Utility of an Image Analysis Method as a Handwashing Measurement Tool

By

Rachel L. Jess
M.A., University of Kansas, 2017
B.S., University of North Texas, 2014

Submitted to the graduate degree program in Applied Behavioral Science and the Graduate Faculty of the University of Kansas in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

Chairperson Claudia Dozier, Ph.D

Pamela Neidert, Ph.D.

Derek Reed, Ph.D.

Christopher Cushing, Ph.D.

Nancy Brady, Ph.D.

Date Defended: 22 July 2021

The dissertation committee for Rachel L. Jess certifies that this is the approved version of the following dissertation:

Utility of an Image Analysis Method as a Handwashing Measurement Tool

Chairperson Claudia Dozier, Ph.D

Date Approved: 22 July 2021

Abstract

Hands are the most common mode of transmission of infection from bacteria and viruses. Washing hands with soap and water is the most effective method for decreasing transmission of infection; however, research suggests that children do not routinely wash their hands using methods that healthcare agencies have determined best practice. Researchers have evaluated various antecedent and consequent strategies to address appropriate handwashing in young children. More research is needed, however, regarding the efficacy and efficiency of teaching and measuring handwashing accuracy and quality in young children. One method for assessing handwashing quality is comparing pre- and post-handwashing levels of proxy contamination using image analysis software. Further evaluation of the correlation between handwashing accuracy and hand cleanliness using proxy contamination should be conducted to determine the validity of this analysis method. The purposes of the current study were to (a) conduct a retrospective data analysis from a series of studies with children on errors made during handwashing and handwashing quality using an index of hand cleanliness and (b) examine the utility of an image analysis method as a measurement tool for hand cleanliness. Overall results suggest that the most important components of handwashing for increasing hand cleanliness include use of soap, amount of vigor, scrubbing the tops and palms of hands, and duration of scrubbing.

Acknowledgements

I would first like to thank my mentor, Dr. Claudia L. Dozier, for her constant guidance and support to help me become the scientist I am today. Second, I would like to thank my lab mates and research assistants who have contributed to and supported the development of this project. Finally, I would like to thank my family, friends, and wife who have all continuously encouraged me to reach my goals, regardless of how difficult the journey may be. I am grateful to have such a supportive environment in which to learn and live.

Table of Contents

Illnesses in Young Children

Several microbiological studies have shown that respiratory droplets and fecal particles, which contain pathogens (i.e., bacteria or viruses) that cause infections, are commonly found on hands (Rabie & Curtis, 2006). Respiratory droplets are produced when an infected person coughs, sneezes, talks, or breathes (Centers for Disease Control and Prevention [CDC], 2020). These infectious respiratory droplets may land on the individual's hands and then transfer to other people or various surfaces (e.g., doorknobs, tables, toys) after contact with hands; respiratory droplets may also land directly on surfaces (CDC, 2020). After handling a contaminated surface, a novel person may become infected after touching their nose, mouth, or eyes. Thus, hands are a common mode of transmission of infection from bacteria and viruses. Young children are highly likely to acquire infections due to the tendency to put their hands or items that have been touched by others in their mouth (Aronson & Shope, 2019; Day et al., 1993; Pickering, 1986). In addition, children who attend out-of-home care (e.g., preschool and daycare) come into close contact with multiple other individuals, such as teachers and other children, which makes them more susceptible to infections than children who do not attend out-of-home care (Niffenegger, 1997).

Most recently in March 2020, the World Health Organization declared the outbreak of COVID-19, a severe respiratory disease caused by a novel coronavirus, a pandemic (WHO, 2020). The virus that causes COVID-19 is currently known to spread rapidly through respiratory droplets of infected individuals. According to the CDC, individuals who are physically near (i.e., within 6 ft) or in direct contact with people with COVID-19 are at highest risk of infection due to close exposure to respiratory droplets. According to the National Institutes of Health (2020), the virus that causes COVID-19 is detectable on many surfaces for several hours. Although COVID-

19 spreads less commonly through contact with surfaces, a healthy individual could become infected after touching a surface containing the virus and then touching their own mouth, nose, or eyes (CDC, 2020). It is currently understood that children with COVID-19 generally have mild symptoms; however, recent research suggests individuals with COVID-19 may spread the virus without showing symptoms (Mizumoto et al., 2020).

Child illnesses result in several negative outcomes. First, infected children are often carriers of infection to family members due to frequent hands-on contact with caregivers (Neuzil et al., 2002). In fact, parents become sick in approximately 40% of child respiratory illness episodes and 36% of acute diarrheal episodes (Sacri et al., 2014). Second, approximately 75% of children's school absences are attributed to illness (Lau et al., 2012; Wang et al., 2017). Missing school, even at an early age, can result in delays in skill acquisition and disrupt a child's routine (Lamdin, 2010). The University of Chicago Consortium on School Research (2013) examined the effects of absenteeism on learning outcomes across eight Chicago preschool programs and found a correlation between the numbers of days missed and scores on end-of-year kindergarten-readiness tests. Specifically, the more days of preschool a student missed during the year, the lower they scored on pre-academic and social-emotional development readiness tests (controlling for entering skills). Overall results suggested that reducing the risk of infection and illness in children would prevent more than half a child's absences from school. To keep children healthy and avoid spreading infection to others, healthcare agencies such as the World Health Organization (WHO) and CDC recommend that children engage in "everyday preventive behaviors," including handwashing.

Importance of Handwashing

Based on decades of microbiological research, healthcare agencies (e.g., CDC, WHO) have determined that the most important components of handwashing include using soap, vigorously scrubbing the hands, and ensuring that all parts of the hands (i.e., tops, palms, in between fingers, and fingernails) are scrubbed for approximately 20 seconds (CDC, 2015; WHO, 2009). Using soap during handwashing is more effective than using water alone due to compounds in soap (i.e., surfactants) that lift pathogens and soil from the hands (Jensen et al., 2015; Luby et al., 2011). Several studies have demonstrated the efficacy of handwashing with soap to remove various viruses and bacterial from the hands, such as *Enterobacter* (i.e., bacteria that cause respiratory tract infection; Burton et al., 2011), *E. coli* (i.e., bacteria that cause gastrointestinal infection; Lin et al., 2003), and adenoviruses (i.e., viruses that cause respiratory infection; Aiello et al., 2008). In a study evaluating handwashing efficacy with and without soap for preventing pneumonia in Pakistani children, Luby and colleagues (2005) found that handwashing with soap, regardless of antibacterial formulation, was effective for preventing pneumonia. The mean incidence of pneumonia (i.e., number of new episodes of illness per 100 person-weeks) for children who washed hands with water only was twice as high (4.40) than in children who washed hands with antibacterial soap (2.42) and plain soap (2.20).

In addition to using soap, thoroughly rubbing hands together creates friction, which helps carry pathogens away from the skin (Hoque, 2003; Luby et al., 2007). In one study, Hoque (2003) evaluated the effects of using various rubbing agents (i.e., handwashing with either soap, ash, soil, or water only) during post-defecation handwashing on the level of fecal contamination on hands with 90 women in rural Bangladesh. Experimenters used microbial sampling to measure the number of fecal coliform units grown from samples of women’s hands. After handwashing, participants rinsed their hands in a container of saline solution to create microbial samples. Experimenters applied these samples to agar plates and counted the number of colonies (i.e., groups of growth) to determine the number of fecal coliform units. Results showed that rubbing both hands together with any of the three agents (i.e., soap, ash, or soil) resulted in lower numbers of fecal coliform units compared to rubbing hands together with water only. That is, washing hands with a rubbing agent that creates friction, such as soap, resulted in less fecal contamination than washing hands without a rubbing agent. With respect to duration of scrubbing, few studies have evaluated the health effects of various handwashing durations; however, evidence from these studies suggests scrubbing hands for 15-30 seconds removes more soil and pathogens from hands than scrubbing for shorter durations (Fuls et al., 2008; Jensen et al., 2015; Todd et al., 2010). In one study, Jensen et al. (2015) used microbial sampling to measure the effects of handwashing without soap for 20 s and 5 s on the colony reduction of bacterial contamination on hands. To control for pre-handwashing levels of bacterial contamination, experimenters instructed participants to spread 5 g of ground beef contaminated with *Klebsiella aerogenes* (i.e., bacteria normally found in human intestines that do not cause infection in healthy individuals) on their hands for 30 s. Participants then scrubbed all areas of the hands under running water for 20 s or 5 s, depending on the condition. Results showed that handwashing without soap for 20 s produced a greater reduction in bacterial contamination (1.1 log colony reduction) than handwashing without soap for 5 s (1.0 log colony reduction). Washing hands with soap and water is more effective than alcohol-based hand sanitizers at removing most types of infectious particles; however, the CDC recommends hand sanitizer may be an appropriate alternative when soap and water are unavailable (CDC, 2020; Charbonneau et al., 2000).

In addition to recommendations for how to wash hands, healthcare agencies also recommend key occasions for when to wash hands to prevent pathogen transmission (CDC, 2020). Given that hands are common carriers of respiratory droplets and fecal particles that transmit infection, hands should be washed with soap and water after contact with sources of these pathogens. Handwashing following toileting or diapering can prevent germs transmitted through feces, such as Salmonella, *E. coli*, and norovirus, that cause diarrhea (Franks et al., 1998). Respiratory droplets, which can transmit adenovirus (i.e., common cold) and enterovirus (i.e., hand-foot-mouth disease), are produced when a person coughs or sneezes; thus, handwashing should occur after coughing, sneezing, or coming into contact with saliva and mucus (Franks et al., 1998). Finally, hands should be washed prior to eating or preparing food to prevent germs from spreading or multiplying on food, which could infect others (Todd et al., 2010). The National Association for the Education of Young Children (NAEYC) Accreditation Standards (NAEYC, 2019) recommends practices for promoting and protecting children's health in early childhood environments, such as preschools and daycares. The NAEYC recommends children thoroughly wash their hands for 20 s with soap upon arrival for the day, after toileting, after handling bodily fluids (e.g., wiping their nose, coughing on hands), before and after eating, and after playing outside (NAEYC, 2019).

Interventions for Addressing Handwashing

Various studies have been conducted to address handwashing in young children. Often, these interventions are packaged interventions that involve both antecedent and consequent manipulations. These antecedent procedures include providing rationales and instructions (e.g., Carabin et al., 1999; Ponka et al., 2004; Rosen et al., 2006), modeling how to correctly wash hands (e.g., Au et al., 2009; Day et al., 1998; Deochand et al., 2019; Jess et al., 2019; Rosen et al., 2011; Rosenberg et al., 2010), and auditory prompts (e.g., Kramer, 1978; Lee et al., 2015). Consequent procedures include providing vocal feedback (e.g., Jess et al., 2019) and visual feedback (e.g., Dingman et al., 2020; Fishbein et al., 2011; Oncu et al., 2018; Snow et al., 2008).

