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Major Article

Antimicrobial performance of two preoperative skin preparation solutions containing iodine and isopropyl alcohol

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A B S T R A C T

Background: Healthcare-associated infections (HAIs) are a persistent clinical challenge caused primarily by bacteria on the skin. Proper utilization of optimized antiseptic skin preparation solutions helps reduce the prevalence and impact of HAIs by decreasing patient skin microorganisms preoperatively. The purpose of this study was to evaluate the efficacy of 2 antimicrobial solutions containing iodine and isopropyl alcohol (IPA): Povidone iodine (PVP-I) with IPA (ie, PVP-I+IPA, PurPrep) and Iodine Povacrylex+IPA (DuraPrep).

Methods: The antimicrobial activity of the test solutions was evaluated *in vitro* by determinations of minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) against 1105 diverse microbial isolates and a time-kill assay to evaluate efficacy against 120 strains of Gram-positive and Gram-negative bacteria and yeasts. Peel tests were performed between skin samples treated with test solutions and representative drape/dressing materials to determine effects of test solutions on the biomechanical adhesion properties. Finally, an Institutional Review Board (IRB)-approved, randomized, controlled, single-center, partially blinded *in vivo* study was performed to assess the immediate and persistent antimicrobial activity of the test solutions on the abdomen and groin.

Results: Both PVP-I+IPA and Iodine Povacrylex+IPA solutions demonstrated broad-spectrum antimicrobial activity with MIC and MBC at less than 1% of the full-strength concentration of each product against a wide variety of microorganisms. In the time-kill tests, both solutions were able to successfully reduce all microbial populations by 99.99% (ie, 4 log₁₀) at the contact times of 30 seconds, 2 minutes and 10 minutes. The 2 solutions showed relatively similar adhesion results when tested with 3 representative operating room materials. Both PVP-I+IPA and Iodine Povacrylex+IPA met the expected Food and Drug Administration (FDA) efficacy requirements at 10 minutes and 6 hours post-treatment for both anatomic sites (ie, groin, and abdomen) in the clinical study, with no safety issues or adverse events.

Conclusions: Analysis of the *in vitro* antimicrobial activity, biomechanical adhesive strength, and *in vivo* efficacy of PVP-I+IPA demonstrated similar results compared to Iodine Povacrylex+IPA. Both products were efficacious at reducing or eliminating a wide range of clinically-relevant microorganisms in lab-based and clinical settings, supporting their use as antiseptic skin preparation solutions to reduce bacteria on the skin that can cause infection.

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Shortridge are employees of JMI Labs, which was contracted by BD to perform the MIC/MBC testing. Dr Zook is an employee of BD. Dr Lake is a consultant for, and Dr Deeken is the owner of, Covalent Bio, LLC, which received funding from BD for this project. All conflicts of interest have been declared in the title page of the manuscript, as well as in the submitted forms.

BACKGROUND

Healthcare-associated infections (HAIs) in hospitals are caused by a wide range of infectious agents through a variety of different entry routes.^{1–3} HAIs are a significant cause of morbidity and mortality, not only in adult populations, but also among pediatric and neonate populations. The incidence rate of HAIs in the United States (US) has been estimated at 3.2%–4.9%.^{2–5} corresponding to as many as 1.7 million affected patients and costs between \$96–147 billion annually.⁵ Compounding efforts to reduce infections, increasing prevalence of multidrug resistant microorganisms and pressure to accelerate treatment to reduce healthcare costs, concerns with HAIs are likely to continue in the foreseeable future.⁶

Extensive evidence suggesting that the most prevalent source of infection related to devices and surgical-site procedures is microorganisms on the patient's skin.^{7–9} The development and implementation of topical antimicrobial products that effectively and efficiently reduce skin microbes prior to initiation of medical procedures continues to be important to reduce the occurrence of HAIs.

Due to their antiseptic properties, products containing iodine, and alcohol (eg, isopropyl alcohol [IPA], ethanol, 1-propanol, etc.) have been used for many years to prepare skin for surgical intervention. Polyvinylpyrrolidone (povidone) iodine (PVP-I) is a complex solution containing iodine as a bactericidal agent and povidone as a carrier molecule. Upon contacting tissues, PVP-I releases free iodine over time, which oxidizes fatty acids in bacteria cell walls and penetrates cell membranes to disrupt/denature proteins and nucleic acids.¹⁰ IPA provides antimicrobial action through coagulation and/or denaturing of soluble proteins, which has been associated with disruption of cytoplasmic integrity, cell lysis, and interference with cellular metabolism.¹¹ Based on their respective mechanisms of action, the combination of PVP-I, and IPA has been shown to be more efficacious than PVP-I alone.^{12,13}

