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Efficacy and safety of a novel antimicrobial preoperative skin preparation

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Abstract

Objective: Alternatives to skin preparation with conventional preoperative antiseptics are required because of adverse reactions and the potential emergence of resistance. Here, we present 2 phase 2 studies of ZuraGard (ZG), a novel formulation of isopropyl alcohol and functional excipients developed for preoperative skin antisepsis.

Methods: Microbial skin flora on abdominal and inguinal sites in healthy volunteers were quantitatively assessed following application of ZG versus a negative control (ZV) and a chlorhexidine/alcohol preparation, Chloraprep (CP). In trial 1, ZG administered for both recommended and abbreviated application times was compared with CP and ZV via bacterial reductions at 10 minutes, and 6 hours, 12 hours, and 24 hours following application. In trial 2, the 10-minute postapplication responder rates (RRs) for ZG, participants with abdominal $\geq 2 \log_{10}$ per cm^2, and inguinal $\geq 3 \log_{10}$ per cm^2 reductions in colony-forming units (CFU) were compared to RRs of participants treated with CP.

Results: In trial 1, ZG at the recommended application time reduced mean bacterial counts by ~3.18 $\log_{10}$ CFU/cm^2 and ~2.98 $\log_{10}$ CFU/cm^2 at abdominal and inguinal sites, respectively. Qualitatively similar reductions were observed for the abbreviated ZG application time and all CP applications. Application of ZV was ineffective. In trial 2, 10-minute RRs for ZG and CP exceeded 90% at abdominal sites. At inguinal sites, RRs were 83.3% for ZG and 86.7% for CP. No skin irritation or other adverse events were observed.

Conclusions: ZG matched CP efficacy under these experimental conditions with immediate and persistent microbial reductions, including abbreviated application times. Further clinical studies of this novel preoperative antiseptic are merited.

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Nearly 21 million surgical procedures are performed annually in the United States.[1] Nearly 500,000 of these procedures (~2%–3%) are complicated by surgical site infections (SSIs) every year.[2] SSIs lead to longer hospital stays, [2] higher rates of unplanned reoperation,[3] higher rates of hospital readmission,[4–7] and a 2- to 4-fold higher risk of death.[8,9] Annually, SSIs are responsible for nearly 10,000 avoidable deaths[10] and $9 billion in excess healthcare costs[11] in the United States.

Endogenous microflora cause a substantial number of these SSIs. Preoperative skin antisepsis, which decreases the concentration of bacteria colonizing the surgical field, is an SSI preventative intervention recommended by public health authorities and professional societies.[12–15] These recommendations call for the use of topical preoperative skin preparations that contain alcohol for immediate cutaneous microbial reduction, combined with another agent with residual antiseptic activity to inhibit microbial regrowth during the surgical procedure.[16] Current evidence does not support the use of one alcohol-containing topical skin antiseptic over another[17]; accordingly, the spectrum of distinct alcohol-containing agents used for skin antisepsis in the United States is small.

ChloraPrep (CP, Becton Dickenson, Franklin Lakes, NJ), 2% chlorhexidine gluconate (CHG) with 70% isopropyl alcohol, is currently one of the most widely used preoperative topical skin preparations in the United States.[18] CHG is active against a wide array of common skin organisms. However, its widespread use may lead to the emergence of clinically significant resistance that can limit its future utility.[19] In addition, rare but serious allergic reactions have been reported with antiseptic products containing CHG.[20] Consequently, continued development of new and effective antiseptic formulations is imperative.

We evaluated the antimicrobial efficacy and basic safety profile of a newly developed topical preoperative skin antiseptic ZuraGard (ZG; Zurex Pharma, Middleton, WI), which contains isopropyl alcohol as an active ingredient as well as the functional excipients citric acid, alkyl para-hydroxybenzoates, methylene blue (as a colorant), and purified water.