Rationales and Instructions

Rationales are statements that describe reasons why an individual should, or should not, engage in a particular behavior (Wilder et al., 2010). Many studies using rationales and instructions for addressing handwashing in children have involved discussing the importance of handwashing (e.g., washing hands can remove germs that get you sick) and describing correct handwashing (e.g., telling children when to wash hands; Carabin et al., 1999; Ponka et al., 2004; Rosen et al., 2006). For example, Ponka et al. (2004) presented preschool children with a video about disease transmission and described how handwashing removes germs. The video stressed the importance of key times to wash hands but did not describe how to appropriately wash hands. The experimenters presented the video and discussed these topics with the preschool staff during an in-service workshop prior to the study. Results showed that children under 3 years in the intervention group missed fewer days of school due to illness than children in the control group; however, children over 3 years showed no statistically significant difference between intervention and control groups. This may be because staff physically assisted younger children during handwashing, whereas older children were not provided physical assistance. Based on the results of studies involving rationales and education, these methods are generally ineffective when implemented alone and should be used in conjunction with other intervention components (Staniford & Schmidtke, 2020). Research is needed to determine the optimal contexts in which rationales and education may promote frequent handwashing; for example, additional research may evaluate the role rationales and education have in maintaining frequent handwashing after using other evidence-based methods.

Modeling

Modeling is a teaching procedure in which a particular behavior is demonstrated for a learner (Miltenberger, 2016). Models can be presented in-person (i.e., live) or via video or other symbolic presentation. Live models involve a learner observing an individual (e.g., teacher, nurse, peer) engaging in a particular behavior. Modeling as a procedure to teach handwashing is mostly used with young children and adults with developmental disabilities, including live models (e.g., in-person; Au et al., 2009; Day et al., 1998; Rosen et al., 2011) and symbolic models (e.g., video models, cartoons, puppets; Deochand et al., 2019; Jess et al., 2019; Rosenberg et al., 2010). With respect to live models, Walmsley et al. (2013) used in-person models as one component in a training procedure to teach handwashing to five young adults with various disabilities. During all handwashing training sessions, experimenters modeled correct handwashing for individual participants at a sink and vocally stated each step as it was completed; handwashing steps included wetting the hands, dispensing soap, scrubbing all areas of the hands for 15 s, and rinsing soap off of hands. After modeling correct handwashing, the experimenter instructed the participant to practice washing their hands. The experimenter implemented least-to-most prompting (i.e., gestural, partial physical, full physical prompts) if a participant omitted a handwashing step or performed a step incorrectly during rehearsal. Handwashing training sessions continued until a participant correctly performed all handwashing steps without prompting for two consecutive sessions. Throughout the study, experimenters used Glo-Germ™ to measure handwashing effectiveness; that is, after handwashing, experimenters observed the amount of remaining Glo-Germ™ on participants' hands and assigned a score based on hand cleanliness. Because handwashing accuracy data were only collected during handwashing training and not throughout the study, the long-term effects of modeling on handwashing accuracy were not determined. Additionally, the modeling procedure was evaluated in conjunction with prompting, so it is unknown what effect modeling alone would have on participants' handwashing. In two similar studies, Day et al. (1998) implemented a package training that included a nurse modeling correct handwashing for thirteen first-grade children. After a discussion about the importance of handwashing at certain times, nurses modeled appropriate handwashing at classroom sinks and instructed the children to practice the handwashing steps. Following the intervention, children's handwashing quality increased and maintained during monthly observations. Again, it is unknown how effective the modeling procedure would be if used without other treatment components. Further research is needed to determine live modeling procedures that are effective when used in isolation to teach handwashing to young children.

Several studies have used video modeling to address handwashing in young children. Some studies using video models have used unknown models (e.g., Deochand et al., 2019; Early et al., 1998), whereas some have used experimenter-created videos showing known models, such as teachers or peers (e.g., Jess et al., 2019, Rosenberg et al., 2010). With respect to unknown models, Deochand et al. (2019) presented a handwashing video created by the World Health Organization to three children with developmental disabilities in an attempt to increase handwashing accuracy and duration. Experimenters presented the video model prior to each handwashing observation; however, all children required individualized error correction and feedback in addition to the video model to increase handwashing duration and accuracy. The additional treatment components were introduced at the same time, so it is unknown which individual or combined components are responsible for the behavior change. In another study, Early et al. (1998) provided a one-time handwashing assembly to all first- and fourth-grade students at two elementary schools. During the assembly, experimenters showed a video of a clown demonstrating accurate handwashing. Following the video presentation, other educational materials were presented, including posters depicting accurate handwashing, a discussion on germ transmission, and stickers for participation. During post-intervention observations, the percentage of children washing their hands before lunch and after bathroom use slightly increased from 64% during pre-intervention observations to 72%. Similar to other studies using models to address handwashing, it is unknown what effect the clown handwashing video model would have on handwashing frequency when presented in isolation.

With respect to known models, Jess et al. (2019) presented a handwashing video to three groups of 14 preschool children showing a known adult engaging in accurate handwashing (i.e., following CDC recommendations) in an attempt to increase handwashing accuracy and quality. The video showed the adult with dirty hands (e.g., covered in washable markers) and showed images of Glo-Germ™-illuminated hands before handwashing (i.e., tops, bottoms, and between fingers of both hands illuminated) while the narrator explained that the illuminated areas were germs that need to be washed off. The narrator described each handwashing step and instructed participants to practice while the known adult engaged in that step in the video. Then, the narrator taught a 20-s handwashing song and instructed participants to sing while the known adult washed their hands. After watching the video model, participants washed their hands. In addition to the video model, experimenters also provided visual feedback regarding handwashing quality by showing children pictures of their Glo-Germ™-illuminated hands. Results of the group analysis showed that the intervention was effective for increasing the number of correctly completed handwashing steps and decreasing the amount of post-handwashing illuminated Glo-Germ™ for all children.

Overall, results of studies involving handwashing models indicate modeling may be an effective component for increasing handwashing in children when combined with other strategies, such as prompting and feedback. However, there are limitations to these studies that should be addressed. First, because many studies included multiple treatment components, it is unknown what combination of additional treatment strategies are necessary and sufficient for modeling to be effective. Second, it is unknown how many model presentations are needed to produce behavior change. That is, some studies presented a model one time, whereas other studies presented a model prior to each handwashing observation. Finally, results of these studies suggest that videos of known models may improve handwashing accuracy better than videos of unknown models. It is unknown, however, which characteristic similarities are responsible for a model to be most effective. Future research should involve component analyses of various treatment components that improve the effectiveness of handwashing models, as well as evaluating the number of presentations necessary to promote accurate handwashing. More studies should also compare the effects of known versus unknown models, as well as live versus symbiolic models, on the accuracy and frequency of children's handwashing.

Auditory Prompts

Prompts are antecedent stimuli intended to promote a specific response in the presence of a discriminative stimulus (S^D; Cooper et al., 2007). Auditory prompts are audible sounds, such as alarms or songs, used to occasion specific responding (Alberto et al., 1999). Several studies have included auditory prompts, such as singing handwashing-related songs, as part of treatment packages (e.g., Lee et al., 2015; Jess et al., 2019; Rosen et al., 2006). In one study, Kramer

(1978) taught 21 preschool-aged children a 10-step handwashing procedure using a handwashing song that described each step in the procedure. Across 10 training sessions, children first sang the handwashing song as a group and then individually practiced washing their hands while experimenters played a recording of the handwashing song. Results showed that the handwashing song was effective for increasing the number of correctly completed handwashing steps from baseline levels for 19 of the 21 participants. In another study, Lee et al. (2015) evaluated the effects of a handwashing teaching package, which included teaching a handwashing-related song, on the handwashing quality of children with disabilities. The teaching package included a handwashing video model, visual prompts in the form of posters depicting correct handwashing steps, teaching children a 20-s song that described areas of the hand to scrub, and prompting children to sing the handwashing song while they washed their hands. Experimenters measured participants' handwashing quality using a 4-point scale rating of post-handwashing levels of illuminated Glo-Germ™. After the handwashing teaching package was introduced, children's handwashing quality ratings increased from baseline levels, suggesting improved handwashing behavior.

Results of studies including songs to address handwashing in young children suggest that singing a song during handwashing may promote and maintain appropriate handwashing. Additionally, singing a song that describes areas of the hands to scrub may function as a self-instruction. That is, the lyrics of a handwashing song may direct children to engage in the specific steps that are described. Various public health agencies (e.g., CDC, WHO) suggest individuals sing a 20-s song (e.g., "Happy Birthday") during handwashing to promote appropriate handwashing duration; however, it is unknown if handwashing-related songs may influence correct handwashing accuracy or quality more than non-handwashing songs. Thus, future research should compare the effects of teaching and singing handwashing-related versus non-handwashing-related songs to determine the extent to which the handwashing song exerts stimulus control over accurate handwashing. Researchers may also be interested in further determining the effects of auditory stimulus control on accurate handwashing. That is, following teaching correct handwashing using treatment packages with auditory prompts (e.g., singing song during handwashing), researchers should evaluate the continued effects of auditory prompts without other treatment components on children's maintenance of accurate handwashing.

Feedback

Feedback is the presentation of stimuli that describe characteristics of a response and the relation between previous responding and a target response, or goal; thus, the parameters of feedback covary with parameters of a response (Mangiapanello & Hemmes, 2015). Various forms or modalities of feedback include performance feedback (e.g., vocal statements and written evaluations; Johnson, 2013) and visual feedback (e.g., viewing recorded videos of responding; Sigurdsson & Austin, 2008; Smith et al., 1960). Many studies have used performance feedback in the form of visual feedback to show children how well they washed their hands using a substitute product for germs, such as common household products (e.g., glitter and petroleum jelly; Oncu et al., 2018; Snow et al., 2008). Other studies have used UV-sensitive lotion, such as Glo-Germ™ (Au et al., 2009; Deochand et al., 2019; Dingman et al., 2020; Fishbein et al., 2011; Jess et al., 2019). The removal of Glo-Germ™ during handwashing corresponds with the removal of actual germs from hands. That is, Glo-Germ™ remaining on hands following handwashing indicates areas that were not adequately cleaned. For example, Snow et al. (2008) implemented a one-time hands-on Glo-Germ™ exercise to increase handwashing accuracy with groups of elementary school children. After modeling appropriate handwashing for children, experimenters dispensed Glo-Germ™ on children's hands and allowed children to observe their "dirty" hands under UV light. Experimenters then instructed children to wash their hands and reminded children to remove the germs from their hands. After handwashing, children again observed their hands under UV light to see the effectiveness of their handwashing. Results showed the Glo-Germ™ demonstration was more effective in promoting handwashing compliance over time compared to instructions and modeling alone; however, visual feedback was only implemented following the single demonstration. One reason why visual feedback may be an effective procedure for promoting effective handwashing is that visual feedback regarding initial handwashing performance may serve as a prompt to scrub specific areas of the hands in the future. For example, if post-handwashing visual feedback shows that a child did not effectively remove Glo-Germ™ from the tops or fingernails of their hands, the child may attend to scrubbing those specific areas during subsequent handwashing opportunities. Although visual feedback may be an effective method to increase accurate handwashing in children, limitations of previous studies should be addressed in further research. Handwashing interventions using visual feedback may be burdensome for teachers or school staff to implement with every student. Researchers may be interested in finding less resource intensive methods for delivering visual feedback in the school environment or identifying the optimal schedule of providing visual feedback. That is, researchers should evaluate the difference between daily, weekly, or intermittently scheduled visual feedback delivery on the accuracy and quality of children's handwashing, as well as the effects of intermittent visual feedback on maintenance of accurate and effective handwashing over time.