Several antiseptic products have been developed containing both iodine and alcohol. DuraPrep Surgical Solution (3M, St. Paul, MN), which combines IPA (74% w/w) with a proprietary iodine carrier called Iodine Povacrylex (0.7% available iodine), was marketed in 1988. This widely used surgical prep solution (Iodine Povacrylex +IPA) is painted on the skin in a single, uniform application and exhibits bactericidal activity and antimicrobial persistence.¹⁴ Prevail-FX (Becton, Dickinson and Company, Vernon Hills, IL) is a solution of PVP-I (8.3% w/w) and IPA (72.5% w/w) contained in a specifically designed applicator that was released in the US in 1998. The applicator contains a sponge tip is used to apply the solution (PVP-I+IPA) onto the skin's surface. A previous study evaluated the antimicrobial activity of PVP-I+IPA and Betadine (PVP-I; Purdue Frederick Co, Norwalk, CT) against normal skin flora on the abdomen and groin at 10 minutes, 30 minutes, 6 hours, and 24 hours after application.¹⁵ The PVP-I+IPA solution was applied using a 1-step, 30-second scrub application compared to a 2-step, 5-minute application with PVP-I solution (ie, scrub and paint). PVP-I+IPA was found to be as efficacious as PVP-I with a shorter, simpler application, demonstrated antimicrobial persistence for 24 hours, and had no reported adverse events.¹⁵ In 2020, a sterilized version of the PVP-I+IPA product containing the same excipients and active ingredients in a well-established applicator was marketed as PurPrep (Becton, Dickinson and Company, Vernon Hills, IL) in accordance with Food and Drug Administration (FDA) recommendations to label skin preparation solutions as sterile or nonsterile.¹⁶ However, the performance of these PVP-I +IPA products has not been directly compared to Iodine Povacrylex +IPA to identify any relevant differences in antimicrobial activity. Thus, the purpose of this study was to evaluate the performance of PVP-I+IPA and Iodine Povacrylex+IPA (as well as a vehicle control solution) as antiseptic skin preparation solutions using a variety of standard *in vitro* and *in vivo* studies.

METHODS

Materials. For the test solutions evaluated in this study, the base materials include PVP-I, IPA, and saline. Specific groups considered include (1) 8.3% w/w PVP-I with 72.5% IPA (Prevail-FX or the sterile version PurPrep; PVP-I+IPA), (2) Iodine Povacrylex (0.7% available iodine) and IPA, 74% w/w (DuraPrep; Iodine Povacrylex+IPA), (3) a vehicle (V) control to evaluate the effect of removing iodine from the formulation (ie, V-PVP+IPA) and (4) a negative control of 0.9% saline.

MIC/MBC. The antimicrobial activity of the test solutions was evaluated by determination of minimum inhibitory concentrations (MICs) and minimum bactericidal concentration (MBCs). MIC and MBC testing for each individual organism was conducted in alignment with published guidelines by the Clinical and Laboratory Standards Institute (CLSI).^{17–22} Detailed methods can be located in the guidelines,^{17–22} and an overview of the methods is presented here. For the MIC/MBC testing, each experimental product was tested against a total of 1105 microbial isolates (464 American Type Culture Collection (ATCC) and 641 clinical) from 22 different microbial species and genera that included Gram-positive and Gram-negative bacteria and yeasts (Fig 1). The MICs and MBCs are reported as percent of isolates that were inhibited and killed, respectively, at specific test-solution dilution. Dilutions of the full-strength products included: 0.008, 0.015, 0.03, 0.06, 0.12, 0.25, 0.5, 1, 2, 4, 8, 16, and 32% (for nonsterile PVP-I+IPA and Iodine Povacrylex+IPA) and 0.8, 1.5, 3, 6, 12, 25, 50% (for V-PVP+IPA). Media use was conducted in accordance with the CLSI guidelines.^{17–22} For all dilutions tested for MIC, 50 μ L of each test solution diluted in water was prepared into a 96 well microtiter plate, and then 10 μ L of inoculum to produce a final density of 5×10^5 CFU/mL was added. After a 15-minute incubation period at room temperature, 50 μ L of species-appropriate broth was added to each well.^{17–22} Wells were then incubated at 35°C overnight for 16–20 hours. If overnight incubation had insufficient growth for anaerobes or yeast based on the positive control, the samples were re-incubated for an additional 16–18 hours. The lowest concentration of test product that did not result in visible growth of the test microorganism was recorded as the MIC according to CLSI standards.²⁰ The MIC₅₀ and MIC₉₀ value reported is the lowest dilution where test product inhibited microbial growth of 50% or 90% of the specified microorganisms, respectively. For the MBC testing, 100 μ L of Dey and Engley (D/E) neutralizing broth was added to the wells prior to analysis to neutralize the biological activity of iodine. The 200 μ L samples were then plated and incubated at 35°C overnight and colonies were counted, if present. If growth wasn't confluent the plates were re-incubated for another 24 hours and colony count was repeated. The MBC value was recorded as the concentration of test product that reduced the viable population of the inoculated test microorganisms by 99.9%. The MBC₅₀ and MBC₉₀ values reported were the lowest dilution of product that killed 50% or 90% of the specified microorganisms respectively.

