates, H. K. et al (1994). Developmental Neurotoxicity Evaluation of Orally Administered Isopropanol in Rats. *Toxicol Sci Toxicological Sciences* 22.1*: 152-58.*

Benigni R, Bossa C, Tcheremenskaia O (2013). Nongenotoxic carcinogenicity of chemicals: mechanisms of action and early recognition through a new set of structural alerts. *Chemical reviews* 113: 2940-2957

Bevan, C. et al (1995). Two-generation reproduction toxicity study with isopropanol in rats. *J. Appl. Toxicol*., 15:117-123.

Beyer, B.K. et al (1992). "Final Report: Multi-Generation Rat Reproduction Study with Isopropanol." Chemical Manufacturers Association.

Boatman, R.J. et al (1998). Dermal absorption and pharmacokinetics of isopropanol in the male and female F-344 Rat," *Drug Metabol Disposit* 26:197-202.

Burleigh-Flayer, H.D et al (1994). Isopropanol 13-week vapor inhalation study in rats and mice with neurotoxicity evaluation in rats. *Fundam App. Toxicol* 23: 421-428.

Burleigh-Flayer, H. et al (1997). Isopropanol vapor inhalation oncogenicity study in Fischer 344 rats and CD-1 mice. *Fundam Appl Toxicol* 36:95-111.

Clayton GD, Clayton FE (1981) *Patty's Industrial Hygiene and Toxicology*, Vol. 2A, 2B, 2C, New York: John Wiley Sons

Clewell, H.J. et al (2001).Development of a physiologically based pharmacokinetic model of isopropanol and its metabolite acetone. *Toxicol Sci* 63:160-172.

Clewell, H.J et al (2004).Evaluation of the potential impact of age- and gender-specific pharmacokinetic differences on tissue dosimetry. *Toxicol Sci* 79: 381-393.

Clode, S.A. et al (1986). A Pilot One-Generation Study with Isopropyl Alconhol in Rats (Final Report). The British Industrial Biological Research Association.

Faber, Willem D. et al (2008). "Review of Reproductive and Developmental Toxicity Studies with Isopropanol." *Birth Defects Research Part B: Developmental and Reproductive Toxicology* 83.5: 459-76.

Food and Drug Administration (FDA), "Notice of Proposed Rulemaking for Alcohol Drug Products for Topical Antimicrobial OTC Human Use", May 21, 1982 47 Fed. Reg.R 22324.

Food and Drug Administration (FDA), "Pre-market safety testing framework for over-the-counter healthcare antiseptic drugs", September, 3, 2014. 20140903-NDAC-B1-01-FDA_Backgrounder.pdf

Food and Drug Administration (FDA), Safety and Effectiveness of Health Care Antiseptics; Topical Antimicrobial Drug Products for Over-the-Counter Human Use; Proposed Amendment of the Tentative Final Monograph; Reopening of the Administrative Record; Proposed Rule. 80 Fed. Reg. 25166 (May 1, 2015).

Gentry, P.R. et al (2002).Application of a physiologically based pharmacokinetic model for isopropanol in the derivation of a reference dose and reference concentration. *Regul Toxicol Pharmacol* 36: 51-68.

Gentry, P.R. et al (2003).Evaluation of the potential impact of pharmacokinetic differences on tissue dosimetry in offspring during pregnancy and lactation. *Regul Toxicol Pharmacol.* 38: 1-16.

Gorkal, A.R., et al. (1989). Effect of isopropanol on rat brain monoamine levels. *Pharmacol Toxicol*. 65: 390-392.

Horton AW et al (1965). Carcinogenesis of the skin. 3. The contribution of elemental sulfur and of organic sulfur compounds. *Cancer research* 25: 1759-1763.

IARC (1999) Isopropanol. Vol. 71, pp 1027-1036. Lyon, France: IARC monographs on the evaluation of carcinogenic risks to humans.

IARC (2012a) Isopropyl Alcohol Manufacture by the Strong-acid Process. In *IARC monographs on the evaluation of carcinogenic risks to humans: Chemical agents and related occupations* Vol. 100F. Lyon, France.