With respect to vocal feedback, Deochand et al. (2019) used Glo-Germ™ to provide visual feedback of initial handwashing attempts and described the areas that were missed with adolescents with intellectual disabilities. That is, experimenters applied Glo-Germ™ to participants' hands, instructed them to wash their hands, and then showed participants their UV-illuminated hands following handwashing. If a participant's hands remained illuminated with Glo-Germ™ after handwashing, experimenters offered participants the opportunity to rewash their hands (error correction). Results of this evaluation showed that the visual feedback and error correction procedure was effective for increasing hand cleanliness over time. In a similar study, Jess et al. (in preparation) provided in-situ feedback to children regarding incorrect handwashing steps. That is, if a child failed to complete or incorrectly completed a handwashing step, the experimenter interrupted handwashing, provided vocal feedback regarding that step, and instructed the participant to continue washing their hands. Although these error correction procedures were implemented in packaged interventions that included video modeling and visual feedback, results suggest error correction and in-situ feedback may be important components for increasing handwashing accuracy and quality. The results of these studies replicate previous research on the effects of in-situ feedback on young children's behavior (e.g., Dib & Sturmey, 2007; Houvouras & Harvey, 2014; Schreibman et al., 1983). The in-situ feedback component implemented in Jess et al. (2019) consisted of delivering verbal feedback, modeling the correct behavior, and having the participant rehearse the correct behavior (i.e., behavioral skills training), which has been shown to be an effective procedure in teaching children a variety of skills (e.g., Miltenberger, 2008; Poche et al., 1981). Furthermore, there are several reasons why these error correction and vocal feedback procedures may be effective. For example, requiring the participant to practice the correct response prior to moving on in the response sequence may function as positive practice or negative reinforcement. With respect to positive practice, repeated practice of a correct response has been shown to be effective in increasing the practiced behavior (e.g., Carey & Bucher, 1981); that is, positive practice may provide more opportunities for the participant to emit the correct response under appropriate stimulus conditions, which may enhance stimulus control over correct responding in the future (e.g., Worsdell et al., 2005). For example, the participants may have engaged in correct handwashing behavior because of the additional practice of the correct responses under similar conditions. With respect to negative reinforcement, in-situ feedback may potentially include sources of negative reinforcement (e.g., Ingvarsson & Hollobaugh, 2010); that is, participants may engage in correct responding to avoid repeated practice of the correct response. For example, the participants may have engaged in correct handwashing behavior to avoid the implementation of error correction or in-situ feedback and avoid a prolonged session. This negative reinforcement contingency would only operate if the implementation of in-situ feedback was nonpreferred or aversive to a participant.

In summary, results of studies addressing handwashing in young children suggest a combination of both antecedent and consequence strategies should be used to teach and maintain appropriate handwashing. In addition to the limitations mentioned above, this literature has several other limitations. Many studies addressing handwashing in young children have used group and pre-post designs to determine the effects of interventions on handwashing (e.g., Au et al., 2009; Day et al., 1998; Early et al., 1998). Therefore, individual effects of the intervention are unknown and repeated measures of the effects are not evaluated. Some studies (e.g., Hagiwara & Myles, 1999; Kissel et al., 1983; Luke & Alavosius, 2011; Rosenberg et al., 2010; Walmsley et al., 2013) have used single-subject designs (e.g., multiple-baseline-across-participants designs) to evaluate the effects of handwashing interventions; however, many of these studies attempted to increase handwashing in adults. Therefore, additional research should be completed using single-subject-design methodology to determine individual effects of handwashing interventions in young children.

In many studies addressing handwashing in young children (e.g., Carabin et al., 1999; Guinan et al., 2002; Roberts et al., 2000), experimenters trained teachers or staff on the intervention components to implement in the schools or day care centers. Of the studies involving teacher or staff implementation of the intervention, most did not collect or report data on treatment integrity (e.g., Harrison, 2012; Ponka et al., 2004); thus, it is unknown whether the teachers or staff implemented the intervention correctly. This is important because treatment integrity of interventions is associated with treatment effects (Gresham et al., 1993); that is, high levels of treatment integrity have been shown to result in greater treatment effects than low levels of treatment integrity (Fiske, 2008). It is possible that the studies would have shown greater treatment effects if the experimenters ensured correct intervention implementation. In future evaluations of handwashing interventions, experimenters should collect and analyze data on treatment integrity to ensure interventions are implemented with high integrity.

More research is still needed regarding the efficacy and efficiency of teaching and measuring handwashing accuracy and quality in young children. One such method for assessing handwashing quality is comparing pre- and post-handwashing levels of proxy contamination using image analysis software. This assessment method has been used in several studies with young children; however, further evaluation of the correlation between handwashing accuracy and hand cleanliness using proxy contamination should be conducted to determine the validity of this analysis method.

Handwashing Measurement

Several measurement methodologies have been utilized in handwashing research to assess the frequency, accuracy, and effectiveness of handwashing behavior (Haas & Larson, 2007). Methods for assessing various dimensions of handwashing include direct measures, such as direct observation of handwashing frequency and accuracy, and indirect measures, such as microbial sampling, incidence of illness, and image analysis (Deochand & Deochand, 2016; Rotter et al., 2017; WHO, 2006).

Direct Observation

Direct observation involves observing an individual during handwashing and recording data on aspects of handwashing behavior (e.g., frequency and accuracy). Observational recording is the most objective and reliable method for assessing handwashing for several reasons (WHO, 2006). First, direct observation allows an observer to record opportunities for handwashing, such as after toilet use, and handwashing behavior during such opportunities to determine appropriate handwashing frequency (Haas & Larson, 2007). Second, observers can use a checklist or other direct observation scoring system to monitor the accuracy of handwashing behavior and specific errors made during handwashing, such areas of the hands scrubbed, amount of vigor used to scrub, duration of scrubbing, and use of soap (Gould et al., 2007). Finally, direct observation of handwashing behavior allows for repeated observations across individuals, which allows observers to determine the effects of interventions at the individual level (Haas & Larson, 2007). However, one disadvantage of conducting repeated observations across individuals is that data collection may require excess time and effort. Additionally, in-person observations may also influence participants' handwashing behavior due to observer reactivity (Pickering et al., 2014). To address these limitations, observers should consider several observation practices, such as probe data collection and conducting covert observations. Collecting probe data on handwashing behavior involves infrequently observing handwashing across various situations, which is a less resource-intensive method for assessing behavior (Lerman et al., 2011). Covert observations involve collecting data on handwashing behavior without informing individuals of the nature of the observation (Franklin et al., 1996).

With respect to studies measuring handwashing in young children, some studies have used direct observation measures, such as the frequency of handwashing (e.g., Early et al., 1998; Rosen et al., 2006); however, it is unclear how accurate children were in washing their hands with respect to best practices. Additionally, increases in the frequency of handwashing in which multiple best-practice steps are missing or done incorrectly likely will not have any influence on infections or the cleanliness of hands. Thus, it is important to measure both the degree of cleanliness and the accuracy of handwashing, and research on which handwashing variables result in hand cleanliness is warranted.

Microbial Sampling

Microbial sampling involves comparing the amount of microbial (i.e., viral or bacterial) colony-forming units (CFU) grown from pre- and post-handwashing samples on agar plates to determine the effectiveness of handwashing (Rotter et al., 2017). Experimenters can collect microbiologic samples by pressing parts of the hand (e.g., fingertips or palms) directly onto an agar plate or by swabbing hands with a moistened cotton swab and applying the swab to an agar plate (Jensen et al., 2015). After at least 24 hr of incubation to allow cultures to cultivate, experimenters count the number of CFU present on the plate (Hautemaniere et al., 2009). This method allows experimenters to determine the quality of handwashing by comparing the number of CFU growth of pre- and post-handwashing samples; reductions in the number of colonies present on post-handwashing samples as compared to pre-handwashing samples indicate handwashing efficacy. This method, however, is expensive to employ, requires a sterile laboratory environment, and takes at least 24 hr for microbes to grow (Hansen & Knochel, 2003). Thus, less resource-intensive methods for accurately measuring hand cleanliness are warranted.

Some studies assessing handwashing in young children have used microbial sampling measures, such as the level of fecal contamination on hands (e.g., Carabin et al., 1999; Randle et al., 2013). For example, Carabin et al. (1998) measured the average number of fecal coliforms (i.e., bacteria found in fecal matter) per pair of hands and per toy in a child care center by rinsing hands and toys in a saline solution and then filtering and incubating the saline solution to identify the particle colonies per ml. This type of outcome measure, however, does not involve directly observing children engaging in handwashing; therefore, it is unknown whether children wash their hands correctly or more often.

Incidence of Illness

Some studies addressing handwashing in young children have measured the incidence of illness or infection (e.g., Nandrup-Bus, 2009; Ponka et al., 2004; Roberts et al., 2000) rather than directly observing or measuring handwashing behavior. For example, Ponka et al. (2004) determined the effects of a handwashing intervention by measuring the number of days children were absent from a preschool program due to various illnesses, including upper respiratory infections, ear infections, pink eye, and diarrhea. One limitation to using the incidence of illness as a measure for handwashing adherence is that it is possible that variables other than handwashing may influence the outcome measure. For example, infections that result in a child being absent from school may be caused by airborne transmission of infectious repiratory droplets rather than the lack of appropriate handwashing. Additionally, as with other outcome measures, measuring the incidence of illness does not allow researchers to determine whether children wash their hands correctly or more frequently.