Time Kill. Time-kill tests were conducted according to American Society for Testing and Materials (ASTM) E2783–11(16): Standard Test Method for Assessment of Antimicrobial Activity of Water Miscible Compounds Using a Time-Kill Procedure to evaluate the efficacy of the 3 test solutions (nonsterile PVP-I+IPA, Iodine Povacrylex+IPA, and V-PVP+IPA) against 120 strains of 17 bacterial and yeast species from ATCC and clinical sources (full list in Fig 3). The percent and log₁₀ reduction of the microbial population of each challenge strain was determined following exposure to each test solution at room temperature for 30 seconds, 2 minutes, and 10 minutes. All samples were neutralized prior to plating and tested in triplicate. A neutralization validation (American Society for Testing and Materials [ASTM] 1054–08: Standard Test Methods for Evaluation of Inactivators of Antimicrobial Agents) was performed to confirm that the neutralizing solution (Butterfield's Phosphate Buffer solution with 100 g/L

Organism	ATCC Strains (#)	Clinical Strains (#)	PVP-I + IPA		Iodine Povacrylex + IPA		V-PVP + IPA		
			MIC50	MIC90	MIC50	MIC90	MIC50	MIC90	
Gram-positive Bacteria	<i>Enterococcus faecalis</i>	24	26	0.25	0.25	0.5	0.5	6	6
	<i>Enterococcus faecium</i>	23	27	0.25	0.25	0.5	0.5	6	6
	<i>Micrococcus</i> spp.	22	28	0.25	0.25	0.25	0.5	3	3
	<i>Staphylococcus aureus</i>	25	26	0.25	0.25	0.5	0.5	6	6
	<i>Staphylococcus epidermidis</i>	25	25	0.25	0.25	0.25	0.5	3	6
	<i>Staphylococcus haemolyticus</i>	9	41	0.25	0.25	0.25	0.5	3	6
	<i>Staphylococcus hominis</i>	9	42	0.25	0.25	0.25	0.25	1.5	3
	<i>Staphylococcus saprophyticus</i>	6	44	0.25	0.25	0.25	0.5	3	6
	<i>Streptococcus pneumoniae</i>	24	26	0.25	0.5	0.25	0.5	1.5	3
	<i>Streptococcus pyogenes</i>	25	25	0.5	0.5	0.5	0.5	1.5	3
Gram-negative Bacteria	<i>Acinetobacter</i> spp.	20	30	0.25	0.25	0.25	0.25	6	6
	<i>Enterobacter</i> spp.	23	27	0.25	0.25	0.25	0.5	6	12
	<i>Escherichia coli</i>	20	30	0.25	0.5	0.25	0.5	6	12
	<i>Haemophilus influenzae</i>	24	26	0.06	0.12	0.12	0.12	1.5	1.5
	<i>Klebsiella pneumoniae</i>	26	24	0.25	0.25	0.25	0.5	6	12
	Other <i>Klebsiella</i> spp.	22	29	0.25	0.25	0.25	0.5	6	12
	<i>Proteus mirabilis</i>	25	25	0.25	0.25	0.25	0.5	6	12
	<i>Pseudomonas aeruginosa</i>	24	26	0.25	0.25	0.25	0.25	3	3
	<i>Serratia marcescens</i>	22	30	0.25	0.25	0.25	0.5	6	12
An-aerobic Bacteria	<i>Bacteroides fragilis</i>	18	32	0.5	1	0.25	1	6	6
Yeast	<i>Candida albicans</i>	25	25	0.06	0.25	0.12	0.25	6	6
	Other <i>Candida</i> spp.	23	27	0.06	0.25	0.06	0.12	6	6

Fig 1. MIC₅₀ and MIC₉₀ of PVP-I+IPA, Iodine Povacrylex+IPA, and V-PVP+IPA: average minimum inhibitory concentration values which prevented visible growth of isolated microorganisms (ATCC and clinical strains) by 50% and 90%, respectively.

Polysorbate 80, 11.67 g/L lecithin, 5 g/L sodium thiosulfate pentahydrate, and 1 mL/L Triton-X-100 effectively neutralized the antimicrobial properties of each test material and was nontoxic to the microorganisms. Neutralizer validation was performed against 5 challenge strains that represent Gram-positive bacteria (*Staphylococcus aureus* ATCC 6538, *Staphylococcus aureus* MRSA ATCC 33591, and *Streptococcus pneumoniae* ATCC 49619), Gram-negative bacteria (*Escherichia coli* ATCC 11229) and yeast (*Candida albicans* ATCC 10231).

Peel adhesion. Simple peel tests were performed between skin samples treated with test solutions and representative drape/dressing materials to determine biomechanical adhesion properties. To this end, 7 × 10-inch pieces of porcine dermis were acquired (BioVT, Westbury, NY), cleaned and patted dry, and then securely mounted between the top and bottom frames of a custom test jig. Three drapes were chosen for evaluation: Ioban, Steri-Drape, and Tegaderm (products of 3M, St. Paul, MN); 1.5-inch-thick strips were cut from each material. A thin, even coat of each test solution (nonsterile PVP-I+IPA or Iodine Povacrylex+IPA) was applied to a 2 × 8-inch area of porcine skin. Five samples (n = 5) were used to evaluate each drape-solution combination, yielding a total of 30 samples (ie, 5 samples x 3 drapes x 2 test solutions). After a dry time of 3 to 5 minutes, drape strips were pressed onto the porcine dermis sample, taking care to remove air bubbles, while leaving a 2-inch-long tab of drape on one end for attachment to an Instron test machine (Instron, Norwood, MA). Incremental displacement was slowly applied until the drape strip was visibly taut, followed by a peel test at a rate of 100 mm/min until the strip was free from the dermis sample or the translation limit was reached. Force and displacement data were acquired at 10 Hz and analyzed to compute peak force (ie, maximum force measured during the test). In addition, the critical peel force, which is the average force required to advance the peeling of drapes from the dermis sample, was computed at the average force value within the range of each test corresponding to force values greater than 25% of the peak force. This threshold, which was different from the value used in our previous work,²³ was determined to be the appropriate level to compute critical force during the portion of the test where peeling was actively occurring. A 2-way analysis of variance (ANOVA) was used to