Methods

Study population

For trial 1 and trial 2, respectively, and prior to the recruitment of study participants, the Gallatin and MicroBiotest institutional

review boards reviewed and approved these study protocols. Healthy, adult male and female participants with skin surfaces free of tattoos, dermatoses, abrasions, cuts, lesions or other skin disorders within 15.24 cm (6 inches) of the test site were recruited at 2 US study sites. Potential participants whose bacterial counts were $<1.3\times10^3$ CFU/cm^2 on the abdomen and/or 1.0×10^5 CFU/cm^2 on the inguinal region during pretreatment screening were excluded. Additional exclusions criteria included a history of skin allergies, skin cancer within 15.24 cm (6 inches) of the study site, or any sensitivity to natural rubber latex, adhesive skin products (eg, adhesive dressings, medical tapes), isopropyl alcohol, or any ZG excipients. Participants currently using products containing CHG were also excluded. All female participants of childbearing potential were required to have negative urine pregnancy test results prior to treatment/testing.

Study design

We conducted 2 phase 2 studies in which ZG was compared to CP and/or an alcohol-free ZG vehicle (ZV). Our overall aim was to assess the immediate and persistent activity of ZG against natural bacterial flora on the skin of adult human participants and to compare these outcomes with those obtained with CP as a reference and ZV as a negative control.

On the day of treatment, participants providing informed consent were assigned a unique code which specified the randomly assigned application locus of test solutions used in these studies. Participants underwent sampling to measure baseline cutaneous microbial counts at abdominal and inguinal sites prior to application of study test solutions. In each study, the primary investigator or a designated clinical team member topically applied test solutions per protocol to each test region by scrubbing over a 3.8×12.7-cm (1.5×5-inch) area at inguinal sites (rich in sebaceous glands) and over a 12.7×12.7-cm (5×5-inch) area at abdominal sites (poor in sebaceous glands). Each test site was air dried, and the posttreatment weight of the applicator was recorded. Following application of test solutions, cutaneous samples for culturing were obtained from study skin sites using the time intervals noted below.

In the first phase 2 study (trial 1), the primary outcome measure was a comparison of mean log$_{10}$ reductions in colony-forming units per cm^2 [log$_{10}$ CFU/cm^2]) from baseline for 2 different ZG application times (recommended and abbreviated) versus equivalent ZV and standardized CP application times. Inguinal and abdominal sites on each side of each participant's body were randomly assigned (1) recommended ZG application times (2-minute inguinal scrub and 30-second abdominal scrub); (2) abbreviated ZG application times (1-minute inguinal scrub and 15-second abdominal scrub); (3) standardized CP application times (2-minute inguinal scrub and 30-second abdominal scrub); or (4) recommended ZV application times (2-minute inguinal scrub and 30-second abdominal scrub). Postapplication cutaneous samples were collected at 10 minutes, 6 hours, and at either 12 or 24 hours after application.

The second phase 2 study (trial 2) was designed similarly with 3 important modifications. First, a negative control was not used, and ZG and CP test solutions were compared directly against each other. Second, the abbreviated 15-second ZG application time was used only for abdominal sites; the recommended 2-minute ZG application time was used for inguinal sites. Third, the antimicrobial effectiveness of the test solutions was assessed as 10-minute responder rates (RR) where RR was the proportion of participants with a ≥2 log$_{10}$ CFU/cm^2 reduction from baseline at the abdominal site and a ≥3 log$_{10}$ CFU/cm^2 reduction from baseline at the

inguinal site 10 minutes after test solution application. Bacterial counts at both loci were required to remain below baseline counts 6 hours later. The primary objective was to meet or exceed a 70% RR.

Immediately after the 10-minute skin sample was taken in both studies, skin testing sites on the abdomen or groin targeted for 6-hour, 12-hour, and 24-hour testing were covered with sterile, semiocclusive gauze taped to the skin to cover and protect the test site. All participants were instructed not to remove the dressing, to limit their physical activity, to avoid sweating, and to avoid any potential test site contamination. This procedure is in line with studies that do not house participants for the entire testing period and consistent with preoperative study designs. No washing of the product application sites and no friction with clothing occurred.

Bacterial sampling procedures

All microbial specimens were collected from each skin site in each study by the testing site's clinical team using a sterile cylinder containing 3.0 mL sterile stripping and suspending fluid with product neutralizers. Once in contact with the skin, the area surrounding the cylinder was massaged to enhance collection of cutaneous flora. All samples were transferred to a sterile counting tube. A second aliquot sample was collected in the same manner immediately afterward. Both were combined and serially diluted in Butterfield's phosphate buffer containing product neutralizers. Plated cultures were prepared from each of these dilutions on tryptic soy agar with product neutralizers (TSA+) and incubated at 30°C for ~72 hours, or until the appearance of sufficient bacterial growth. Colonies were counted on culture plates by technicians blinded to the sample origin. Cutaneous bacterial counts at baseline and at each postapplication time were recorded for each test area of skin surface in colony-forming units (CFU) per square centimeter (cm^2).