IARC (2012b) Mists from Strong Inorganic Acids In *IARC monographs on the evaluation of carcinogenic risks to humans: Chemical agents and related occupations* Vol. 100F. Lyon, France.

International Conference on Harmonization(ICH) (1995). ICH Harmonized Tripartite Guideline: Guideline on the Need for Carcinogenicity Studies of Pharmaceuticals S1A , available athttp:/www.ich.org/fileadmin/PublicWebSite/ICHProducts/Guidelines/Safety/S1A/Step4/S1AGuideline.pdf, 1995.

Jones AW (2000) Elimination half-life of acetone in humans: case reports and review of the literature. *Journal of analytical toxicology* 24: 8-10.

Kapp Jr., R.W. et al (1993). In vitro and in vivo assays of isopropanol for mutagenicity. *Environ. Mol. Mutagen*. 22: 93–100.

Kapp Jr., R.W. et al (1996). Isopropanol: Summary of TSCA Test Rule Studies and relevance to hazard identification. *Regul Toxicol Pharmacol* 23:183-192.

Lehman, AJ and HF Chase (1944). The acute and chronic toxicity of iso-PrOH. *J Lab Clin Med* 29: 561-567.

Lehman, AJ at al (1945). Isopropyl alcohol. Acquired tolerance in dogs, rate of disappearance from the blood stream in various species, and effects on successive generation of rats. *J Pharmacol Exp ther* 85: 61-69.

Martinez TT et al (1986). A comparison of the absorption and metabolism of isopropyl alcohol by oral, dermal and inhalation routes. *Veterinary and human toxicology*28: 233-236

Natowicz, M. et al (1985). Pharmacokinetic analysis of a case of isopropanol intoxication. *Clinical chemistry* 31: 326-328

OECD (1997). Standard Information Data Set: 2-Propanol. UNEP Publications.

Pilegaard, K and O Ladefoged (1993). Toxic effects in rats of twelve weeks' dosing of 2-propanol, and neurotoxicity measured by densitometric measurements of glial fibrillary acidic protein in the dorsal hippocampus. *In vivo (Athens, Greece)* 7: 325-330

Sarangapani, R. et al (2003). Evaluation of the potential impact of age- and gender-specific lung morphology and ventilation rate on the dosimetry of vapors. *Inhal Toxicol* 15:987-1016.

Slauter, RW et al (1994). Disposition and pharmacokinetics of isopropanol in F-344 rats and B6C3F1 mice. *Fundamental and applied toxicology : official journal of the Society of Toxicology*23: 407-420

Tyl, R.W. et al (1994).Developmental toxicity evaluation of isopropanol by gavage in rats and rabbits. *Fundam Appl Toxicol* 22: 139-151.

US EPA (1991). Isopropanol; Final Test Rule. 54. Fed. Reg. 43252 (October 23, 1989).

USEPA. (1991) Alpha2u-globulin: Association with chemically induced renal toxicity and neoplasia in the male rat. Risk Assessment Forum, USEPA, Washington, DC.

USEPA. (2003a) Toxicological review of acetone (CAS No. 67-64-1): In support of summary information on the Integrated Risk Information System (IRIS). Washington, DC.

USEPA. (2003b) Toxicological review of Methyl Ethyl Ketone (CAS No. 78-93-3): In support of summary information on the Integrated Risk Information System (IRIS). . Washington, DC.

USEPA. (2011) Toxicological review of n-butanol (CAS No. 71-36-3): In support of summary information on the Integrated Risk Information System (IRIS). Washington, DC.

US EPA (2015). Endocrine Disruptor Screening Program: Weight of Evidence Conclusions on the Tier 1 Screening Assays for the List 1 Chemicals, available athttp://www2.epa.gov/ingredients-used-pesticide-products/weight-evidence-edsp-acetone.

Van Duuren, BL et al (1978) Mouse skin carcinogenicity tests of the flame retardants tris(2,3-dibromopropyl)phosphate, tetrakis(hydroxymethyl)phosphonium chloride, and polyvinyl bromide. *Cancer research* 38: 3236-3240.