statistically compare the critical peel-force values and identify any significant differences due to drape or treatment.

In vivo Clinical Efficacy. The 3 skin preparation solutions (sterile PVP-I+IPA, Iodine Povacrylex+IPA, and saline) were tested against normal skin flora in an IRB-approved (MBT IRB #102219-1), randomized, controlled, single-center, partially blinded study to assess the immediate and persistent antimicrobial activity of single-use applicators containing one of the test solutions according to FDAs Tentative Final Monograph for Over-the-Counter Topical Antimicrobial Drug Product testing standards.²⁴ All human subjects provided written, informed consent prior to inclusion in the study.

Prior to the start of the efficacy study, the effectiveness of the neutralizer was validated to ensure that the neutralization solution had no effect on the growth of microorganisms and the test solutions' active ingredients were effectively inactivated by the neutralizer. Six healthy human volunteers (n = 6; mean age = 41.7±4.5 years; 4 males, 2 females) were recruited for the efficacy study according to inclusion/exclusion criteria (see below). Each subject's abdomen was treated in 2 × 5 inch surface areas using either PVP-I+IPA or Iodine Povacrylex+IPA, followed by initiation of sample collection 30±5 seconds later using the cup scrub technique in an area of 1 inch².^{15,25,26} Three challenge microorganisms were used for evaluation: *Staphylococcus epidermidis* (ATCC 12228), *Staphylococcus epidermidis* (ATCC 51625) and methicillin-resistant *Staphylococcus aureus* (MRSA; ATCC 33592). In accordance with ASTM E1054-08,²⁷ samples (collected from volunteers or aliquots of the neutralizer) were inoculated with a challenge microorganism to test neutralizer effectiveness and toxicity, with aliquots plated in duplicate after 1 and 30 minutes and incubated for 72 hours at 30°C. The number of surviving challenge microorganisms were then counted, converted to colony forming units (CFU) per mL, transformed to log₁₀, and then compared to test-microorganism viability populations.

Prior to the start of the efficacy study, spore-recovery validation was performed to confirm the ability of the sampling procedures to recover a population of spores applied to clean skin. Ten healthy human volunteers (n = 10; mean age = 35.1±17.3 years; 7 males, 3 females) were recruited for this study according to the efficacy study's inclusion/exclusion criteria (see below). The volar surface of

subjects' forearms was cleaned with IPA swabs and then marked in 3 areas of 0.6 inch². Each site was independently inoculated with spores of *Bacillus atrophaeus* ATCC 9372 using a pipette and a glass rod to spread the inoculum. After 10 minutes of drying, baseline samples were collected from each forearm, followed by randomized treatment of remaining sites with 0.03 mL of PVP-I+IPA, Iodine Povacrylex+IPA, or saline. After 3 minutes, samples were acquired using the cup scrub technique, diluted, plated on agar, and incubated for 72 hours at 30°C. The number of microorganisms were then counted, converted to CFU per mL, transformed to log₁₀, and then compared to baseline sample results.

For the clinical efficacy study, testing followed ASTM Standard Test Method E1173-15.²⁸ Healthy human volunteers, 18 years of age or older, with no dermatologic conditions or known history of sensitivity to natural rubber latex, adhesive skin products (eg, Band-Aids, medical tapes), PVP-I, IPA, or common personal-care or beauty products were considered for this study. A total of 119 volunteers were consented, enrolled, and screened for baseline counts of resident skin microbes $\geq 1.0 \times 10^3$ CFU/cm² (3.00 log₁₀/cm²) per abdominal site (left and right) and $\geq 1.0 \times 10^5$ CFU/cm² (5.00 log₁₀/cm²) per groin site (left and right). Individuals who meet screening day microbial requirements on the left and right side of the abdomen and groin were eligible for treatment. A total of 80 volunteers (n = 80; mean age = 40.4±15.2 years; 45 males, 35 females) were randomized, treated, and completed the study. On the day of treatment, baseline samples were collected from both the left and right abdomen and groin anatomic sites. Each individual anatomic site with microbial treatment day baseline counts of $\geq 1.0 \times 10^3$ and $\leq 3.2 \times 10^5$ CFU/cm² (3.00 to 5.50 log₁₀/cm²) on the abdomen and $\geq 1.0 \times 10^5$ and $\leq 3.2 \times 10^7$ CFU/cm² (5.00 to 7.50 log₁₀/cm²) on the groin were considered evaluable and included in the modified intent to treat (mITT) analysis. Anatomic sites outside this specified range on treatment day were excluded from the efficacy analysis.