Image Analysis

Another method used to assess handwashing quality is image analysis of fluorescence remaining on hands following handwashing. This method involves applying an ultraviolet (UV) sensitive substance (e.g., Glo-Germ™) to participants' hands prior to handwashing and comparing the surface area of UV-illuminated areas prior to and following handwashing. Experimenters can determine an index of hand cleanliness based on the reduction in fluorescence following handwashing; the greater the reduction in post-handwashing fluorescence, the cleaner the hands. Some researchers have measured handwashing efficacy by assigning rating scores of hand cleanliness based on post-handwashing levels of illumination (e.g., Lee & Lee, 2014; Walmsley et al., 2013); that is, experimenters view participants' hands under UV light after handwashing and assign a score related to the amount of illumination remaining on hands. This visual analysis method, however, can require extensive observer training and reliability checks to ensure accurate and valid measurement. Other researchers (e.g., Deochand & Deochand, 2016; Jess et al., 2019) have used image-analysis software to quantify the amount of illumination remaining on hands; the image-analysis software can measure the surface area of illuminated areas of the hands prior to and following handwashing to determine handwashing effectiveness. One image-analysis software that has been used in behavior-analytic research is ImageJ (Jess et al., 2019). ImageJ, developed by the National Institutes of Health, can be calibrated to calculate dimensional measurements, such as the surface area of an item in an image. One limitation of this software for analyzing images of hands is that there may be surfaces of the hand (e.g., under fingernails, between fingers) that are not easily visible in an image. That is, there may be some areas of the hands where the software can not adequately measure. The utility of the ImageJ image analysis assessment method as a handwashing measurement tool should be examined by evaluating how the analysis correlates with handwashing factors that may influence hand cleanliness (e.g., duration, vigor, use of soap). That is, researchers should conduct a parametric analysis of various handwashing steps and determine the effects of those steps on the outcome of handwashing. Results of such research may inform which handwashing components are most important to teach young children.

Purpose

In an attempt to address some limitations of handwashing teaching and measurement procedures previously evaluated with young children, we conducted a series of studies in which we used Glo-Germ™ as a tool to teach handwashing to young children and as an additional measure of handwashing accuracy. In these studies, we measured the percentage of CDC handwashing steps children performed correctly and the percentage of hands illuminated by Glo-Germ™ prior to and following handwashing. In our first study, we implemented a handwashing treatment package consisting of a handwashing rule, singing a handwashing song, video modeling, and visual feedback of hand cleanliness with groups of children. Although we showed positive effects for handwashing accuracy and hand cleanliness with the handwashing treatment package, our treatment package was comprised of multiple components; thus, we did not know which treatment components were necessary and sufficient for behavior change. To address some of these limitations, we conducted a second study to evaluate the separate and combined effects of the handwashing treatment package components using an additive component analysis with groups of children. We later modified procedures to conduct treatment package sessions with participants individually and observed more robust treatment effects than when we conducted sessions with groups of participants. Given this outcome, it was unclear whether we would have observed different results of the separate handwashing treatment package components if we conducted those sessions individually. Therefore, in our third study, we conducted a component analysis of the handwashing treatment package components across five different individual participants to determine the components that were necessary and sufficient for behavior change at the individualized level.

Overall results of these three studies suggested that the entire handwashing treatment package was effective and necessary to show the largest effect for increasing accurate handwashing and hand cleanliness. We observed similar results across studies, in that moderate changes in correct handwashing resulted in large changes in post-handwashing illumination. Specifically, when correct handwashing reached approximately 60%, we observed a large decrease in the percentage of hands illuminated by Glo-Germ™ following handwashing. Therefore, to evaluate the validity of Glo-Germ™ illumination as a handwashing measurement procedure, in Study 1 of the current study we decided to conduct a retrospective analysis that involved comparing handwashing errors to post-handwashing illumination levels during baseline and treatment package sessions across all three previously conducted studies. Based on the outcomes of the retrospective analysis, we were interested in further validating the Glo-Germ™ illumination measurement procedure in the current Study 2 by evaluating the degree to which the CDC's recommended handwashing procedure (i.e., using soap to scrub all areas of both hands for 20 s) resulted in hand cleanliness with adult participants. We then conducted a parametric analysis of the handwashing steps included in the CDC handwashing procedure (i.e., duration of scrubbing, amount of vigor, use of soap, areas of hands scrubbed) to determine the components necessary and sufficient for optimal hand cleanliness.

Study 1 Method: Error Analysis

Purpose

The purpose of this study was to conduct a retrospective analysis of data collected from a series of previously conducted studies in which we used Glo-Germ™ as an additional measure of handwashing accuracy using image analysis software. We conducted a retrospective analysis of the data to evaluate the relation between handwashing accuracy and hand cleanliness by comparing errors made during handwashing to post-handwashing illumination levels during baseline and treatment package session across all three studies conducted with children.

Participants, Setting, and Materials

Participants across previously conducted Studies 1, 2, and 3 were 21 typically developing children who ranged in age from 3-years-old to 6-years-old and attended the Educare preschool program of the Edna A. Hill Child Development Center at the University of Kansas. All participants could follow multi-step instructions (e.g., “stand up and walk to the sink”) as reported by classroom supervisors. The participants in the previously conducted Study 1 (n = 10) and Study 2 (n = 6) were quasi-randomly assigned to one of three experimental groups prior to the beginning of the studies. The participants in the previously conducted Study 3 (n = 5) were not assigned to groups. Experimenters conducted one to two handwashing sessions with each participant every day the preschool classrooms were open. During the first and second studies, there were at least two children present for every handwashing session across all groups.

Experimenters conducted sessions in the library area and handwashing areas in one of the university-based preschool classrooms. Prior to some handwashing sessions, the experimenters presented an instructional video to participants using a laptop computer in the library area. During all sessions, experimenters provided a “waiting box” that contained toys and activities commonly available in the classroom (e.g., paper and crayons, toy cars, and action figures) to the participants while they waited in the library area to wash their hands. The handwashing area of the classroom contained one child-sized sink, an automatic soap dispenser, one paper towel dispenser, and one trashcan.

Experimenters used Glo-Germ™, a non-hazardous clear cosmetic lotion that illuminates under UV light, during each session. Glo-Germ™ contains substances that simulate germs found on hands prior to handwashing; the formulation requires a similar amount of effort to remove Glo-Germ™ during handwashing to that of removing most germs from the hands. Experimenters also used an alcohol-based hand sanitizer to remove remaining Glo-Germ™ on participants' hands following experimental sessions. The Glo-Germ™ and hand sanitizer were stored in a locked cabinet to prevent unsupervised child exposure. The lead experimenter built a UV-light box to illuminate the Glo-Germ™ and take pictures of participants' hands. The UV-light box was 33 cm x 24 cm x 24 cm with (a) an opening in the front for participants to place their hands, (b) a UV light inside to illuminate the Glo-Germ™, (c) an opening at the top of the box for the camera, and (d) a metric ruler on the interior bottom to calibrate the photo (Appendix A).

Response Measurement

For the series of three studies, we collected data on each participant's correctly completed handwashing steps. Experimenters collected data on correct handwashing steps completed by each participant using a pencil and the Handwashing Checklist (Appendix B), which was based on steps included in the CDC's recommendated handwashing procedure (CDC, 2020). During handwashing sessions, experimenters stood approximately 2 ft behind the participant in the handwashing area of the classroom to observe each participant washing their hands. The experimenter recorded whether the participants correctly completed each step as described on the

Handwashing Checklist on a step-by-step basis. The participant was required to perform Steps 1 and 3-8 with both hands to be scored as correct. For example, if a participant lathered the fingernails of his right hand but not his left hand, that step would be scored as incorrect on the Handwashing Checklist.

For our retrospective analysis of errors, experimenters analyzed each participant's Handwashing Checklists from each baseline and treatment package session to determine the Handwashing Checklist steps with errors. We analyzed these data at the overall level (i.e., all participants), the study level (i.e., participants in each study), and the individual level. At the overall level, for each step, we divided the number of baseline or treatment package sessions across all participants with an error by the total number of baseline or treatment package sessions across all participants. At the study level, for each step, we divided the number of baseline or treatment package sessions across participants in a study with an error by the total number of baseline or treatment package sessions across participants in a study. We also analyzed the study-level data for the final baseline and treatment package sessions using the same calculations.

In the series of studies, experimenters also measured each participant's percentage of hands illuminated by Glo-Germ™ prior to and following handwashing with ImageJ (ImageJ, 2017), a visual-editing software program (Appendix C). After dispensing Glo-Germ™ on participants' hands, experimenters took one photo of each participants' hands (one palm facing up and one palm facing down) in the UV-light box prior to and following handwashing. Data collectors uploaded each image to ImageJ and adjusted the software's measurement scale to the correct pixel-to-centimeter ratio by drawing a line over a 1 cm section of the ruler and selecting "Set Scale." Experimenters then drew around the perimeter of hands with the freehand selection tool such that the entire hands were selected, cut out the image of the hands, and pasted into a new image window. To set measurement specifications, experimenters selected “Set Measurements” and checked “Area” and “Limit to Threshold” options. First, to calculate the surface area (cm2) of the entire hands, experimenters adjusted the threshold of the image to show the entire hands, then selected “Measure” setting to analyze visible areas of the image. Then, to calculate then surface area (cm2) of the illuminated areas of hands, experimenters selected “Process” and “Make Binary” to automatically adjust the threshold of image to show only the illuminated areas of hands and selected the “Measure” setting to analyze visible areas of image. Finally, to calculate the percentage of hands illuminated by Glo-Germ™, the surface area of illuminated areas was divided by the total surface area of hands and multiplied by 100%. This process was conducted for each picture (i.e., pre- and post-handwashing) for each participant during all sessions.

For our retrospective analysis of hands illuminated, experimenters analyzed each participant’s percentage of hands illuminated pre- and post-handwashing across each baseline and treatment package session. We analyzed these data at the overall level (i.e., all participants), the study level (i.e., participants in each study), and the individual level. At the overall level, we summed the percentage of hands illuminated pre-handwashing for all participants during baseline sessions and divided by the total number of participants; these procedures were also used for post-handwashing percentages and for data from treatment package sessions. At the study level, we summed the percentage of hands illuminated pre-handwashing for participants in a study during baseline sessions and divided by the total number of participants in a study; these procedures were also used for post-handwashing percentages and for data from treatment package sessions. We also analyzed the study-level data for the final baseline and treatment package sessions using the same calculations.

Interobserver Agreement and Procedural Integrity

A second observer simultaneously but independently collected data during at least 30% of sessions across participants in each study. To calculate interobserver agreement (IOA) for correct handwashing, we divided the number of steps with agreement (i.e., both observers scored the same response for a step) by the total number of steps and multiplied by 100%. Mean IOA for Study 1, 2, and 3 was 94% (range, 90%-100%), 95% (range, 83%-100%), and 96% (range, 87%-100%), respectively.