Ward, JM et al (1986). Pathology of aging female SENCAR mice used as controls in skin two-stage carcinogenesis studies. *Environmental health perspectives* 68: 81-89.

Zakova, N et al (1985). Evaluation of skin carcinogenicity of technical 2,2-bis-(p-glycidyloxyphenyl)-propane in CF1 mice. *Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association* 23: 1081-1089.

Zeiger, E et al (1992). Salmonella mutagenicity tests: V. Results from the testing of 311 chemicals. *Environmental and molecular mutagenesis* 19 Suppl 21: 2-141.

APPENDICES

Appendix A-1. *In Vitro* Efficacy: MIC/MBC STUDY PROPOSAL

Active Ingredient: 60% isopropyl alcohol

Study Title: Determination of the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of one (1) Test Material 609 JPD

Study Rationale: The 1994 Tentative Final Monograph (TFM) requires that the finished product be challenged with 25 ATCC strains and 25 fresh clinically isolated strains of each of 10 Gram-negative and 10 Gram-positive bacterial species and 2 yeast groups of species (*Candida albicans* and *Candida* ssp., other than *albicans*). The number of bacterial species and strains tested in this proposed study will be established based on existing data available in published literature. Where data exists in published literature; further testing will not be conducted and the published data will be submitted to supplement the report.

Design: This study, a Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) evaluation of one (1) Test Material (60% IPA) 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 Material will be evaluated using one thousand one-hundred and seventy-five (1,100) different microorganism strains. Twenty-five (25) American Type Culture Collection (ATCC) strains, as available, 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 *Acinetobater baumannii*
2 *Bacteroides fragilis*
3 *Haemophilus influenza*
4 Enterobacter species
5 *Escherichia coli*
6 Klebsiellaspecies
7 *Klebsiella pneumoniae*
8 *Pseudomonas aeruginosa*
9 *Proteus mirabilis*
10 *Serratia marcescens*
11 *Staphylococcus aureus*
12 *Staphylococcus epidermis*
13 *Staphylococcus hominus (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*

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 each test product will be reported.

A detailed protocol will be submitted to FDA for review prior to initiating testing.

Appendix A-2. *In Vitro* Efficacy: TIME KILL STUDY PROPOSAL

Active Ingredient: 60% Isopropyl Alcohol

Study Title: An *In-Vitro* evaluation of Isopropyl Alcohol 60% for antimicrobial properties using the standardized ASTM E2783 Time-Kill Method.

Study Rationale: The 1994 TFM requires that the microorganisms tested in the time kill study are to be the 22 organisms identified in paragraph (a)(1)(ii) of the *in vitro* section plus five microorganisms of clinical concern including *Burkholderia cepacia*, *Staphylococcus aureus* (MRSA), *Staphylococcus epidermidis*, *Enterococcus faecalis* (VRE) and *Enterococcus faecium* (VRE).

Design: This study will use an *in vitro* time-Kill Method to evaluate the antimicrobial properties of one test method when challenged with suspensions of the twenty-two (22) microorganism specifically called out in the 1994 FDA TFM and select clinical isolates as noted above.

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 $\log_{10}$ 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 in triplicate 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 marcscens* (ATCC #14756) will be used as the challenge species in the neutralization study.

Efficacy Evaluation: A single replicate of the procedure will be performed for each individual evaluation. A dilution/aliquot of 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 and 30 seconds with a validated neutralizing technique. The test material will be neutralized at the sampling time and the surviving microorganisms enumerated. The percent of $\log_{10}$ reduction, from an initial microbial population will then be calculated.

A detailed protocol will be submitted to FDA for review prior to initiating testing.

Appendix B-1. *In Vivo* Efficacy: Healthcare Personnel Hand Rub (Pilot)

Active Ingredient: 60% Isopropyl Alcohol

Study Title: Pilot study to evaluate the dose/concentration and subject size numbers for use in the pivotal study using the standardized ASTM E2755-15.

Study Rationale: This study is being conducted to evaluate the antimicrobial effect of 60% isopropyl alcohol for use as a health care personnel hand rub in order to select the appropriate application conditions for a pivotal efficacy study, and inform the sample size calculation for the pivotal trial. A positive control using an NDA approved Healthcare Personnel Handrub will be included.