Each volunteer was randomized to receive 2 treatments to bilateral sites of the abdomen and groin (n = 31–33 per treatment per anatomic site), where applications were on a 5 × 5 and a 2 × 5-inch area on the abdomen and groin, respectively. Randomized treatments included: (1) a 10.5 mL applicator of PVP-I+IPA applied on the abdomen and groin, (2) a 6 mL applicator of Iodine Povacrylex+IPA applied on the abdomen and 6 groin, (3) a 10.5 mL applicator of 0.9% normal saline applied on the abdomen and groin, and (4) an exploratory treatment arm of a sub-filled 6 mL applicator of PVP-I+IPA applied to the abdomen with a 10.5 mL applicator of PVP-I+IPA applied to the groin for a shorter application time than specified by the manufacture (short application). The PVP-I+IPA and saline solutions were applied by scrubbing the skin back and forth for 30 seconds on the abdomen and 30 seconds (short application) or 2 minutes on the groin, completely wetting the treatment area, while the Iodine Povacrylex+IPA was painted on using a single, uniform application; a drying time of 3 minutes was allowed following application of all solutions. Measures of antimicrobial efficacy included microbial reductions of resident skin microbes at timepoints of 10 minutes and 6 hours after product application, in order to evaluate requirements for preoperative skin preparation as described in guidelines established by the FDA.²⁴ The log₁₀ CFU/cm² reductions were calculated by subtracting the post-product application log₁₀ recovery from the pre-product data for all products for both the groin and the abdomen at each sample time (10 minutes and 6 hours). Two-sided 95% confidence intervals (CIs) were calculated for the mean log reductions for each product at each post-application time point on each anatomic area and compared using 1-sample t-tests (2-sided). Specifically, to assess the immediate antimicrobial effect, the lower bound 95% CI from the test solutions were compared to the FDA requirement of achieving greater than 2 and 3 log₁₀ CFU/cm² reductions on the abdomen and groin, respectively, 10 minutes after application. To assess the

persistence of the antimicrobial response, the lower bound 95% confidence intervals of the log₁₀ CFU/cm² reductions for each test solution were compared to the FDA requirement of ≥ 0 on both the abdomen and the groin after 6 hours. The safety of each product was determined by tracking skin-irritation scores- and the incidence of adverse events reported during the study.

RESULTS

MIC. Across all test solutions, inhibition of ATCC isolates and clinical isolates was similar, so results were calculated across all isolates within a given group of microorganisms. For MIC evaluations, PVP-I+IPA was effective at inhibiting growth of Gram-positive bacteria, Gram-negative bacteria, and yeasts with MIC₅₀ and MIC₉₀ concentration values (minimum inhibitory concentration for 50% and 90% of the isolates, respectively) ranging between 0.06% and 0.5% and 0.12% and 1%, respectively (Fig 1). Similarly, Iodine Povacrylex+IPA inhibited growth with MIC₅₀ and MIC₉₀ values ranging between 0.06% and 0.5% and 0.12% and 1%, respectively. Compared to Iodine Povacrylex+IPA, MIC values for PVP-I+IPA were lower for 14 species, identical for 6 species, and higher for only 2 species (the anaerobic bacteria *Bacteroides fragilis* and other species of *Candida* yeast) of microorganisms. Of note, all evaluated microorganisms were inhibited by $\leq 1\%$ PVP-I+IPA and $\leq 1\%$ Iodine Povacrylex+IPA. In contrast, the vehicle control V-PVP+IPA was less active than Iodine+IPA test products, with MIC₅₀ and MIC₉₀ values between 1.5% and 6% and 1.5% and 12%, respectively.

MBC. For MBC evaluations, PVP-I+IPA was effective at killing Gram-positive bacteria, Gram-negative bacteria, and yeasts with MBC₅₀ and MBC₉₀ values ranging between 0.06% and 0.5% and 0.12% and 1%, respectively (Fig 2). Similarly, Iodine Povacrylex+IPA killed the microorganisms evaluated, with MBC₅₀ and MBC₉₀ values ranging between 0.12% and 0.5% and 0.25% and 1%, respectively. Compared to Iodine Povacrylex+IPA, MBC₅₀/MBC₉₀ values for PVP-I+IPA were lower for 17 species, identical for 4 species, and higher for only 1 species (*Bacteroides fragilis*) of microorganisms. Of note, all evaluated microorganisms were inhibited by $\leq 1\%$ PVP-I+IPA and $\leq 1\%$ Iodine Povacrylex+IPA. In contrast, the vehicle control V-PVP+IPA was less active than the Iodine+IPA test products, with MBC₅₀ and MBC₉₀ values between 1.5% and 6% and 1.5% and 12%, respectively.

Taken together, the results of the MIC/MBC assays demonstrate the potent antimicrobial activity of both PVP-I+IPA and Iodine Povacrylex+IPA with prolonged exposure to a wide variety of microorganisms.

