

# A Randomized Trial of the Efficacy of Hand Disinfection for Prevention of Rhinovirus Infection

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**Background.** Hand disinfection is frequently recommended for prevention of rhinovirus (RV) infection and RV-associated common colds. The effectiveness of this intervention has not been established in a natural setting. The purpose of this study was to determine the effect of hand disinfection on RV infection and RV-associated common cold illness in a natural setting.

**Methods.** A controlled clinical trial was done in young adult volunteers during 9 weeks of the fall 2009 RV season. Volunteers were randomized to either an antiviral hand treatment containing 2% citric acid and 2% malic acid in 62% ethanol (n = 116) or to a no-treatment control group (n = 96). The hand treatment was applied every 3 hours while the subjects were awake. All volunteers kept a daily diary of symptoms and had a nasal lavage for polymerase chain reaction once each week and 2 additional lavages around the time of each common cold illness. The primary endpoint was the number of RV-associated illnesses. The incidence of RV infection and of common cold illnesses were evaluated as secondary endpoints.

**Results.** The hand treatment did not significantly reduce RV infection or RV-related common cold illnesses. The total number of common cold illnesses was significantly reduced in the intent-to-treat analysis, but this effect was not seen in the per protocol analysis.

**Conclusions.** In this study, hand disinfection did not reduce RV infection or RV-related common cold illnesses.

**Clinical Trials Registration.** NCT00993759.

Rhinovirus (RV) infections are the most frequent cause of common cold illnesses. These upper respiratory infections are generally mild and self-limited, but they are associated with exacerbations of underlying lung disease in predisposed patients and may be associated with severe disease in the elderly [1–4]. Successful prevention or treatment of RV infections with resulting prevention of these complications would be important with regard to both medical morbidity and economic cost.

Previous studies identified hand-to-hand transfer of RV as a likely mechanism of transmission for this pathogen, suggesting that inactivation of RV on the hands might be an effective method for prevention of these infections [5]. A series of studies has established that lowering the pH on the hands provides virucidal activity against RVs and prevents infection in the experimental setting [6–8]. Ethanol hand sanitizers effectively remove RV from the hands, and addition of organic acids to the sanitizer provides an additional antiviral effect that lasts for up to 4 hours after the application [7]. The purpose of this study was to evaluate the effect of a hand treatment with persistent virucidal activity for prevention of RV infection and RV-associated common cold illnesses in the natural setting.

## METHODS

### Volunteers

Healthy adult volunteers aged >18 years were recruited from the University of Virginia community. Subjects

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with skin conditions that would interfere with safety evaluations or medical conditions that could impact the subject's well-being or affect study results and subjects whose occupations required frequent hand washing were excluded. Written informed consent was obtained, and volunteers were compensated for participation.

### **Study Treatment**

Volunteers were randomly assigned to either the active hand treatment group or to a no-treatment control group. Subjects in the control group were asked to follow their normal daily hand-washing routine. The active hand treatment was a lotion containing 62% ethanol, 2% citric acid, and 2% malic acid that had been previously shown in the experimental setting to prevent RV infection for up to 4 hours after application [7].

### **Randomization and Masking**

A randomization code generated using commercially available software was provided by the sponsor. Staff at the study site assigned sequential subject numbers as they enrolled volunteers into the study, and treatment assignment was determined by the subject number. The clinical staff and subjects were not blinded to study treatment. Personnel who conducted the laboratory assays were blinded to study group and to whether the specimen was from a routine or illness-related visit.

### **Study Design**

All subjects were enrolled in the study in the last week of August 2009. Volunteers randomized to the treatment group were asked to apply the product every 3 hours while awake and after hand washing for the 9-week duration of the study. Volunteers also kept a daily diary of the time of each product application and of common cold symptoms. All volunteers were seen weekly for nasal lavage and to assess compliance with product use and symptom recording, to replenish supplies of the hand treatment, and to monitor for adverse events. In addition to these weekly visits, volunteers using the hand treatment also came to the study site for an additional visit each week for the first 5 weeks of the study to assess and reinforce compliance with the study treatment. Whenever a volunteer reported a common cold illness, 2 additional nasal lavage specimens were collected within 72 hours. All nasal lavage specimens were assayed by polymerase chain reaction (PCR) for the presence of RV.

### **PCR Assay**

Polymerase chain reaction using AmpliTaq Gold DNA Polymerase from Applied Biosystems was used to detect RV infections. The forward primer was HRV5' (CCC CTG AAT G[C/T]G GCT AAC C) and the reverse primer was HRV3' (CAA AGT AGT [C/T]GG TCC C[A/G]T CC).