Observers also collected procedural integrity data on experimenter implementation of treatment components during at least 30% of sessions across participants in each study. During baseline and treatment package sessions, observers recorded whether the experimenter correctly stated the handwashing rules prior to handwashing and delivered praise following correct handwashing. During treatment package sessions, observers recorded whether the experimenter presented the handwashing video model, sang the handwashing song during handwashing, and showed and described a participant’s pre- and post-handwashing pictures. To calculate procedural integrity, the number of correct procedural integrity steps were divided by the total number of procedural integrity steps and multiplied by 100%. Procedural integrity for all studies was 100%.

Procedures

General procedures were similar in all studies. Prior to each session, experimenters instructed a group of participants (Study 1 and 2) or all participants (Study 3) to walk toward the library area of the classroom. The experimenters provided the participants with the waiting box and allowed the participants to engage with the items in the box while they waited to wash their hands. The experimenters called one child at a time to walk to the sink area for their session. To determine the order in which experimenters conducted sessions with participants in each group in Study 1 and 2 and with all participants in Study 3, experimenters selected a number from a cup that corresponded to one of five pre-made data sheets. The order of participants across data sheets was different and was created using a random-list generator.

Before each participant washed their hands, the experimenter dispensed a dime-sized drop of Glo-Germ™ on both of the participant's hands and rubbed the Glo-Germ™ on all areas of both hands (i.e., between fingers, on fingernails, and on tops and palms of hands). Following the application of Glo-Germ™, the experimenter instructed the participant to place their hands into the UV light box with the right palm facing up and the left palm facing down. If a participant failed to comply with these procedures following the first instruction, the experimenter provided a model prompt for the participant to comply. That is, the experimenter showed the participant how to comply with the instruction by doing it herself. The experimenter turned on the UV light and took a photo of the participant's hands using the camera affixed to the top of the UV light box. Across all phases, if a participant correctly completed all steps of handwashing, the experimenter delivered a statement of praise to the participant following completion of handwashing (e.g., "Great job washing your hands! You did all the steps right!"). Following the participant washing his or her hands, the experimenter took the post-handwashing picture using the procedures described above. To ensure complete removal of Glo-Germ™ after the session (i.e., after the post-handwashing picture), the experimenter dispensed a dime-sized drop of hand sanitizer on the participant's hands and instructed them to rub both hands together.

After delivering hand sanitizer, the experimenter instructed the participant to return to the library area of the classroom until all sessions were complete.

Baseline

During each session in this condition, the experimenters instructed the participant to wash their hands with a reminder to use soap. That is, the experimenters said, “Walk to the sink and wash your hands. Remember to use soap and don’t rinse the soap until you are all done!” Experimenters did not provide any additional instructions or feedback to the participants during handwashing (except praise if they were to complete all handwashing steps correctly).

Treatment Package

During each session in this phase, the experimenter presented a 2.5-min handwashing video model prior to handwashing and provided visual feedback regarding hand cleanliness to the participants. The video model (see script in Appendix D) was narrated by an adult familiar to the participants (i.e., the lead experimenter) and provided a rationale for correct and frequent handwashing and a model for correct handwashing as described by the Handwashing Checklist. In the video, the narrator described why correct and frequent handwashing is important (e.g., “Washing your hands will remove germs that may get you sick!”). The video then showed images of Glo-Germ™-illuminated hands before proper handwashing (i.e., tops, bottoms, and between fingers of both hands completely illuminated) while the narrator explained that the illuminated areas depict germs that need to be washed off. The narrator described each handwashing step portrayed in child-appropriate language while engaging in that step at a sink. The narrator instructed the participants to rehearse the steps depicted and described in the video following the presentation of each step. The experimenters rehearsed each step alongside the video and verbally prompted the participants to physically rehearse each step the video depicted.

If the participants physically rehearsed the steps with the video, the experimenter intermittently provided descriptive praise to the participants (e.g., "Great job pretending to wash your hands!"). The experimenters did not provide any other attention to the participants during the video.

Following the instruction of all handwashing steps, the narrator in the video taught a 20-s song set to the tune of "Frere Jacques" that lasts the duration of correct handwashing and describes what areas of the hands the participants should wash (see song lyrics at end of Appendix D). The video then showed the adult singing the song while washing her hands according to the steps that were previously described; the narrator in the video prompted the participants to sing along with the song. Following the presentation of handwashing steps and the handwashing song, the video displayed Glo-Germ™-illuminated hands following proper handwashing (i.e., tops, bottoms, and between fingers of hands were be minimally illuminated) while the narrator explained that the illuminated areas depicted germs that were not washed off during handwashing.

The experimenter provided the waiting box to the participants after presenting the video. After taking a participant's pre-handwashing picture, the experimenter showed the picture to the participant. While presenting the pre-handwashing picture, the experimenter pointed out the areas of the participant's hands that were illuminated by Glo-Germ™ and explained that the illuminated areas represent germs that the participant needed to wash off. For example, the experimenters might have said, "Let's see what your hands look like before you wash your hands. There are germs all over the tops and bottoms of your hand. When you wash your hands, make sure you wash the germs off those areas." The experimenter then instructed the participant to wash their hands with a reminder to use soap. That is, the experimenters said, "Walk to the sink and wash your hands. Remember to use soap and don't rinse the soap until you are all done!" During handwashing, the experimenters sang the handwashing song while the participants washed their hands. The experimenter began singing the song after the participant dispensed soap; the experimenter sang the entire song even if the participant rinsed his or her hands before the experimenter had completed the song. Experimenters did not provide any other instructions or feedback to the participant (except praise if they were to complete all handwashing steps correctly).

Following handwashing, the experimenter instructed participants to place their hands back into the UV-light box to take the post-handwashing picture. The experimenter showed the post-handwashing picture to the participant and provided descriptive feedback regarding the amount of remaining illuminated Glo-Germ™ in the post-handwashing picture. That is, the experimenter described the areas of the hand that were still illuminated by Glo-Germ™ and explained to the participant that they did not wash the germs off those areas. For example, the experimenter might have said, "Look at the picture of your hands after you washed them. There are less germs than before you washed your hands, but there are still germs between your fingers and under your nails."

Study 1: Results

Results of Study 1 are depicted in Figures 1-8. Figure 1 depicts overall handwashing error data. Handwashing steps are scaled to the x-axis and the mean percentage of sessions across participants with an error is scaled to the y-axis. Data for baseline sessions are depicted by grey bars and data for treatment package sessions are depicted by white bars; error bars depict the range. During baseline, 24.2% of sessions across participants had an error on wetting hands prior to dispensing soap. Approximately 50% of sessions across participants had errors on dispensing soap, rinsing hands, and drying hands. Most sessions across participants had errors on scrubbing tops (82.1%), palms (90.2%), between fingers (91.6%), and fingernails (93.3%) of the hand. During treatment package sessions, more sessions across participants (40.4%) had an error on wetting hands prior to dispensing soap. Across all other steps, fewer sessions across participants had errors on dispensing soap (8.3%), scrubbing the tops (45.4%), palms (51.8%), between fingers (53.6%), and fingernails (62.4%), rinsing hands (18.2%), and drying hands (36%).

Figure 2 depicts handwashing error data at the study level; graphing conventions are identical to Figure 1. Data for Study 1 are shown on the top panel, data for Study 2 are shown in the middle panel, and data for Study 3 are shown in the bottom panel. Across all studies, during baseline, some sessions across participants had errors on wetting hands, dispensing soap, rinsing hands, and drying hands. During some or most sessions across participants, there were errors on scrubbing the tops, palms, between fingers, and fingernails of hands. In the treatment package, more sessions across participants in Study 1 and 3 had an error on wetting hands prior to dispensing soap. Across all three studies, fewer sessions across participants had errors on scrubbing the tops, palms, between fingers, and fingernails of the hand, rinsing hands of soap, and drying hands compared to baseline

Figure 3 depicts handwashing error data at the study level during the final baseline and treatment package sessions. Handwashing steps are scaled to the x-axis and the percentage of participants with an error is scaled to the y-axis. Across all studies, during the last baseline session, some participants made errors wetting their hands, dispensing soap, rinsing hands of soap, and drying hands. Most or all participants made errors when scrubbing the tops, palms, between fingers, and fingernails of hands. During the last treatment package session, more participants in Study 1 and 3 made an error wetting hands prior to dispensing soap. Across all three studies, fewer participants made errors scrubbing the tops, palms, between fingers, and fingernails of the hand, rinsing hands of soap, and drying hands compared to baseline. No participants made errors dispensing soap.

Figure 4 depicts overall hands illuminated pre- and post-handwashing data. Pre-handwashing percentages are depicted by grey columns and post-handwashing percentages are depicted by white columns; error bars depict the range. During baseline, the mean percentage of hands illuminated pre-handwashing was 92.1% (range, 89.2%-94.2%); the mean percentage of hands illuminated post-handwashing was 64.3% (range, 60.4%-73.8%). During the treatment package, the mean percentage of hands illuminated pre-handwashing was 93.6% (range, 90.0%-95.7%); the mean percentage of hands illuminated post-handwashing was 21.7% (range, 1.3%-40.3%).

Figure 5 depicts hands illuminated data at the study level. Across all studies, the mean percentage of hands illuminated pre-handwashing across baseline and treatment package sessions was high (*M* = 92.8%). During baseline sessions across studies, the mean percentage of hands illuminated post-handwashing decreased slightly from pre-handwashing levels (*M* = 64.3%). During treatment package sessions for each study, the mean percentage of hands illuminated post-handwashing decreased greatly from pre-handwashing levels (*M* = 21.7%).

Figure 6 depicts hands illuminated data at the study level data during the final baseline and treatment package sessions. Across all studies, during the final baseline sessions, the mean percentage of hands illuminated post-handwashing decreased slightly from pre-handwashing levels. During the final treatment package session, the mean percentage of hands illuminated post-handwashing decreased greatly from pre-handwashing levels. Our overall results suggest that there is a correlation between the number of handwashing errors and the percentage of hands illuminated by Glo-Germ™. That is, the higher number of handwashing steps with errors is correlated with a higher mean percentage of post-handwashing illumination. Additionally, when the number of errors decreases, the percentage of hands illuminated following handwashing also decreases.