Time Kill. Prior to execution of time-kill experiments, neutralization validation was performed. The neutralizing solution was verified to effectively neutralize each test solution when evaluated with the 5 representative challenge strains (data not shown). For the time-kill examination, PVP-I+IPA was found to successfully reduce all microbial populations by 99.99% when tested against 120 isolates of various bacteria and yeast species when evaluated at 3 specific time points: 30 seconds, 2 minutes and 10 minutes (Fig 3). Similarly, Iodine Povacrylex+IPA and V-PVP+IPA also successfully reduced all populations of microorganisms by 99.99%. Thus, each of the 3 test solutions including the vehicle control (which contains IPA but no iodine) was sufficient to kill microorganisms of various categories at 30 seconds, 2 minutes, and 10 minutes. Results for ATCC isolates and clinical isolates were similar; therefore, results were averaged across all isolates within a given species of microorganism.

Peel adhesion. Critical peel-force values showed some subtle variability with different treatment solutions and drapes/dressings (Fig 4). Adhesion of loban drape strips to the porcine dermal samples yielded mean adhesion forces of 1.70 N (0.77, 2.63; 95% confidence intervals [CI]) and 1.23 N (0.80, 1.65) for PVP-I+IPA and Iodine Povacrylex+IPA, respectively. Similarly, Steri-Drape samples yielded critical peel-force values of 0.91 N (0.60, 1.21) and 1.47 N (1.18, 1.76) for

Organism	ATCC Strains (#)	Clinical Strains (#)	PVP-I + IPA		Iodine Povacrylex + IPA		V-PVP + IPA		
			MBC50	MBC90	MBC50	MBC90	MBC50	MBC90	
Gram-positive Bacteria	<i>Enterococcus faecalis</i>	24	26	0.25	0.5	0.5	1	12	12
	<i>Enterococcus faecium</i>	23	27	0.5	0.5	0.5	0.5	12	12
	<i>Micrococcus spp.</i>	22	28	0.25	0.5	0.5	1	6	6
	<i>Staphylococcus aureus</i>	25	26	0.25	0.5	0.5	0.5	12	12
	<i>Staphylococcus epidermidis</i>	25	25	0.25	0.25	0.25	0.5	6	12
	<i>Staphylococcus haemolyticus</i>	9	41	0.25	0.5	0.25	0.5	6	6
	<i>Staphylococcus hominis</i>	9	42	0.25	0.25	0.25	0.5	6	12
	<i>Staphylococcus saprophyticus</i>	6	44	0.25	0.5	0.5	0.5	3	6
	<i>Streptococcus pneumoniae</i>	24	26	0.25	0.5	0.25	0.5	1.5	3
<i>Streptococcus pyogenes</i>	25	25	0.5	0.5	0.5	1	1.5	3	
Gram-negative Bacteria	<i>Acinetobacter spp.</i>	20	30	0.25	0.25	0.25	0.5	6	6
	<i>Enterobacter spp.</i>	23	27	0.25	0.5	0.5	0.5	12	12
	<i>Escherichia coli</i>	20	30	0.25	0.5	0.25	0.5	12	12
	<i>Haemophilus influenzae</i>	24	26	0.06	0.12	0.12	0.25	1.5	1.5
	<i>Klebsiella pneumoniae</i>	26	24	0.25	0.5	0.5	0.5	12	12
	Other <i>Klebsiella spp.</i>	22	29	0.25	0.5	0.5	0.5	12	12
	<i>Proteus mirabilis</i>	25	25	0.25	0.25	0.25	0.5	12	12
	<i>Pseudomonas aeruginosa</i>	24	26	0.25	0.5	0.25	0.5	6	6
<i>Serratia marcescens</i>	22	30	0.25	0.25	0.25	0.5	12	12	
An-aerobic Bacteria	<i>Bacteroides fragilis</i>	18	32	0.5	1	0.25	1	12	25
Yeast	<i>Candida albicans</i>	25	25	0.12	0.25	0.25	0.5	12	25
	Other <i>Candida spp.</i>	23	27	0.12	0.5	0.25	0.5	12	25

Fig 2. MBC₅₀ and MBC₉₀ of PVP-I+IPA, Iodine Povacrylex+IPA, and V-PVP+IPA: average minimum bactericidal concentration values at which isolates (ATCC and clinical strains) were reduced by 50% and 90%, respectively.

PVP-I+IPA and Iodine Povacrylex+IPA, respectively. Lastly, the Tegaderm samples adhered with peel-force values of 1.10 N (0.75, 1.45) and 1.14 N (0.76, 1.52) for PVP-I+IPA and Iodine Povacrylex+IPA, respectively. The 2-way ANOVA found non-significant main effects of treatment ($P = .23$) and drape/dressing material ($P = .15$) but yielded a significant interaction effect ($P = .02$). Thus, the 2 evaluated test solutions showed relatively similar adhesion results when tested with 3 representative operating-room materials, while the interplay between these 2 factors led to subtle changes in critical peel force (ie, PVP-I+IPA had slightly

higher adhesion for Ioban but lower adhesion for Steri-Drape compared to Iodine Povacrylex+IPA).