## **Statistical Analysis**

### **Sample Size**

The sample size was determined for the primary efficacy endpoint—comparison of the number of RV-associated illnesses per 100 subjects in the control group with that in the treatment group. The sample size calculation used the following 4 assumptions: (1) The incidence of cold illnesses in the fall RV season is approximately 0.7 illnesses per 100 persons per day or 44 illnesses per 100 subjects over the 63-day course of the study [9]; (2) 65% of these illnesses are caused by RV and so are available for prevention [10]; (3) the efficacy of the hand treatment for prevention of RV-associated illnesses is 75%; and (4) there is no placebo effect in the no-treatment control group. Using these assumptions, a sample size of 92 subjects per arm was calculated to have 90% power to detect the treatment effect with a 2-sided  $P_{\alpha} = .05$ . The randomization scheme was designed to provide additional subjects in the treatment group in anticipation that some subjects would be removed from the study due to hand irritation.

### **Evaluation of Efficacy**

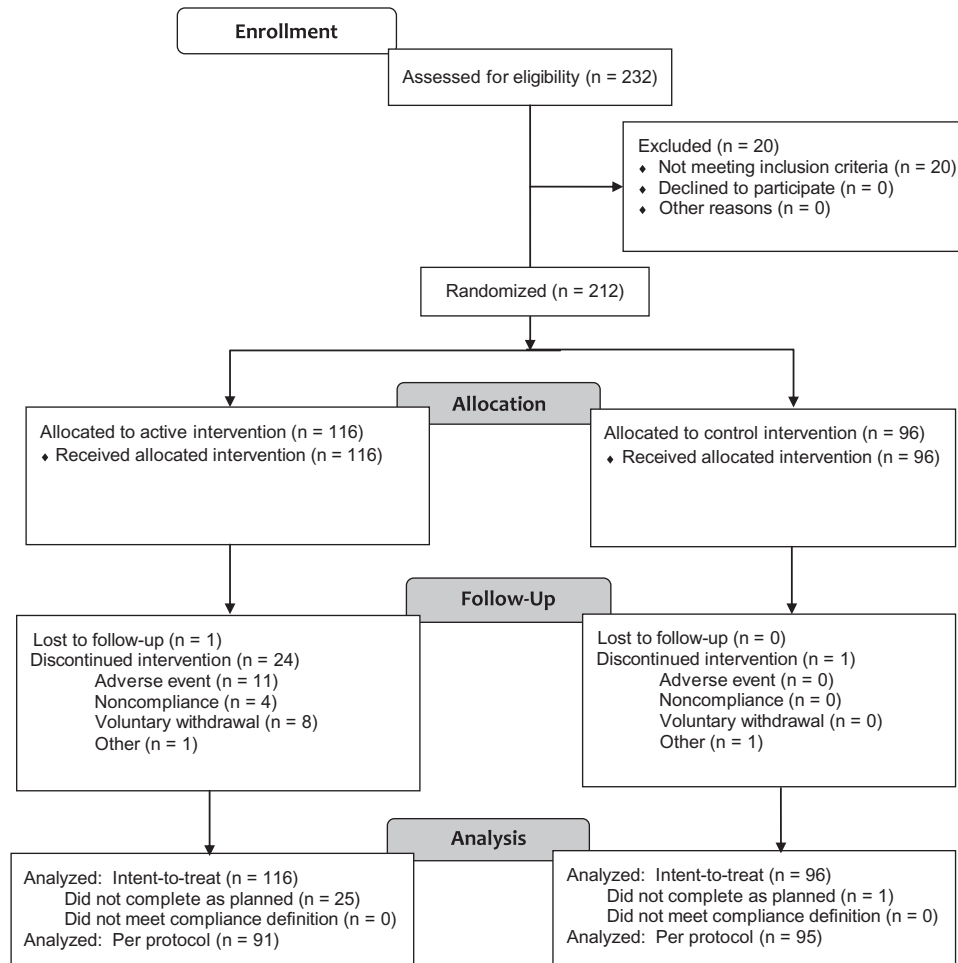
All analyses were performed on both the intent-to-treat (ITT) and per protocol (PP) populations. The ITT analysis included the available data from all randomized subjects. Data were not collected from subjects after they discontinued participation in the study. The PP population was defined a priori as subjects who completed the study and used at least 90% of the amount of hand treatment expected to be used if all applications were made as directed.

The primary efficacy endpoint was assessed by a between-treatment Poisson regression. Secondary analyses consisting of the comparison of the incidence of common cold illnesses in each treatment group and the incidence of RV infections in each treatment group were done with  $\chi^2$  tests.