Individual results for handwashing errors and hands illuminated across baseline and treatment package sessions are shown in Figures 7-9. Handwashing Checklist steps with errors are depicted by filled grey boxes on the top panels and percentages of hands illuminated pre- and post-handwashing are shown on the bottom panels; pre-handwashing percentages are depicted by closed black circles and post-handwashing percentages are depicted by open circles. Participant names are followed by the numbered study in which they participated. Figure 7 depicts the data for participants who made zero errors on scrubbing steps (i.e., Steps 3-6) during the final treatment package sessions; these graphs show data for Josh, Quentin, Ed, Larry, Max, and Beck. As shown on the top panels, results show that multiple errors occurred during baseline for Josh (M = 6), Quentin (M = 6.1), Ed (M = 6), Larry (M = 5.9), and Beck (M = 5.8); these participants made errors on Steps 2-7 (i.e., dispensing soap, scrub all areas of hands, rinse hands). Max made multiple errors (M = 4), primarily on Steps 4-6 (i.e., scrub tops, between fingers, and fingernails). As shown in the bottom panels, all six participants demonstrated only a slight decrease in post-handwashing illumination during baseline (M = 69.2%, range = 88.2% - 41.9%). During treatment package sessions, Ed made no errors. Five participants initially performed multiple handwashing steps incorrectly including Josh (M = 3.2), Quentin (M = 4), Larry (M = 3), Max (M = 2.4), and Beck (M = 2.1). After a few treatment package sessions, these five participants made fewer handwashing errors but continued to err on Steps 5 and 6 (scrub between fingers and fingernails). During the final treatment package sessions, all participants engaged in zero errors on scrubbing steps; however, two participants (Josh and Beck) made errors when wetting hands prior to dispensing soap. We observed a large change in the percentage of post-handwashing illumination during the final treatment package sessions to a mean of 10.2% (range, 2.7% – 14.2%) for all of these participants.

Figure 8 depicts the data for participants who made one to two errors on scrubbing steps (i.e., Steps 3-6) during the final treatment package sessions; these graphs show data for Kate, Garth, Ken, Ellie, Ann, Jade, and Lin. As shown on the top panels, results show that multiple errors occurred during baseline for Kate (*M* = 6.8), Garth (*M* = 6.6), Ken (*M* = 5.7), Ellie (*M* = 4.9), Ann (*M* = 4.5), Jade (*M* = 6), and Beck (*M* = 5.1). Ann made errors on Steps 4-6 (i.e., scrub tops, between fingers, and fingernails); all other participants made errors on Steps 1, 3-6, and 8 (i.e., wet hands, scrub all areas of hands, dry hands). As shown in the bottom panels, all seven participants demonstrated only a slight decrease in post-handwashing illumination during baseline (*M* = 78.2%, range = 86.6% - 47.1%). During treatment package sessions, participants continued to perform some handwashing steps incorrectly, including Kate (*M* = 3.6), Garth (*M* = 4.3), Ken (*M* = 3.9), Ellie (*M* = 2.8), Ann (*M* = 3.1), Jade (*M* = 3.4), and Beck (*M* = 3.3). During the final treatment package sessions, these participants made fewer handwashing errors but continued to err on Steps 5 and 6 (scrub between fingers and fingernails). We observed a moderate change in the percentage of post-handwashing illumination during the final treatment package sessions to a mean of 15.9% (range, 4.6% – 20.2%) for Kate, Garth, Ken, Ellie, Ann, and Lin. The data for Jade show that although he made some errors during scrubbing steps in the treatment package, we did not observe a large change in post-handwashing illumination to a mean of 40.6%. This may be due to other handwashing variables (e.g., duration, vigor) on which we did not collect data during these studies.

Figure 9 depicts the data for participants who made multiple errors on scrubbing steps (i.e., steps 3-6) during the final treatment package sessions; these graphs show data for Andy, Lisa, Tad, Jay, Hudson, Mel, Mark, and Briggs. As shown on the top panels, results show that multiple errors occurred during baseline for Andy (M = 3.6), Lisa (M = 5.2), Tad (M = 7), Jay (M = 5.6), Hudson (M = 6.3), Mel (M = 5), Mark (M = 6), and Briggs (M = 5.5). Andy made errors on Steps 3-6 (i.e., scrub palms, tops, between fingers, and fingernails). Tad, Jay, Hudson, Mel, and Mark made errors on Steps 2-7 (i.e., dispensing soap, scrub all areas of hands, rinse hands). Lisa and Briggs made errors on Steps 1, 3-6, and 8 (i.e., wet hands, scrub all areas of hands, dry hands). As shown in the bottom panels, all eight participants demonstrated only a slight decrease in post-handwashing illumination during baseline (M = 69.9%, range = 87.1% - 41.8%). During treatment package sessions, participants continued to perform multiple handwashing steps incorrectly, including Andy (M = 3.3), Lisa (M = 4.1), Tad (M = 4.7), Jay (M = 4.8), Hudson (M = 4), Mel (M = 3.9), Mark (M = 4.5), and Briggs (M = 5). We observed a moderate decrease in the percentage of post-handwashing illumination during treatment package sessions to a mean of 21.6% (range, 4.1% – 36.3%) for six of these participants (Tad, Jay, Hudson, Mel, Mark, and Briggs); we observed a minimal decrease in post-handwashing illumination for Andy and Lisa to a mean of 55.2% (range, 39.9% – 73%).

Study 2 Method: Utility of Image Analysis Method

Purpose

The purpose of Study 2 was two-fold. First, we evaluated the degree to which the CDC handwashing procedure (i.e., using soap to scrub all areas of both hands for 20 s) resulted in hand cleanliness using post-handwashing image analysis of fluorescence. Then, we conducted a parametric analysis of the handwashing steps included in the CDC procedure (i.e., duration of scrubbing, amount of vigor, use of soap, areas of hands scrubbed) to determine the components necessary and sufficient for optimal hand cleanliness.

Participants, Setting, and Materials

A convenience sample of 12 adults associated with the Edna A. Hill Child Development Center at the University of Kansas participated in the current study. We chose to include adult participants in this evaluation for several reasons. First, because our measures were highly sensitive to variations in procedural integrity, adults were more likely to perform the specific and varied handwashing components with high procedural integrity. Second, our procedures included manipulations of decreased handwashing integrity. Thus, we did not want to inadvertently teach poor handwashing skills to young children. Participants included two undergraduate students, six graduate students, one faculty member, and three staff members; participants ranged in age from 21 to 38 years. Two participants were male and the remaining participants were female. The lead experimenter included a brief questionnaire regarding skin sensitivities with the informed consent documentation (Appendix E-F) to all potential participants. Individuals who reported sensitivity to UV light or ingredients contained in Glo-Germ™ or cracked or broken skin were to be excluded from participating due to potential risk for dermatological irritation or reaction. No participants were excluded due to their responses on the skin sensitivity questionnaire.

This study received approval from the Human Research Protection Program (HRPP) of the University of Kansas to conduct in-person research with added health and safety measures during the COVID-19 pandemic (Appendix G). Prior to and following each session, experimenters sprayed an Environmental Protection Agency (EPA) approved odorless sanitizer on all surfaces of the sink area (i.e., faucet handles, countertops, soap dispenser, paper towel dispenser) and wiped dry with a paper towel. All experimenters, participants, and observers wore cloth or surgical face masks and plastic face shields during all sessions. Experimenters wore disposable latex-free gloves during all sessions and disposed of gloves in a trash can immediately following each session.

Experimenters conducted sessions with individual participants in a private sink area adjacent to a conference room in the child development center. The sink area contained one sink, one push-style foaming soap dispenser, one paper towel dispenser, and a trash can. Experimenters posted condition-specific Handwashing Checklists (Appendix H-P) in a plastic page protector above the sink and video recorded all sessions using an iPod. Experimenters used a pencil and condition-specific Handwashing Checklist to record procedural integrity data from video-recorded sessions. The lead experimenter created a 1-min video model for each experimental condition. Each condition-specific video model depicted the lead experimenter washing her hands at a sink according to the steps described in the corresponding Handwashing Checklist. All videos showed the experimenter turning on the water and wetting hands under the stream of water for 5 s, and drying hands with a paper towel for 5 s. The Correct Handwashing video showed the experimenter dispensing soap, scrubbing the tops, palms, between fingers, and fingernails of both hands with moderate vigor for 20 s, and rinsing hands under the water for 5 s. The No Soap video showed the experimenter scrubbing the tops, palms, between fingers, and fingernails of both hands with moderate vigor for 20 s. The Light Vigor video showed the experimenter dispensing soap, scrubbing the tops, palms, between fingers, and fingernails of both hands with low vigor for 20 s, and rinsing hands under the water for 5 s; the High Vigor video was similar to Low Vigor except the experimenter scrubbed hands with high vigor. The 10 Seconds video showed the experimenter dispensing soap, scrubbing the tops, palms, between fingers, and fingernails of both hands with moderate vigor for 10 s, and rinsing hands under the water for 5 s; the 5 Seconds video was similar to 10 Seconds except the experimenter scrubbed hands for 5 s. The Missing Tops video showed the experimenter dispensing soap, scrubbing the, palms, between fingers, and fingernails of both hands with moderate vigor for 20 s, and rinsing hands under the water for 5 s. The Missing Between Fingers video showed the experimenter dispensing soap, scrubbing the tops, palms, and fingernails of both hands with moderate vigor for 20 s, and rinsing hands under the water for 5 s. Finally, the Missing Fingernails video showed the experimenter dispensing soap, scrubbing the tops, palms, and between fingers of both hands with moderate vigor for 20 s, and rinsing hands under the water for 5 s. Experimenters used a laptop computer to present the condition-specific video models to participants prior to and during each session.

Experimenters used Glo-Germ™ as described in Study 1. The experimenters replaced the original Glo-Germ™ lid with clean pump-top lid from a lotion bottle to control for the amount of Glo-Germ™ dispensed (i.e., approximately ½ tsp per pump). Experimenters used a soft shell, foldable UV-light box (Appendix Q) to take pictures of participants' hands illuminated by Glo-Germ™ prior to and following handwashing. The UV-light box was a 30cm x 30cm x 30cm fabric box with (a) an opening in the front for participants to place their hands, (b) an opening at the top of the box for a UV light to illuminate Glo-Germ™, (c) an opening at the top of the box for an iPod to take pictures, and (d) a metric ruler on the interior bottom to calibrate the photo.

Image and Data Analysis

Image analysis procedures were similar to those described in Study 1; however, experimenters took two photos of each participant's hands (one photo with both palms facing up and one photo with both palms facing down) in the UV-light box prior to and following handwashing. We used these conservative image analysis measures because we were interested in collecting more complete and accurate data on hand cleanliness. Data for the percentage of hands illuminated per condition were analyzed at the individual and group level. At the individual level, experimenters depicted the percentage of hands illuminated for all four pictures (i.e., pre-palms up, pre-palms down, post-palms up, post-palms down) on a session-by-session basis for each individual participant. At the group level, experimenters used GraphPad Prism software to determine the mean, standard deviation, and correlation coefficient of pre- and post-handwashing illumination across participants for each condition; we used these data to calculate the within-subjects effect size of each condition. The within-subjects effect size corresponds to the effect of the intervention between pre- and post-handwashing illumination; that is, the larger the effect size, the larger the difference between pre- and post-handwashing illumination.

