**Recontamination of hands over 24 hours using a quaternary ammonium hand sanitiser with residual effect**

Running title: Recontamination of hands after using a hand sanitiser with residual effect

Marco-Felipe King1\*, Amanda M. Wilson2, Paz Aranega Bou3, Martín López-García4, Charles P. Gerba5

1. School of Civil Engineering, University of Leeds, Woodhouse Lane, LS29JT, Leeds, UK
2. Mel and Enid Zuckerman College of Public Health, University of Arizona, Tucson, AZ, USA
3. School of Mathematics, University of Leeds, Woodhouse Lane, LS29JT, Leeds, UK
4. Department of Soil, Water and Environmental Science, University of Arizona, Tucson, AZ, USA

\*Please address correspondence to m.f.king@leeds.ac.uk

**Keywords:** hand recontamination, residual effect, quaternary ammonium compound, hand hygiene, surface contact, hospital infection

# **Abstract:**

Recontamination of hands after hygiene is important for estimating the risk of infection transmission from surface contacts. Quaternary ammonium compounds (QAC) have a residual effect which was found to continue to reduce total aerobic colony counts (ACC) on participants’ hands 2hours after application. This study presents results from recontamination of participants’ hands treated with 1%w/w QAC vs control. Recontamination of the treated group never reaches baseline at 24h(p=.009023). Untreated samples averaged a 2.5log10 ACC/hour increase higher than treated after the residual effect wore off. At 6h treated hands were 1.9log10 lower than baseline, whilst untreated were 0.5log10 higher.

# **Introduction**

Many communicable diseases are known to have multiple routes of transmission (e.g. influenza, norovirus and rotavirus) and can be acquired from self-inoculation with a contaminated finger[1]. A central tenet of infection prevention therefore lies in effective hand hygiene[2]. Quaternary ammonium compounds (QACs/quats) are listed on the USA’s environmental protection agency’s list of efficacious disinfectant products against COVID-19 (List N) whilst 70% ethanol alcohol gel is promoted by the World Health Organisation as a viable method of reducing enteric transmission[3] and currently a preferred method used globally to curtail the spread of COVID-19[4]. However, the recontamination of the general public’s hands through surface contacts throughout the day is poorly understood both qualitatively and quantitatively. QACs are known to have a residual biocidal effect over time [5] so may help reduce overall hand recontamination[6]. This article presents an experimental study of hand recontamination over 24hours after hand washing with non-antimicrobial soap and water which is contrasted against the effect of handwashing with non-antimicrobial soap and water and then hand hygiene with a 1% benzyl‐C12-C16‐alkyldimethyl chloride (ADBAC) hand sanitiser. The principal aim is to study hand recontamination quantitatively for use in mathematical risk analysis scenarios.

# **Methods**

Ten participants agreed to be in this study. Participants were divided into two groups, since sampling would occur in frequent intervals over 24 hrs. This allowed the burden of time commitment and disruption of usual behaviours to be divided among the two groups. All ten participants washed their hands for 1 minute with soft soap and then used a paper towel to dry their hands. Participants were then asked to rub their fingertips and thumb of each hand for 1 minute onto two separate Petri dishes containing 10 mL of Tryptone Soy Broth (TSB). This was done to quantify the amount of endogenous bacteria that could be recovered with this method. To quantify the recovered bacteria, 1 mL of the TSB was then eluted into a separate Petri dish containing 10 to 15 mL of sterile Tryptone Soy Agar. Once plates were solidified, they were incubated for 24 hours at 37°C.

For all participants, one hand served as the control while the other was treated with the intervention and was randomly chosen. On the intervention hand, 3 mL of the hand sanitiser containing a quaternary ammonium compound: benzalkonium chloride (<1%w/w) was applied and rubbed in for 30 seconds on the hand up to the wrist by a researcher wearing gloves. The hand was allowed to air dry for 5 minutes at room temperature, after which participants rubbed each hand onto separate Petri dishes containing 10 mL of Maximum Recovery Diluent (MRB) for one minute. One mL was then aliquoted into a Petri dish with 15 mL of TSA. Once plates were solidified, they were incubated for 24 hours at 37°C. Colonies were then enumerated.

One group of five subjects were then instructed to resume usual behaviours and routines, with the exception to limit hand washing. After 2 hrs, the same sampling procedure was used to measure bacteria counts on both the control and intervention hand. This was repeated at 4 hrs and 6 hrs from the hand sanitizer application time. The second group of five participants were sampled at 18 hrs, 20 hrs, 22 hrs, and 24 hrs. Bacteria counts on hands at each time interval were then compared for the control and intervention hands.