A common cold illness was defined as the presence of any of the symptoms of nasal obstruction, rhinorrhea, sore throat, or cough on at least 3 consecutive days. Illnesses separated by at least 3 symptom-free days were considered separate illnesses. Illnesses occurring within 4 days of enrollment were excluded from the analysis as likely preexisting infections. Rhinovirus infection was defined as the detection of RV in nasal lavage. Polymerase chain reaction–positive specimens separated by at least 8 days and at least 1 negative PCR specimen were considered separate infections. Infections detected in the nasal lavage collected at the first visit were excluded as preexisting infections. Rhinovirus-associated illness was defined as a common cold illness occurring within 8 days of detection of RV in nasal lavage by PCR.

## **RESULTS**

A total of 212 subjects were enrolled in this study (116 in the treatment group and 96 in the control group) and included



**Figure 1.** Flow diagram depicting the passage of participants through the study.

in the ITT analysis (Figure 1). The mean age of the subjects was 21.8 (standard deviation [SD], 4.6) in the treatment group and 22.5 (SD, 4.8) in the control group. The sex distribution (male to female) was 44 to 72 and 30 to 66 in the 2 groups, respectively. One hundred eighty-six (88%) subjects, 91 in the treatment group and 95 in the control group, were included in the PP analysis. Twenty-five subjects in the treatment group and 1 subject in the control group did not complete the study as planned. All subjects who finished the study applied at least 90% of the expected amount of hand treatment and were included in the PP analysis.

There was no treatment effect on RV infection or RV-associated common cold illnesses in either the ITT or the PP analyses (Table 1). All RV-associated illnesses were based on detection of RV either at the time of the illness or at the first weekly visit after the illness. In the ITT analysis, 45 of the 116 (39%) treated subjects had at least 1 RV infection compared with 47 of the 96 (49%) control subjects ( $P = .3$ ). Twenty-six (22%) of the treated subjects had at least 1 RV-associated

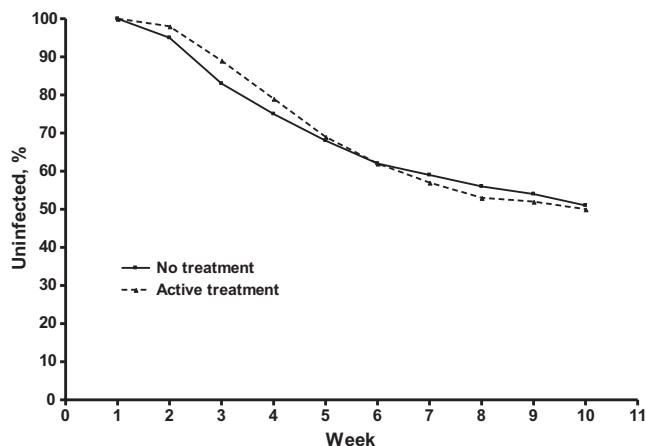
illness compared with 23 (24%) subjects in the control group ( $P > .5$ ). Similar results were seen in the PP analysis. There

**Table 1. Comparison of the Antiviral Hand Sanitizer Treatment Group and the No-Treatment (Control) Group**

Type of Analysis	Antiviral Treatment	No Treatment
Intent-to-treat analysis	(n = 116)	(n = 96)
Common cold illnesses	56 (48; 39–57)	72 (75; 65–83) <sup>a</sup>
Rhinovirus infections	49 (42; 34–51)	49 (51; 41–61)
Rhinovirus-associated illnesses	26 (22; 16–31)	24 (25; 17–35)
Per protocol analysis	(n = 91)	(n = 95)
Common cold illnesses	50 (55; 45–65)	71 (75; 65–82)
Rhinovirus infections	45 (50; 39–60)	49 (52; 42–61)
Rhinovirus-associated illnesses	25 (28; 19–37)	24 (25; 18–35)

Data are presented as no. (no. per 100 subjects; 95% confidence interval).

<sup>a</sup>  $P = .01$  for comparison to active treatment.



**Figure 2.** Comparison of the time to first rhinovirus-associated illness in the treatment and no-treatment (control) groups.

was no difference in the time to first RV-associated illness between the treatment and control groups (Figure 2).

The hand treatment did reduce the number of common cold illnesses in the ITT analysis. There were 48 illnesses per 100 subjects in the treatment group compared with 75 illnesses per 100 subjects in the control group ( $P = .01$ ). Forty-five of the 116 (39%) subjects in the treatment group had at least 1 illness over the course of the study compared with 56 of the 96 subjects (58%) in the control group ( $P = .005$ ). In the PP analysis, the illness rate was 55 per 100 subjects in the treatment group and 75 per 100 subjects in the control group ( $P = .09$ ). Thirty-nine of the 91 treated subjects (43%) had at least 1 illness compared with 55 of the 95 (58%) subjects in the control group ( $P = .06$ ) in the PP analysis.