Procedural Integrity & Interobserver Agreement

Data collectors observed and recorded participant and experimenter behavior using a condition-specific data sheet and pencil during video-recorded sessions. Observers recorded whether a participant completed a step (a) independently correct, (b) correctly after receiving feedback, (c) incorrectly after receiving feedback, or (d) incorrectly without any feedback. To calculate procedural integrity, the number of handwashing steps completed correctly, regardless of receiving feedback, were divided by the total number of handwashing steps and multiplied by 100%. A second observer independently recorded data for at least 30% of sessions for all participants. Interobserver agreement was calculated by dividing the number of handwashing steps with an agreement (i.e., both observers scored the same response) by the total number of handwashing steps and multiplying by 100%. Interobserver agreement for all sessions across all participants was 100%.

Procedures

Experimenters conducted two sessions of each condition with all participants. The order of conditions were counterbalanced across participants; for each participant, the lead experimenter typed all condition names into a random list generator and used the outcome to determine condition order. Each session lasted approximately 5 min. Experimenters scheduled all research sessions for particular participants for the same time of day. For example, all sessions with Participant A occurred at 9:00 am and all sessions with Participant B occurred at 1:30 pm. Prior to all sessions, experimenters instructed participants to remove any hand jewelry (e.g., rings, bracelets, watches) prior to delivering Glo-Germ™ to reduce variability of images across participants. The experimenters put on gloves, dispensed one pump of Glo-Germ™ onto one of the participant's palms, and instructed them to rub the substance over all surfaces of both hands (i.e., palms, tops, between fingers, on fingernails); experimenters provided feedback if areas of the hand were not rubbed with Glo-Germ™. That is, the experimenter would instruct the participant to continue rubbing their hands together to spread Glo-Germ™ across all areas. Participants then placed their hands into the UV-light box with both palms facing up and the experimenter took the first pre-handwashing photo. Next, the experimenter instructed participants to turn their hands over with both palms facing down and took the second pre-handwashing photo. After the participant removed their hands from the UV light box, the experimenter wiped the inside of the UV light box with a paper towel to remove any Glo-Germ™ that may have transferred from the participant's hands.

After taking the pre-handwashing pictures, the experimenter provided the participant with a copy of the condition-specific Handwashing Checklist and read the checklist items aloud while the participant looked at the checklist. Participants then had an opportunity to review the Handwashing Checklist and ask the experimenter questions for 2 min or until the participant stated readiness to begin. Next, the experimenter positioned the laptop in front of the participant and presented the condition-specific handwashing video model; the experimenter described the handwashing steps depicted in the video. During this initial video model presentation, the experimenter instructed the participant to rehearse the handwashing steps depicted by the video as they were displayed on the screen. After the initial video model presentation, the experimenter positioned the laptop near the sink to play the video model during the handwashing observation. The experimenter instructed the participant to walk to the sink, started the video model, and instructed the participant to wash their hands following the steps with the video. During handwashing, if a participant performed any step of the condition-specific Handwashing Checklist incorrectly, the experimenter immediately provided vocal feedback describing the error and instruct the participant to perform the step correctly. For example, if a participant only scrubbed the top of the right hand during the Correct Handwashing condition, the experimenter would say, “Make sure you scrub the top of your left hand before rinsing off the soap.”

Following handwashing, the experimenter instructed participants to place their hands into the UV-light box with both palms facing up and the experimenter took the first post-handwashing photo. Next, the experimenter instructed participants to turn their hands over with both palms facing down and took the second post-handwashing photo. Experimenters did not show participants their pre- or post-handwashing pictures.

Correct Handwashing

Experimenters instructed participants to wash hands using all aspects of correct handwashing. That is, the Correct Handwashing Checklist (Appendix H) steps included wetting both hands under running water (any temperature) from wrist to tip, dispensing one pump of soap, scrubbing all areas of the hands (i.e., rub tops of each hand with palm of opposite hand, rub palms of both hands together, rub between fingers with fingers of opposite hand, scrub fingernails of each hand in palm of opposite hand) outside the stream of water for 20 s, rinsing soap off of hands under running water for 5 s, and drying hands with paper towels for 5 s. The video model provided included all steps, and feedback was provided if any step was not initially completed.

No Soap

Experimenters instructed participants to wash hands using all aspects of correct handwashing except dispensing soap and rinsing soap off of hands. That is, the No Soap Handwashing Checklist (Appendix I) steps included wetting both hands under running water (any temperature) from wrist to tip, scrubbing all areas of the hands (i.e., rub tops of each hand with palm of opposite hand, rub palms of both hands together, rub between fingers with fingers of opposite hand, scrub fingernails of each hand in palm of opposite hand) outside the stream of water for 20 s, and drying hands with paper towels for 5 s. The video model provided included all steps except dispensing soap and rinsing soap off of hands, and feedback was provided if any step was not initially completed. Experimenters removed the soap dispenser during this condition to promote procedural integrity.

Light Vigor

Experimenters instructed participants to wash hands using all aspects of correct handwashing using the least amount of force necessary to spread soap across the hands. That is, the Light Vigor Handwashing Checklist (Appendix J) steps included wetting both hands under running water (any temperature) from wrist to tip, dispensing one pump of soap, scrubbing all areas of the hands (i.e., rub tops of each hand with palm of opposite hand, rub palms of both hands together, rub between fingers with fingers of opposite hand, scrub fingernails of each hand in palm of opposite hand) outside the stream of water using light vigor for 20 s, rinsing soap off of hands under running water for 5 s, and drying hands with paper towels for 5 s. The video model provided included all steps, and feedback was provided if any step was not initially completed or if participants scrubbed their hands too vigorously.

High Vigor

Experimenters instructed participants to wash hands using all aspects of correct handwashing using strong force to vigorously scrub their hands. That is, the High Vigor Handwashing Checklist (Appendix K) steps included wetting both hands under running water (any temperature) from wrist to tip, dispensing one pump of soap, scrubbing all areas of the hands (i.e., rub tops of each hand with palm of opposite hand, rub palms of both hands together, rub between fingers with fingers of opposite hand, scrub fingernails of each hand in palm of opposite hand) outside the stream of water using high vigor for 20 s, rinsing soap off of hands under running water for 5 s, and drying hands with paper towels for 5 s. The video model provided included all steps, and feedback was provided if any step was not initially completed or if participants did not use enough vigor to scrub their hands.

Ten Seconds

Experimenters instructed participants to wash hands using all aspects of correct handwashing and scrub for only 10 s. That is, the Ten Seconds Handwashing Checklist (Appendix L) steps included wetting both hands under running water (any temperature) from wrist to tip, dispensing one pump of soap, scrubbing all areas of the hands (i.e., rub tops of each hand with palm of opposite hand, rub palms of both hands together, rub between fingers with fingers of opposite hand, scrub fingernails of each hand in palm of opposite hand) outside the stream of water for 10 s, rinsing soap off of hands under running water for 5 s, and drying hands with paper towels for 5 s. The video model provided included all steps and depicted scrubbing for 10 s, and feedback was provided if any step was not initially completed or if the participant scrubbed their hands for longer than 10 s.

Five Seconds

Experimenters instructed participants to wash hands using all aspects of correct handwashing and scrub for only 5 s. That is, the Five Seconds Handwashing Checklist (Appendix M) steps included wetting both hands under running water (any temperature) from wrist to tip, dispensing one pump of soap, scrubbing all areas of the hands (i.e., rub tops of each hand with palm of opposite hand, rub palms of both hands together, rub between fingers with fingers of opposite hand, scrub fingernails of each hand in palm of opposite hand) outside the stream of water for 5 s, rinsing soap off of hands under running water for 5 s, and drying hands with paper towels for 5 s. The video model provided included all steps and depicted scrubbing for 5 s, and feedback was provided if any step was not initially completed or if the participant scrubbed their hands for longer than 5 s.

Missing Tops

Experimenters instructed participants to wash hands using all aspects of correct handwashing except scrubbing the tops of the hands. That is, the Missing Tops Handwashing Checklist (Appendix N) steps included wetting both hands under running water (any temperature) from wrist to tip, dispensing one pump of soap, scrubbing all areas except the tops of the hands (i.e., rub palms of both hands together, rub between fingers with fingers of opposite hand, scrub fingernails of each hand in palm of opposite hand) outside the stream of water for 20 s, rinsing soap off of hands under running water for 5 s, and drying hands with paper towels for 5 s. The video model provided included all steps except scrubbing the tops of the hands, and feedback was provided if any step was not initially completed or if the participant scrubbed the tops of the hands.

Missing Between Fingers

Experimenters instructed participants to wash hands using all aspects of correct handwashing except scrubbing between fingers. That is, the Missing Between Fingers Handwashing Checklist (Appendix O) steps included wetting both hands under running water (any temperature) from wrist to tip, dispensing one pump of soap, scrubbing all areas except between fingers of the hands (i.e., rub tops of each hand with palm of opposite hand, rub palms of both hands together, scrub fingernails of each hand in palm of opposite hand) outside the stream of water for 20 s, rinsing soap off of hands under running water for 5 s, and drying hands with paper towels for 5 s. The video model provided included all steps except scrubbing between fingers of the hands, and feedback was provided if any step was not initially completed or if the participant scrubbed between the fingers.

Missing Fingernails

Experimenters instructed participants to wash hands using all aspects of correct handwashing except scrubbing fingernails. That is, the Missing Fingernails Handwashing Checklist (Appendix P) steps included wetting both hands under running water (any temperature) from wrist to tip, dispensing one pump of soap, scrubbing all areas except fingernails of the hands (i.e., rub tops of each hand with palm of opposite hand, rub palms of both hands together, rub between fingers with fingers of opposite hand) outside the stream of water for 20 s, rinsing soap off of hands under running water for 5 s, and drying hands with paper towels for 5 s. The video model provided included all steps except scrubbing the fingernails of the hands, and feedback was provided if any step was not initially completed or if the participant scrubbed the fingernails.