In vivo Clinical Efficacy. Results of the neutralization study indicated that the antimicrobial was effectively neutralized, and there was no effect on the growth of microorganisms. Surviving populations of the challenge microorganisms were not more than 0.2 log₁₀ less than the corresponding viability populations (data not shown). In addition, results of the spore-recovery study indicated that the testing method was adequate to recover microorganisms from the skin after application of the investigational products. Surviving

Organism	ATCC Strains (#)	Clinical Strains (#)	PVP-I + IPA	Iodine Povacrylex + IPA	V-PVP + IPA	
Gram-positive Bacteria	<i>Enterococcus faecalis</i>	4	4	99.99%	99.99%	99.99%
	<i>Enterococcus faecalis VRE</i>	4	0	99.99%	99.99%	99.99%
	<i>Enterococcus faecium</i>	4	4	99.99%	99.99%	99.99%
	<i>Enterococcus faecium VRE</i>	0	4	99.99%	99.99%	99.99%
	<i>Staphylococcus aureus</i>	4	4	99.99%	99.99%	99.99%
	<i>Staphylococcus aureus MRSA</i>	4	4	99.99%	99.99%	99.99%
	<i>Staphylococcus epidermidis</i>	4	4	99.99%	99.99%	99.99%
	<i>Staphylococcus epidermidis MDR</i>	3	0	99.99%	99.99%	99.99%
	<i>Staphylococcus epidermidis MRSE</i>	1	4	99.99%	99.99%	99.99%
	<i>Streptococcus pneumoniae</i>	4	4	99.99%	99.99%	99.99%
<i>Streptococcus pyogenes</i>	4	4	99.99%	99.99%	99.99%	
Gram-negative Bacteria	<i>Burkholderia cepacia</i>	4	4	99.99%	99.99%	99.99%
	<i>Escherichia coli</i>	4	4	99.99%	99.99%	99.99%
	<i>Klebsiella pneumoniae</i>	4	4	99.99%	99.99%	99.99%
	<i>Pseudomonas aeruginosa</i>	4	4	99.99%	99.99%	99.99%
<i>Serratia marcescens</i>	4	4	99.99%	99.99%	99.99%	
Yeast	<i>Candida albicans</i>	4	4	99.99%	99.99%	99.99%

Fig 3. Time-kill experimental data for PVP-I+IPA (8.3%PVP-I in 72.5% IPA), Iodine Povacrylex+IPA, and V-PVP+IPA at full strength concentration, where data represent percent reduction in organisms (for ATCC and clinical strains) at 30 seconds, 2 minutes and 10 minutes.

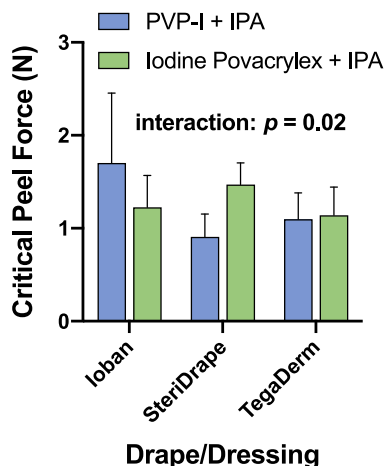


Fig 4. Critical peel force values for PVP-I+IPA (8.3%PVP-I in 72.5% IPA) and Iodine Povacrylex+IPA for 3 different drapes/dressings: Ioban, SteriDrape and TegaDerm; a 2-way ANOVA indicated a significant interaction between factors ($P = .02$).

populations of the cultured microorganisms were not more than 0.3 \log_{10} less than the mean \log_{10} of the baseline sample (data not shown).

For the abdomen (Fig 5), PVP-I+IPA achieved mean log reductions of 2.95 (2.78, 3.13; 95% CI) and 1.89 (1.63, 2.14) at 10 minutes and 6 hours, respectively, while the application of Iodine Povacrylex+IPA resulted in reductions of 2.80 (2.59, 3.01) and 1.64 (1.39, 1.89) at the same time points. The negative control group (saline) had moderate mean log reductions of 0.98 (0.85, 1.12) and 0.77 (0.62, 0.92) on the abdomen at 10 minutes and 6 hours, respectively. For the exploratory evaluation of a sub-filled applicator of PVP-I+IPA (6 mL application), mean log reductions of 2.53 (2.32, 2.75) and 1.70 (1.47, 1.92) were achieved at 10 minutes and 6 hours, respectively.

For the groin (Fig 5), PVP-I+IPA achieved mean log reductions of 3.65 (3.34, 3.96) and 2.41 (2.17, 2.64) at 10 minutes and 6 hours, respectively, while application of Iodine Povacrylex+IPA resulted in reductions of 3.22 (2.94, 3.51) and 2.18 (1.97, 2.40) at the same time points. Samples from saline-treated tissues showed mean log reductions of 1.15 (1.03, 1.27) and 1.11 (0.95, 1.28) on the groin at 10 minutes and 6 hours, respectively. For the exploratory group with a short-application time of PVP-I+IPA (30 seconds), the mean log reduction of microorganism was 3.25 (3.01, 3.48) and 2.26 (2.05, 2.46) at 10 minutes and 6 hours, respectively.