### Safety Analysis

The use of the active hand treatment consistently for the 9-week study period was associated with hand irritation. Eleven of the 116 volunteers (9%) in the treatment group met protocol criteria for removal from the study due to hand irritation. An additional 8 subjects who did not meet these protocol criteria voluntarily withdrew due to hand irritation. There was no hand irritation in the control group. No other adverse effects of the study treatment were noted.

## DISCUSSION

This study found that use of a virucidal hand treatment had no significant impact on the incidence of RV infection or RV-associated illness. The hypothesis that hand disinfection would prevent transmission of RV was based on a series of previous studies that suggested that direct contact was the predominant mechanism of spread of RV [5]. Studies in the experimental setting suggest that effective prevention of RV

infection requires complete eradication of the virus from the hands [11, 12]. Both routine hand washing and ethanol containing hand sanitizers are effective for removal of RV from the hands but have no persistent activity against subsequent hand contamination [7]. A hand treatment that has persistent antiviral activity for some time after application would be expected to be more effective than simply removing the virus present on the hands.

The inactivation of RV by acids is well known, and early experiments suggested that treatment of the hands with organic acids might combine potent virucidal activity with a persistent antiviral effect [6]. Subsequent studies in the experimental setting confirmed these initial observations and demonstrated the effectiveness of organic acid-containing hand sanitizers for prevention of RV infections transmitted by direct contact [8]. The hand treatment used in this study, 62% ethanol with 2% malic acid and 2% citric acid, was effective for immediate removal of virus from the hands and inactivated virus contaminating the hands for up to 4 hours after application [7].

There are several potential explanations for the difference in results between the studies in the experimental setting and those reported here in the natural setting. The conditions of the experimental studies—enforced compliance, exposure to virus in a liquid medium, and exposure to virus by only the direct inoculation route—cannot be replicated in the natural setting. Compliance with the study regimen, defined as using at least 90% of the predicted volume of hand treatment over the course of the study, was 100%. Post hoc analyses, however, suggested an inverse correlation between the volume of the active hand treatment used and the likelihood of developing a common cold illness.

A second variable that may explain the difference in results between the studies conducted in the experimental and natural settings is the potential effect of mucus on the virucidal activity of the acids. In the experimental setting, RV is generally applied to the hands in liquid medium [7, 8]. In the natural setting, virus that contaminates the hands is presumably contained in small particles of nasal secretions. It is possible that the presence of the nasal secretions protects the virus from the action of the hand treatment. This possibility could be investigated in the experimental setting, but detection of such an effect would suggest that a low pH hand treatment for prevention of natural RV infection will not be possible.

A final difference between the natural and the experimental setting is the potential for routes of transmission other than direct-contact self-inoculation. The widely held opinion that RV is transmitted by direct contact rests on a combination of data from studies in the experimental setting and a single study using an iodine hand treatment in

the natural setting [5]. Other studies in the experimental setting have suggested alternate routes of transmission [13]. Our study did not directly address the route of transmission of RV, although the results of the study suggest that this should be addressed by future research.

Attempts to evaluate the effect of hand disinfection, whether hand washing or use of a hand sanitizer, for common cold illnesses have produced mixed results [14–19]. Our results are similarly mixed, although our study was not optimally designed to assess effects on illness because of the potential for introduction of bias due to the fact that the symptom scores are subjective and the subjects were not blinded to the study treatment. There was a statistically significant reduction in common cold illness in the volunteers treated with the virucidal hand treatment in the ITT analysis, although this significance was lost with the smaller sample sizes in the PP analysis. The modest effects on illness are not explained by an impact of the treatment on pathogens other than RV. Polymerase chain reaction assays for RSV, coronavirus, and influenza A and B virus were done on RV-negative specimens associated with common cold illnesses, but treatment effects on these other viruses were not demonstrated.

This study is the result of an effort that began >30 years ago with the suggestion that RVs were efficiently spread by direct contact. The disparity between the results of this study and the earlier study in the natural setting is unexplained. The earlier study focused on preventing infection in mothers in contact with children in the home setting, and it is possible that the nature of the interpersonal interaction and, therefore, the route of viral transmission, are different in the home compared with the adult populations included in our study. Regardless, the results of our study call into question commonly held assumptions about the route of spread of RV infection and suggest that studies to define the route of spread in different populations in the natural setting are needed.

## Notes

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**Potential conflicts of interest.** R. B. T. is a consultant to Henkel and received grant funding to conduct these studies. All other authors are current or former employees of Henkel.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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