Design: Baseline values and microbial $\log_{10}$ reductions for each test configuration will be calculated for each subject. The indicator microorganism with be *Serratia marcescens* (ATCC #14756). The testing methods are based on ASTM E 2755-15 *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 isopropyl alcohol concentration and/or doses.

Duration: Single application

Data Analysis:

Study Objective: The purpose of this study is to determine the test conditions for use in the pivotal study.

Criteria for Inclusion:

1. Subjects may be of either gender, at least 18 years of age, and of any race.
2. Subjects must possess both hands.
3. Subjects must 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.

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 prolapsed, 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.

Number of Subjects: TBD

Study Drug and Administration: 60% Isopropyl alcohol

Efficacy Evaluation:

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. The positive control will meet the minimum performance criteria.

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 will be documented. 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.

A detailed protocol will be submitted to FDA for review prior to initiating testing.

Appendix B-2. *In Vivo* Efficacy: Healthcare Personnel Hand Rub (Pivotal)

Active Ingredient: 60% Isopropyl alcohol

Study Title: Pivotal study to determine the antimicrobial efficacy of one IPA concentration compared to a negative (saline) control using the standardized ASTM E2755-15 test method and a positive control using an NDA approved Healthcare Personnel Handrub.

Study Rationale: This study is being conducted to support Isopropyl Alcohol as an effective antimicrobial active for the health care personnel hand rub indication.

Trial Design: A minimum number of subjects will be evaluated based on statistical requirements for meeting performance criteria. 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 E2755-15 *Determining the Bacteria-Eliminating Effectiveness of Hand Sanitizer Formulations Using Hands of an Adult*. 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. The positive control will meet the performance criteria for log reductions and statistical criteria of responder rate.

Treatment Duration: Single application

Study Objective: The purpose of this study is to determine the antimicrobial effectiveness of 60% Isopropyl Alcohol 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 gender, 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 and Informed Consent Form.

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 or 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 ay.

5. Known allergies to latex (rubber), alcohols, to common antibacterial agents found in soaps or lotions, particularly chlorohexadine 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 prolapsed, 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, 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: The same size calculation has the formula:

$$n \geq \frac{x s^2 (z_{\alpha/2} + z_{\beta})^2}{d^2}$$

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

A calculation will be performed using data from the pilot evaluation to determine subject numbers.

Study Drug(s) and Administration: One IPA concentration with a negative control (saline) and a positive control using an NDA approved product.

Efficacy Evaluation:

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.

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% confidence interval.

Positive control meets minimum criteria.

Use of an appropriate neutralizer in all recovery media (i.e., sampling solution, dilution fluid, and plating media) and a demonstration of neutralizer validation will be performed. 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.

A detailed protocol will be submitted to FDA for review prior to initiating testing.

Appendix C: ASTM E2755-15

Designation: E2755 – 15

Standard Test Method for Determining the Bacteria-Eliminating Effectiveness of Healthcare Personnel Hand Rub 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 healthcare personnel hand rubs, (also known as hand rubs, hygienic hand rubs, hand sanitizers, or hand antiseptics) against transient microbial skin flora on the hands after a single application and after repeated applications.

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

E2756 Terminology Relating to Antimicrobial and Antiviral Agents

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:* For definitions of terms used in this document, see Terminology E2756.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *healthcare personnel handrub, n*—an antimicrobial gel, foam, liquid, spray, or wipe, applied by rubbing to reduce the transient microbial skin flora on hands that are not visibly soiled, and which does not require a post-treatment water rinse. Such agents may also be referred to as hand rubs, hygienic hand rubs, or hand antiseptics.

3.2.2 *healthcare personnel handwash, n*—a cleanser or waterless agent intended to reduce transient microbial skin flora on the hands.

3.2.3 *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 topical transient microbial contaminant. This may also be referred to as a test organism, marker organism, simulant, or contaminant.

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

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

Current edition approved May 15, 2015. Published June 2015. originally approved in 2010. Last previous edition approved in 2010 as E2755–10. DOI: 10.1520/E2755–15

[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.