## **Statistical Analysis**

A mixed effects general linear model was fitted to the data using the BRMS (REF: Bürkner P. C. (2017). brms: An R Package for Bayesian Multilevel Models using Stan. *Journal of Statistical Software*. 80(1), 1-28. [doi.org/10.18637/jss.v080.i01](http://doi.org/10.18637/jss.v080.i01)

) package in R (version 4.1.2). The AM and the PM groups were combined to gain more information when modelling the correlation structure for the repeated measurements over time assuming that the random-effects structure was the same in the two groups. However, in the fixed effects the two groups were considered separately because of the difference in the time periods of sampling. The regression model used had the following form: CFU~group+time\*treatment+(1|id), which accounted for a multiplicative effect of treatment over time.

# **Results and Discussion**

In the treatment group there was an initial 1.2log10 reduction (p=.009023 compared to control), with a continued residual effect for 2-4hours after application with a further 0.95log10 reduction. At 24 hours, there was a 2.1log10 difference between groups (see Figure 1). It is noted that there appears to be no equilibrium of hand contamination at any point in time, which suggest that the hands can become increasingly contaminated over a 24h period. However, we do see a plateau effect between 6h and 16h after the start of the study, which might be explained by participants not touching surface whilst asleep.

The rate of recontamination per hour was not constant and, after the residual effect had worn off, differed marginally between groups (0.04log10 /hour vs. 0.05log10/hour). At 6h, ACC on treated hands were 1.9log10 lower than baseline, whilst untreated were 0.5log10 higher. Hand hygiene compliance in healthcare settings has often been reported as sub-optimal, [7] so a residual effect may be of benefit in terms of reducing risk of healthcare acquired infections. However, due to the residue of compound on the skin, potential Gram-positive and Gram-negative bacteria have been seen to develop resistance over time[8,9] therefore QACs are a possible issue for healthcare use[8]. Since QACs have been shown to be effective against enveloped and non-enveloped viruses, the general population may benefit from a residual effect without antimicrobial tolerance issues. A false sense of security might, however, be counterproductive in the long run.



Figure 1 Comparison of total aerobic colony count on hands over 24 hours: red = untreated, blue = QAC treated.

During non-eating activities, the general public has been seen to touch their nose or mouth on average 2.5 times per hour (ranging from 0 to 14)[10] suggesting that a proportion of infections could be avoided by hand hygiene once every two hours. Wilson et al. [3] shows quantitatively how the effect of a residual sanitiser can reduce the risk from norovirus, even 4h post application and as a result, upholds findings in this study. QAC based sanitisers are typically well tolerated by users but environmental toxicity may be a drawback at high concentrations. Additionally, 3ml of product applied per participant may be higher than typically used and application may not be as thorough as conducted in this study.

Bacterial regrowth on hands inside surgical gloves has demonstrated the benefit of residual hand sanitisers for 6h post-hand sanitiser application[6], which indicated a 2log10 /6hours regrowth rather than recontamination, which is in-line with the current findings. Nevertheless, this suggests that re-growth is the dominant factor over adsorption for exogeneous microorganisms and therefore controversial.

However, these previous studies were limited in that they did not account for the potential loss of hand sanitiser residue during hand-to-surface contacts that may occur over a day. This study improves upon that work by capturing the effect of hand-to-surface contacts on log10 reductions offered by residual hand sanitisers over time.

Application was randomised to either right or left hand. Contact patterns between the left and right hands may vary, influencing differences between the control and intervention results in this study. More data are needed to further describe increases in bioburden on hands over time and reductions offered by residual or non-residual hand sanitisers. This would not only inform infection control decision-making but also would inform mathematical models that describe accruement of pathogens on hands and the risks this accruement poses. This could provide further insight into mechanisms of other interventions that have lasting effects, such as surface disinfectants with residual properties, and how these reductions combined with the potential for bacterial growth explain observed fluctuations in microbial bioburden on hands or fomite surfaces after intervention applications.

**Code, Materials**

Under a Creative Commons Zero v1.0 Universal license (CC-BY), data can be accessed at: DOI

**Conflicts of Interest**

CG has received funding previously from manufacturers for the study of disinfectants, including the Clorox Company, Allied Biosciences, AK Steel, the Consumer Specialty Products Association, the American Water Works Research Foundation, and the University of Arizona transfer research initiative fund.

**Funding Source**

A.M. Wilson was supported by the University of Arizona Foundation and the Hispanic Women’s Corporation/Zuckerman Family Foundation Student Scholarship Award through the Mel and Enid Zuckerman College of Public Health, University of Arizona. M-F. King and C.J. Noakes were funded by the Engineering and Physical Sciences Research Council, UK: Healthcare Environment Control, Optimisation and Infection Risk Assessment (https://HECOIRA.leeds.ac.uk) (grant code: EP/P023312/1). M. López-García was funded by the Medical Research Council, UK (MR/N014855/1).

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