Thus, for both PVP-I+IPA and Iodine Povacrylex+IPA solutions, the expected efficacy standards were met at 10 minutes (ie, more than 2 and 3 \log_{10} reductions for the abdomen and groin, respectively) and 6 hours (ie, ≥ 0 \log_{10} reductions for both anatomic sites). All test

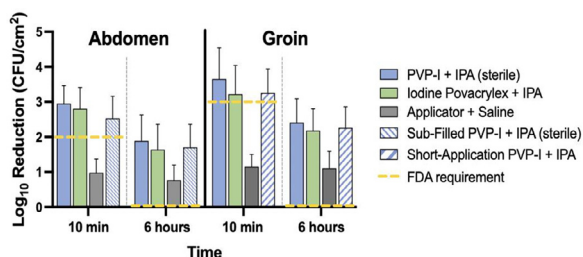


Fig 5. Reduction in resident skin microbes on the abdomen and groin measured 10 minutes and 6 hours after application of PVP-I+IPA (8.3%PVP-I in 72.5% IPA), Iodine Povacrylex+IPA, or saline (with the standard applicator); exploratory groups of sub-filled applicator (6 mL on abdomen) and short-application (30 seconds on groin) of PVP-I+IPA also shown; dashed lines indicate the FDA requirement for anti-microbial activity.

solutions were well tolerated with no safety issues. No skin irritation (ie, erythema, edema, rash, dryness) or other adverse events were reported for any subject after receiving treatment with PVP-I+IPA, Iodine Povacrylex+IPA or saline.

DISCUSSION

The results of this study demonstrate that PVP-I+IPA is equally or more effective as Iodine Povacrylex+IPA at inhibiting growth of (or killing) a wide range of ATCC and clinical isolates of Gram-positive and Gram-negative bacteria and yeasts. Specifically, *in vitro* evaluation determined that MIC/MBC values were generally equal to or smaller (ie, more effective) for PVP-I+IPA compared to Povacrylex+IPA, while results of time-kill studies showed rapid microbicidal efficacy with 99.99% (4-log) reduction in the microbial population, thus both formulations are highly effective antimicrobial solutions. Both skin preparation solutions yielded similar critical peel-force values when evaluated with 3 representative drape/dressing materials, demonstrating similar adhesion strength when attached to dermal surfaces *in vitro*. Finally, results of *in vivo* evaluation showed that PVP-I+IPA and Povacrylex+IPA were both able to outperform the FDA requirements for \log_{10} reduction of resident skin microbes on the abdomen and groin of healthy volunteers after 10 minutes and 6 hours. Overall, PVP-I+IPA was found to equal – or occasionally, surpass – the antimicrobial performance of Povacrylex+IPA when evaluated using a variety of standard *in vitro* and *in vivo* experimental protocols.

Two exploratory groups using PVP-I+IPA were included to consider other aspects of treatment. First, a sub-filled applicator group reduced the applied amount of PVP-I+IPA from 10.5 mL to only 6 mL, which is the same volume that is used for Iodine Povacrylex+IPA treatment. Even with this reduced quantity, treatment with PVP-I+IPA was able to successfully reduce skin microflora below the levels of the FDA requirement. Second, a short-time group reduced the application time of the PVP-I+IPA solution from 2 minutes down to 30 seconds, which matches the application duration of several approved sterile solutions. Even with this reduced application time, the log reduction of the test solution was sufficient to pass the FDA regulatory standard. Thus, the PVP-I+IPA sterile solution is capable of yielding satisfactory results even when administered with a sub-filled volume or short-application time.

A limited series of additional time-kill experiments considered whether diluted PVP-I+IPA would still prove efficacious in eliminating microbes. PVP-I+IPA diluted to 50% still yielded a 99.99% reduction, while a highly diluted solution (0.0001% strength) was ineffective (data not shown). Also, PVP-I+IPA tested in the presence of 5% serum reduced the populations of all tested microorganisms by 99.99% (data not shown). Thus, PVP-I+IPA is still effective in conditions that might occur in the operating room, such as dilution with irrigation solution or mixing with patient fluids (eg, serum); unsurprisingly, at extreme dilution (0.0001% strength), PVP-I+IPA loses its antiseptic efficacy.

In the clinical evaluation, the application of the vehicle (saline) solution resulted in ~ 1 \log_{10} reduction in microbial load for both anatomic sites and at both timepoints (Fig 5). Since saline has no inherent antimicrobial properties, this result suggests that the application technique itself may have contributed to microorganism depletion. Specifically, saline (as well as PVP-I+IPA) was applied using a rigorous, back-and-forth scrubbing technique that differs from the single, uniform layer painting technique recommended for the Iodine Povacrylex+IPA solution. Since the top layers of the epidermis are not all aligned in the same direction,²⁹ solution application using a more rigorous scrubbing technique that includes motion in multiple directions may provide better penetration into all areas of the skin surface.

In conclusion, analysis of the *in vitro* antimicrobial activity, mechanical adhesive strength, and *in vivo* efficacy of PVP-I+IPA

demonstrated similar results compared to Iodine Povacrylex+IPA. Both products were shown to be efficacious at reducing or eliminating a wide range of microorganisms in lab-based and clinical settings, supporting their use as antiseptic skin preparation solutions to reduce microorganisms that cause skin infection.

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