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 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 after multiple consecutive contamination/application cycles in a single day. Evaluating effectiveness after multiple applications simulates repeated use of hand rubs in clinical settings and determines whether progressive build-up of non-volatile ingredients from the test material inhibits the antimicrobial action. An abbreviated test measuring activity of the test material following a single application may be used to simulate situations where high frequency use is not expected.

5. Significance and Use

5.1 Hand hygiene is considered one of the most important measures for preventing the spread of infectious microorganisms. Hand rubs 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. Alcohol-based hand rubs are recommended in healthcare settings 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 designed specifically to evaluate hand rubs for efficacy in eliminating bacteria from experimentally-contaminated hands. It is designed as an alternative to Test Method E1174, which was intended 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 rubs, inadequate drying of the hands after contamination dilutes the test material and can compromise activity, to result in an underestimation of effectiveness. Additionally, because hand rubs are not rinsed after product use, activity can be further degraded by build-up of soil from the contaminating broth and inactivated challenge bacteria on the hands.

5.2.1 In this method, application to the hands of a small volume of high-titer test bacteria suspension minimizes soil load such that the skin is completely dry prior to application of the test material. Further, by applying the bacterial suspension only prior to those test material application cycles followed by sampling, excessive buildup of killed bacteria on the hands is avoided, and the potential impact of non-volatile test product ingredients on bacteria-eliminating effectiveness after ten consecutive applications can be specifically assessed.

5.3 A reference control is evaluated for each subject prior to evaluation of the test material. Data from the reference control helps to control for inter-subject variability, inter-experimental variability, and inter-laboratory variability; and enables improved statistical comparison of test materials evaluated in the same experiment.

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

5.5 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.

5.6 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 rubbing.

5.7 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 *Plastic Bags*—May be used in place of gloves (6.4). Bags should be approximately 29 by 31 cm, possess no antimicrobial properties, and have a low bioburden. Perform a zone of inhibition test, such as AATCC Test Method 147, to evaluate the antibacterial activity

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

6.10 *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.

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

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

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

6.14 *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*, available from Hardy Diagnostics, 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[6] (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), 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 6.9).[7]

NOTE 3—A neutralizer validation should be conducted according to Test Methods 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 rubs) 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.

7.9 *Reference Control*—60% isopropanol in water (v/v).

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 microbial skin flora (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 susceptibility 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

[6] 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.

[7] 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.

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, 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.

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.

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 have or apply nail polish, artificial nails, or nail polish remover, or have undergone nail treatment during the 7-day pre-test conditioning period or on the single test day. 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.

11.2.6 After completing each cleansing wash, have each test subject wait five min prior to the next phase of the study. After completing the cleansing wash following use of the reference control, the wait time is extended to 20 min.

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 test material is evaluated after a single application and after ten consecutive applications. Table 1 illustrates the experimental design. The subject first completes a cleansing wash to remove oil and dirt (see 11.2). The subject's hands are then contaminated with the test bacteria (see 11.3) followed by baseline recovery (see 11.5). After a second cleansing wash to remove residual sampling solution and neutralizers, the subject's hands are again contaminated and the subject applies the reference control (see 11.6). The subject's hands are then sampled for reference control recovery followed by a cleansing wash. The subject's hands are contaminated a third time with the test bacteria, the subject applies the test material (see 11.7), hands are sampled for test material application 1 recovery, and a cleansing wash is performed. The subject then performs 9 consecutive test material applications (test applications 2-10). The subject's hands are contaminated a fourth time with the test bacteria, the subject applies the test material, and hands are sampled for test material application 11 recovery

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—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 11.8 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 *Reference Control Application:*

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

11.6.1.1 Within 10 s, instruct the subject to distribute the reference control over all surfaces of the hands, and fingers, 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.

11.6.1.2 Have subject hold hands upright and motionless prior to bacterial recovery (see 11.8).

11.7 *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.

11.7.1 *Liquid, Gel and Spray Hand Rubs:*

11.7.1.1 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.

11.7.1.2 Within 10 s, instruct the subject to distribute test material over all surfaces of the hands and fingers 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.