Statistical methods

A modified intent to treat (mITT) approach, restricted to participants with baseline cutaneous microbial counts $>1.3\times10^3$ CFU/cm^2 at abdominal sites and $>1.0\times10^5$ CFU/cm^2 at inguinal sites on the day of treatment was used for statistical analysis. Differences in counts between baseline and each programmed postapplication period were calculated as log$_{10}$ CFU/cm^2 data. Descriptive statistics for bacterial count reductions were computed for each sampling site and for each postapplication assessment period using mean, median, standard deviation (SD), and minimum/maximum recovery count. In trial 2, the log recovery counts were converted to binary measures for each participant in the primary analyses to achieve a binomial distribution for RR data. The RR itself was derived from the number of successes at a given time point divided by the number of measurable values for that time point (eg, 18 successes at 24 time points = 75% RR). All results were reported as a net change and 95% confidence intervals (CI) calculated using least-square means. Minitab and the SAS version 8.2 statistical software (SAS Institute, Cary, NC) were used for all statistic calculations.

Safety

Safety was monitored for both studies by cutaneous reactivity at each test site. All local and systemic adverse events (AEs) observed or reported to the investigators were evaluated and followed to resolution along with intensity, duration, and causal relationship to the tested agent. Adhesive reactions as well as acute responses to the sampling techniques were likely and expected and included mild skin irritation and erythema, along with possible allergic reactions.

Table 1. Log$_{10}$ Reductions from Baseline, Inguinal Site

Results for ZG applied for 2 min to the inguen						
Variable	Sample	*N*	Mean	SD	Min	Max
Reduction	10 min post prep	20	2.975	0.979	1.348	4.333
	6 h post prep	20	3.328	1.275	1.257	5.492
	12 h post prep	8	4.081	1.266	2.694	6.038
	24 h post prep	12	3.477	1.518	1.124	5.429
Results for ZG applied for 1 min to the inguen						
Variable	Sample	*N*	Mean	SD	Min	Max
Reduction	10 min post prep	21	3.061	1.102	1.239	5.489
	6 h post prep	21	3.161	1.168	1.328	5.334
	12 h post prep	8	4.326	1.082	3.012	5.666
	24 h post prep	13	3.714	1.682	1.251	6.280
Results for ZV applied for 2 min to the inguen						
Variable	Sample	*N*	Mean	SD	Min	Max
Reduction	10 min post prep	24	1.234	0.580	0.112	2.536
	6 h post prep	24	1.673	0.856	0.276	3.517
	12 h post prep	9	2.111	0.977	0.349	3.974
	24 h post prep	15	1.365	0.646	0.164	2.481
Results for CP applied for 2 min to the inguen						
Variable	Sample	*N*	Mean	SD	Min	Max
Reduction	10 min post prep	11	2.754	0.369	2.277	3.444
	6 h post prep	11	3.520	1.322	1.564	5.880
	12 h post prep	5	3.244	1.069	2.383	5.089
	24 h post prep	6	2.653	1.120	1.502	4.318

Note. SD, standard deviation; Min, minimum; Max, maximum; ZG, ZuraGard; ZV, ZuraGard vehicle; CP, Chloraprep.
[a]Log$_{10}$ reductions from CFU baseline counts assessed 10 minutes, 6 hours, 12 hours, and 24 hours after exposure of the inguinal test area to either ZG, ZG-abbreviated, ZV, and CP in trial 1.

Results

Overall, 100 participants were enrolled and randomized. Notably, 4 participants enrolled in trial 1 who had previously met the necessary baseline cutaneous microbial count thresholds during the screening visit had microbial counts below these required thresholds on the day of treatment and were excluded from the final analyses. Consequently, 96 participants were included in the final mITT analyses.