11.7.1.3 Have subject hold hands upright and motionless prior to bacterial recovery (see 11.8).

11.7.2 *Foaming Formulations:*

11.7.2.1 Dispense approximately 1.5 mL 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.

NOTE 8—The volume output from a foaming dispenser can be calculated by measuring the mass dispensed (g) and dividing by the density of the test material (g/ml). If the density of the test material is unknown, a mass of 1.3 g is approximately equal to 1.5 mL for formulations containing between 60% and 90% ethanol.

11.7.2.2 Within 10 s, instruct the subject to distribute test material over all surfaces of the hands and fingers, 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.

11.7.2.3 Have subject hold hands upright and motionless prior to bacterial recovery (see 11.8).

11.7.3 *Hand Sanitizing Wipes (Towelettes):*

11.7.3.1 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.

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

TABLE 1 Hand Contamination, Product Application and Recovery Schedule

Name	Contamination	Type of Application	Recovery
Cleansing Wash	No	Cleansing Wash	No
Baseline	Yes	None	Yes
Cleansing Wash	No	Cleansing Wash	No
Reference Control	Yes	Reference Control	Yes
Cleansing Wash	No	Cleansing Wash	No
Test Application 1	Yes	Test Material	Yes
Cleansing Wash	No	Cleansing Wash	No
Test Applications 2–10	No	Test Material	No
Test Application 11	Yes	Test Material	Yes

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

11.7.3.4 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 until all liquid has evaporated.

11.7.3.5 Have subject hold hands upright and motionless prior to bacterial recovery (see 11.8).

11.8 *Bacterial Recovery:*

11.8.1 Within one minute after the initial baseline contamination reference control application and test material applications 1 and 11 (Table 1), place gloves (see 6.4) or plastic bags (see 6.8) 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.

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

11.8.3 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.

11.8.4 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 11.8.3) 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 11.8.3) 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:*

13.2.1 Enumerate the *S. aureus* in the recovered sampling solution (see 11.8.3) 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 11.8.3) 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 for the reference control and test material at both Application I and Application 11 for each test subject using the following formulae:

$$\text{Log}_{10}\text{ Reduction for Reference Control} = \text{Log}_{10}\text{ Baseline Recovery} - \text{Log}_{10}\text{ Reference Control Recovery}$$

$$\text{Log}_{10}\text{ Reduction for Test Material}_{App1} = \text{Log}_{10}\text{ Baseline Recovery} - \text{Log}_{10}\text{ Test Material}_{App11}\text{Recovery}$$

$$\text{Log}_{10}\text{ Reduction for TestMaterial}_{App11} = \text{Log}_{10}\text{ Baseline Recovery} - \text{Log}_{10}\text{ Test Material}_{App11}\text{Recovery}$$

where:

App1 = Application 1
App11 = Application 11

15. Comparison of Different Test Materials

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

15.2 Calculate the mean difference in $\log_{10}$ reduction (ΔLR) for each Test Material and the corresponding Reference Control at both Application 1 and Application 11 for each test subject using the following formula:

$$\Delta\text{Log}_{10}\text{ Reduction}_{App1} = \text{Log}_{10}\text{ Reduction for Reference Control} - \text{Log}_{10}\text{ Reduction for Test Material}_{App1}$$

$$\Delta\text{Log}_{10}\text{ Reduction}_{App11} = \text{Log}_{10}\text{ Reduction for Reference Control} - \text{Log}_{10}\text{ Reduction for Test Material}_{App11}$$

where:

App1 = Application 1
App11 = Application 11

15.3 Perform appropriate statistics such as an unpaired T-test (for 2 test materials) or Analysis of Variance (ANOVA) (for ≥3 test materials) to compare mean ΔLog_{10}, $Reduction_{App1}$, and ΔLog_{10} $Reduction_{App11}$ between test materials to identify significant differences in test material performance.

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; alcohol foam; antimicrobial; antiseptic wipe; contaminant; efficacy; hand antiseptic; hand sanitizer; healthcare personnel handrub; *Serratia marcescens*; *Staphylococcus aureus*