Trial 1 participants were 47 males and 17 females aged 18–74 years. Most (ie, 52) were of European-American (white) ethnicity, 1 was African-American (black), 6 were Hispanic, 2 were Asian, and 3 self-identified as "other" ethnicities. Trial 2 participants comprised 20 males and 16 females aged 20–67 years. Furthermore, 20 participants self-identified as European-American, 2 as African-American, 2 as Hispanic, 10 as Asian, and 2 as "other."

Trial 1

In trial 1, 60 participants were randomized and treated. We report those treated sites which passed baseline entry criteria and were included for analysis. Because the database used was acceptable sites rather than participants, there were frequently a different number of sites for each time point in each trial conducted. As shown in Table 1, 10-minute postapplication cutaneous microbial counts at inguinal sites dropped an average of 2.97 log$_{10}$ CFU/cm^2 following ZG application for the recommended time, and an average of 3.061 log$_{10}$ CFU/cm^2 following the abbreviated (1-minute) ZG application. Average reductions in inguinal counts remained at ≥3.0 log$_{10}$ CFU/cm^2 at 6 hours, 12 hours, and 24 hours after ZG application. Although both applications of ZG produced greater mean reductions in microbial flora than did a 2-minute application of CP, these differences did not achieve statistical significance (Fig. 1).

As shown in Table 2, at the 10-minute postapplication period, cutaneous microbial counts at abdominal sites were reduced by an average of 3.181 log$_{10}$ CFU/cm^2. This period followed the recommended (30-second) ZG application time. A reduction of 2.707 log$_{10}$ CFU/cm^2 followed the abbreviated (15-second) ZG application time. Average reductions in abdominal counts exceeded 2.0 log$_{10}$ CFU/cm^2 at 6 hours, 12 hours, and 24 hours.

Application of the CP test solution resulted in a mean reduction in abdominal cutaneous microbial counts of 2.07 log$_{10}$ CFU/cm^2 after 10 minutes. Although reductions in cutaneous microbial counts were below 2.0 log$_{10}$ CFU/cm^2 12 hours following

Table 2. Log$_{10}$ Reductions from Baseline, Abdominal Site[a]

Results for ZG applied for 30 s to the abdomen						
Variable	Sample	N	Mean	SD	Min	Max
Reduction	10 min post prep	15	3.181	1.083	0.266	4.824
	6 h post prep	15	2.977	0.890	1.425	4.464
	12 h post prep	8	3.016	1.447	0.775	5.000
	24 h post prep	7	2.584	1.385	0.490	4.464
Results for ZG applied for 15 s to the abdomen						
Variable	Sample	N	Mean	SD	Min	Max
Reduction	10 min post prep	12	2.707	1.053	0.654	4.260
	6 h post prep	12	2.562	1.158	0.023	4.366
	12 h post prep	5	3.317	0.223	3.038	3.576
	24 h post prep	7	2.437	1.002	1.026	3.760
Results for ZV applied for 30 s to the abdomen						
Variable	Sample	N	Mean	SD	Min	Max
Reduction	10 min post prep	15	1.038	0.869	−0.583	2.590
	6 h post prep	15	1.303	1.230	−0.784	3.750
	12 h post prep	7	1.505	1.029	0.054	3.084
	24 h post prep	8	1.377	1.214	−0.721	3.386
Results for CP applied for 30 s to the abdomen						
Variable	Sample	N	Mean	SD	Min	Max
Reduction	10 min post prep	8	2.070	1.448	0.048	4.062
	6 h post prep	8	1.758	1.181	0.448	3.475
	12 h post prep	4	2.545	1.720	0.378	4.476
	24 h post prep	4	1.453	1.665	−0.520	3.549

Note. SD, standard devtiation; Min, minimum; Max, maximum ZG, ZuraGard; ZV, ZuraGard Vehicle; CP, Chloraprep.
[a]Log$_{10}$ reductions from CFU baseline counts assessed 10 min, 6 h, 12 h, and 24 h after exposure of the abdominal test area to either ZG, ZG-abbreviated, ZV, or CP in trial 1.
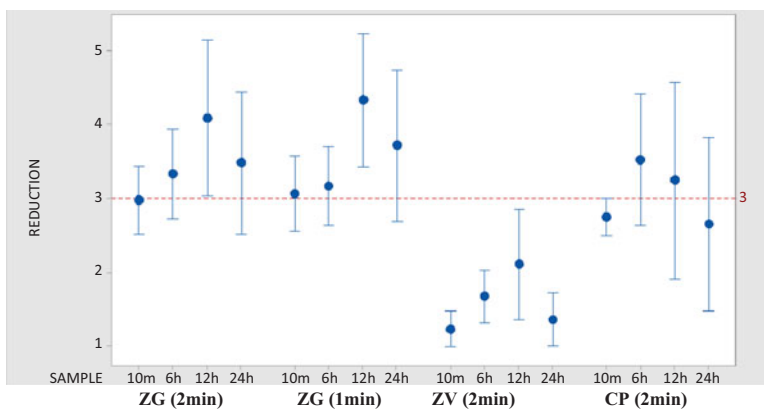

Fig. 1. Comparative interval plot of CFU reductions from baseline at 95% CI for the mean, inguinal site. Log$_{10}$ CFU/cm^2 reductions in CFU from inguinal site baseline are shown. Calculations were made at inguinal site 10 minutes, 6 hours, 12 hours, and 24 hours following application of the active agent. Note. CFU, colony-forming units; CI, confidence interval; ZG, ZuraGard; ZV, ZuraGard vehicle; CP, Chloraprep.

application, reductions exceeding threshold were observed at 6 hours and 24 hours, respectively. Thus, although average abdominal reductions with ZG exceeded those with CP at all time points, these benchmark differences were not statistically significant (Fig. 2). Application of ZV did not achieve target reductions in

cutaneous microbial counts at either the inguinal or abdominal sites.

No skin irritation or other AEs were observed before baseline, after test agent application, or before sample collection time points for all treated participants.

Table 3. Mean Log$_{10}$ CFU Counts and Responder Rates at 10 Minutes[a]

Test Site	Test Agent	Baseline Count	10-Min Count	10-Min Reduction (95% CI)	10 Min % Responder Rate (counts)
Abdominal	CP	3.35	0.48	2.88 (2.5017–3.1936)	90.00 (27/30)
Abdominal	ZG	3.36	0.28	3.08 (2.7036–3.3955)	90.00 (27/30)
Inguinal	CP	5.39	1.28	4.11 (3.7464–4.4383)	86.67 (26/30)
Inguinal	ZG	5.43	1.17	4.27 (3.9042–4.5961)	83.33 (25/30)

Note. CFU, colony-forming units; CI, confidence interval; ZG, ZuraGard; CP, Chloraprep.
[a]Mean Log10 CFU counts and responder rates at 10 min as calculated for ZG and CG in both abdominal and inguinal sites at baseline and 10 min after application. Reduction, change, and CI were calculated using least-squares means and are thus slightly different than numerical mean values.
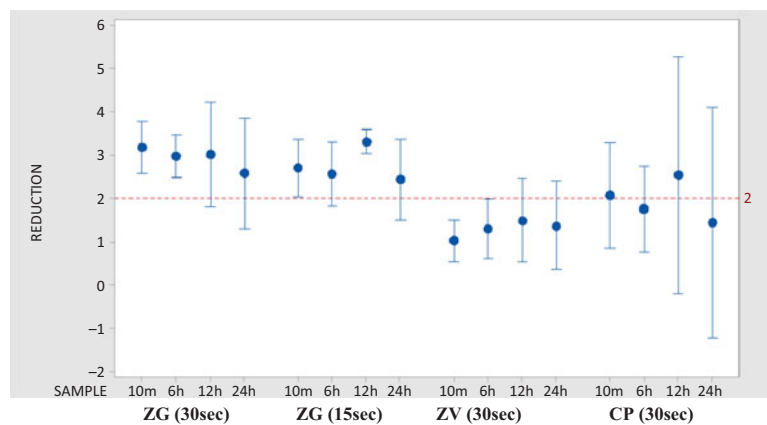

Fig. 2. Comparative interval plot of CFU reductions from baseline at 95% CI for the mean, abdominal site. Log$_{10}$ CFU/cm^2 reductions in CFU from abdominal site baseline are shown. Calculations made at abdominal site 10 minutes, 6 hours, 12 hours, and 24 hours following application of the active agent. Note. CFU, colony-forming units; CI, confidence interval; ZG, ZuraGard; ZV, ZuraGard vehicle; CP, Chloraprep.

Trial 2

In trial 2, 36 participants were randomized and treated. At the 10-minute sampling period, abbreviated ZG abdominal application times yielded an average reduction in cutaneous microbial counts of 3.1 log$_{10}$ CFU/cm^2 from baseline, although recommended ZG application to inguinal sites yielded an average reduction of 4.3 log$_{10}$ CFU/cm^2 from baseline (Table 3). Standardized abdominal and inguinal CP applications yielded comparative count reductions of 2.9 and 4.1 log$_{10}$ CFU/cm^2, respectively. Both ZG and CP applications exceeded the primary end-point objective of a 70% responder rate (see Table 3). No AEs were reported, and there was no evidence of an increase in cutaneous irritation after application of either test solution.

Discussion

Reducing cutaneous bacterial counts at procedural sites is an effective strategy for reducing SSI risk. Existing guidelines[12–15] recommend the application of alcohol-containing products to prepare the skin prior to any surgical procedure because they act more rapidly than do other aqueous solutions.[16,24] Here, we describe the results of 2 phase 2 trials that demonstrate the effectiveness of a novel preoperative antiseptic, ZuraGard, in reducing bacterial counts on the skin of healthy volunteers. ZuraGard reduced microbial counts at both inguinal (≥3 log$_{10}$ CFU/cm^2 reduction) and abdominal (≥2 log$_{10}$ CFU/cm^2 reduction) test sites, caused no observable skin irritation or other AEs, and performed equivalently to CP, currently the most widely used presurgical antiseptic skin preparation in the United States.[18]

Perhaps most notably, ZG achieved targeted 10-minute postapplication microbial reductions regardless of whether a 1- or 2-minute application time was employed at inguinal sites or a 15- or 30-second application time was employed at abdominal sites. The reductions in cutaneous bacterial counts following application of ZG were sustained for up to 24 hours, and no skin irritation was observed. These findings may have important practical significance because shorter application times have been correlated with higher levels of staff adherence to surgical site preparation protocols in at least 1 study.[38]

In view of the potential for regrowth of skin bacteria during the surgical procedure, and given the short duration of alcohol-based antiseptic activity, guidelines recommend that agents used for surgical site preparation contain an additional component to promote prolonged residual activity, typically povidone-iodine or CHG. Although CHG may possesses residual activity that is more pronounced than povidone-iodine,[16,39] there is insufficient evidence to preferentially support one type of alcohol-containing preparation over another or to suggest that addition of another antimicrobial could contribute to initial efficacy.[17,26] Nevertheless, alcohol and CHG combinations have become the preferred surgical site preparation in the United States.[18] There are compelling reasons to pursue the development and evaluation of alternatives to conventional preoperative antiseptics. For example, IgE-mediated anaphylactic/anaphylactoid reactions triggered by CHG exposure,[27] although rare, are increasingly described in association with surgical procedures.[28] Hypersensitivity reactions mediated by other mechanisms are also well known,[29] and given the myriad of opportunities for CHG exposure, there is a legitimate concern that the number of surgery patients with an allergy to this agent will increase.[30] Moreover, the burgeoning number of CHG-containing healthcare products, including medical devices, hand soaps, body washes, surgical irrigation products, and oral rinses, raise concerns about the

potential for selection and spread of resistance to this particular biocide. Emerging CHG resistance has already been suggested in several outbreaks of healthcare-associated infections in the United States,[31,32] and there are indications that high-frequency exposure to sublethal concentrations of CHG may enhance acquired resistance in organisms such as *Acinetobacter* spp, *K. pneumoniae*, and *Pseudomonas* spp, all of which are known for their virulence and adaptability to antibiotics.[32] Notably, in terms of functional excipients used to support persistence and shelf-life, ZG contains a citric acid and sodium citrate solution as well as trace methyl- and propyl-parabens, all of which exhibit mild antimicrobial properties. Taken together, these findings strongly support the need for continued diversification of our topical antiseptic armamentarium.

In summary, ZG is a novel preoperative skin antiseptic that, in preliminary studies, reduces bacterial contamination of the skin and preforms similarly to CP.[21] These results justify larger-scale studies to examine the effectiveness and safety of this product in randomized clinical trials and in more diverse patient populations.

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