Study 2: Results

Results for the pre- and post-handwashing percentages of hands illuminated for all participants across all conditions in Study 2 are shown in Figure 10. Illumination percentages for the tops of hands for each participant are depicted by upward triangles and illumination percentages for the palms of hands for each participant are depicted by downward triangles. Before handwashing, the mean percentage of tops and palms of hands illuminated by Glo-Germ™ across all conditions was high, ranging between 94.8%-97.2%. Following correct handwashing, the mean percentage of tops and palms of hands illuminated across participants was 3.9% and 2.4%, respectively. Following handwashing without soap, the mean percentage of tops and palms of hands illuminated across participants was 24.9% and 17.2%, respectively. Following handwashing with low vigor, the mean percentage of tops and palms of hands illuminated across participants was 37.1% and 23.7%., respectively Following handwashing with high vigor, the mean percentage of tops and palms of hands illuminated across participants was 1.8% and 1.3%, respectively. Following handwashing for 10 s, the mean percentage of tops and palms of hands illuminated across participants was 30.8% and 21.4%, respectively. Following handwashing for 5 s, the mean percentage of tops and palms of hands illuminated across participants was 45.8% and 28.8%, respectively. Following handwashing missing tops of hands, the mean percentage of tops and palms of hands illuminated across participants was 45 % and 4.5%, respectively. Following handwashing missing between fingers, the the mean percentage of tops and palms of hands illuminated across participants was 8.2% and 3%, respectively. Finally, following handwashing missing fingernails, the mean percentage of tops and palms of hands illuminated across participants was 8.9% and 3.2%, respectively. Overall, results from Study 2 suggest vigorously scrubbing all areas of the hands (i.e., rub tops of each hand with palm of opposite hand, rub palms of both hands together, rub between fingers with fingers of opposite hand, scrub fingernails of each hand in palm of opposite hand) with soap for 20 s results in the largest change in cleanliness from pre-handwashing levels.

Procedural integrity data for all participants across all conditions in Study 2 are shown in Figure 11. Conditions are scaled to the x-axis and the mean percentage of procedural integrity is scaled to the y-axis. During all conditions except 5 Seconds, the mean percentage of procedural integrity was 100%; that is, all participants correctly completed all handwashing steps in these conditions. During the 5 Seconds condition, the mean percentage of procedural integrity was 89.9%. In this condition, two participants made errors of omission during Step 6, in which both participants did not scrub the fingernails of one hand. This was likely due to the short duration in which the participants had to scrub all areas of the hands.

The within-subjects effect size for Correct Handwashing was 35.01. The effect size for No Soap was 27.06. The effect sizes for Light Vigor and High Vigor were 12.19 and 55.92, respectively. The effect sizes for 10 s and 5 s were 14.99 and 8.65, respectively. Finally, the effect sizes for Missing Tops, Missing Between Fingers, and Missing Fingernails were 21.03, 30.44, and 32.41, respectively. These results suggest the largest effects for the Correct Handwashing and High Vigor conditions, as well as the smallest effects for the Light Vigor, 10 s, and 5 s conditions.

Across all participants in Study 2, the smallest decrease in mean percentage of tops and palms illuminated was observed following handwashing with light vigor and the largest decrease was observed after handwashing with high vigor. These findings are not surprising, given the results of previous research evaluating the importance of friction to remove pathogens from hands (e.g., Hoque, 2003; Luby et al., 2007). Across all conditions for most participants, the percentage of illumination of palms was lower than the tops of hands. This may be due to the multiple handwashing components performed with the palms; that is, the palms of hands are used to scrub the tops and fingernails of the opposite hand, which may result in the removal of more Glo-Germ™ from the palms.

General Discussion

The purpose of Study 1 was to conduct a retrospective analysis of data collected from a series of studies in which we used Glo-Germ™ as an additional measure of handwashing accuracy using image analysis software. We conducted a retrospective analysis of the data to evaluate the relation between handwashing accuracy and hand cleanliness by comparing errors made during handwashing to post-handwashing illumination levels during baseline and treatment package session across all three studies conducted with children. Overall results of our retrospective analysis showed that the handwashing treatment package was effective for reducing the number of errors during handwashing and decreasing post-handwashing illumination with all participants. For six participants, the treatment packpage was highly effective for reducing errors during handwashing and decreasing post-handwashing illumination. For seven participants, the treatment package was moderately effective for reducing handwashing errors and decreasing post-handwashing illumination. For the remaining eight participants, the treatment package reduced most handwashing errors; however, these participants continued to make errors on important handwashing steps (e.g., scrubbing most areas of the hands) and demonstrated only a moderate decrease in post-handwashing illumination.

The results of Study 1 yielded several interesting findings. One finding is that some participants who made only few handwashing errors demonstrated a small decrease in post-handwashing illumination. For example, during treatment package sessions in which Jade, Ann, Lin, and Andy made errors scrubbing between fingers and fingernails, post-handwashing illumination percentages ranged from 42.6%-60.3%, 30.3%-39.7%, 28.3%-32.2%, and 40.1%-47.2%, respectively. This finding suggests that these participants may have scrubbed the tops and palms of hands for a short duration or with low vigor. Another interesting finding is that some participants who continued to make errors on important handwashing steps during treatment package sessions demonstrated a large decrease in post-handwashing illumination. For example, during the last treatment package session, Jay, Hudson, and Mel made errors scrubbing the tops of hands, between fingers, and fingernails, but post-handwashing illumination percentages were 3.6%, 9.2%, and 11.1%, respectively. This finding suggests that these participants may have scrubbed these areas of the hands after rinsing soap, only scrubbed these areas of one hand, or scrubbed the palms of hands for a long duration or with high vigor. Another interesting finding is that, during treatment package sessions, many participants across all three studies made an error on Step 1 (i.e., wetting hands before dispensing soap). For Step 1 to be scored as correct, the participant had to complete the step prior to Step 2 (i.e., dispensing soap). Participants may have failed to complete Step 1 correctly because, prior to handwashing, the experimenter reminded the participants to use soap during handwashing. This reminder may have served as a prompt for the participants to immediately dispense soap prior to wetting their hands under the water.

Based on the outcomes of the retrospective analysis, we were interested in further validating the Glo-Germ™ illumination measurement procedure with adult participants. The purpose of Study 2 was to first evaluate the degree to which the CDC's recommended handwashing procedure (i.e., using soap to scrub all areas of both hands for 20 s) resulted in hand cleanliness. We then conducted a parametric analysis of the handwashing steps included in the CDC handwashing procedure (i.e., duration of scrubbing, amount of vigor, use of soap, areas of hands scrubbed) to determine the components necessary and sufficient for optimal hand cleanliness. For all participants, handwashing during the correct and high vigor conditions produced the most substantial change in post-handwashing illumination, whereas handwashing with low vigor, shortened durations (i.e., 10 s and 5 s), and without soap produced the least amount of change in post-handwashing illumination. Further, missing scrubbing some areas of the hands, including between fingers and fingernails, showed similar results to correct handwashing. Our results suggest missing scrubbing some areas of the hand during handwashing does not affect hand cleanliness as much as the amount of vigor, duration, and use of soap.

The results of Study 2 yielded several interesting findings. One finding is that in the Correct Handwashing and High Vigor conditions, we observed very little variability of post-handwashing illumination for tops and bottoms of hands across participants. This may be due to participants engaging in similar handwashing procedures when washing their hands outside of the experimental sessions. That is, if participants typically wash their hands using procedures similar to our Correct Handwashing procedures in their daily environments, they would have repeated practice with those procedures. Another interesting finding is that we observed greater variability of post-handwashing illumination for tops and bottoms of hands in the No Soap, Light Vigor, 10 Seconds, and 5 Seconds conditions across participants, as well as for tops of hands in the Missing Tops, Missing Between Fingers, and Missing Fingernails conditions across participants. This finding may be due to the novelty of the handwashing procedures.

These studies support outcomes of previous research and recommendations from healthcare agencies regarding the importance of different parameters of handwashing, including duration, use of soap, amount of vigor, and areas scrubbed, to increase an index of hand cleanliness (CDC, 2015; Fuls et al., 2008; Jensen et al., 2015; WHO, 2009). With respect to the applied implications of these studies, these results suggest that caregivers (e.g., parents, teachers) may not need to direct their efforts to teaching young children the entire best-practice handwashing procedure to produce appropriate hand cleanliness. Instead, based on our results of our evaluations showing that scrubbing between fingers and fingernails may be less important for influencing hand cleanliness, caregivers may wish to teach young children to, at minimum, use soap and vigorously scrub the tops and bottoms of the hands for 20 s during handwashing. It is important to note, however, that any infectious particles remaining on hands may lead to infection. That is, if an individual does not wash off dangerous substances or particles from hands and touches their eyes, nose, or mouth, they could aquire an infection.

These studies also offer support for the use of image analysis of post-handwashing illumination as a handwashing measurement tool. Although the image analysis procedures we used require some training, effort, and time to perform, the methods are not as limited as conducting microbial sampling in the daily environment. In particular, results from the image analysis procedures for Study 2 took approximately 20 min per participant, whereas results from microbial sampling can take approximately 24 hr after sample collection. Thus, compared to other handwashing measurement procedures, image analysis of post-handwashing illumination is a practical and efficacious measurement tool, particularly if used on a probe basis. Additionally, if the image analysis procedures are used in conjunction with visual feedback to teach handwashing to young children, experimenters could both assess hand cleanliness and provide visual feedback of post-handwashing illumination on an intermittent schedule.

One limitation across our studies is that we used different procedures to capture our hands illuminated data. In Study 1, we only captured a sample of hand cleanliness; that is, because the participants placed hands with one palm up and one palm down for the pre- and post-handwashing pictures, we did not assess complete hand cleanliness following handwashing. It is possible that we may have observed different results if we had used a more conservative measure of hand cleanliness, such as taking pictures of the tops and palms of both hands as done in Study 2. In Study 2, we used these conservative image analysis measures because we were interested in determining more complete and accurate data on hand cleanliness. Additionally, we included adult participants in Study 2 because our measures were highly sensitive to variations in procedural integrity and adults were more likely to perform the specific and varied handwashing components with high procedural integrity. Additional evaluations should compare levels of post-handwashing illumination from samples of hands (as done in Study 1) and separate images of tops and palms of hands (as done in Study 2) to determine the outcomes of using the various methods.

One limitation of our studies with young children is that our measurement system did not capture complete effects of all relevant handwashing variables on hand cleanliness. First, our criteria for correct handwashing may have been too stringent. For Step 1 (i.e., wet hands), the participant had to complete the step prior to Step 2 (i.e., dispense soap) to be scored as correct. For example, if a participant dispensed soap before wetting their hands under the water, Step 1 would be scored as incorrect. For most steps, the participant had to complete the step with both hands for that step to be scored as correct. For example, if a participant scrubbed the nails of the right hand but not the left, Step 6 would be scored as incorrect. Additionally, for all scrubbing steps, the participant had to keep soap on their hands and not rinse it off while completing the step to be scored as correct. For example, if a participant rinsed the soap off their hands and continued scrubbing between their fingers, Step 5 would be scored as incorrect. Second, across our studies with young children, we did not collect data on the duration of scrubbing or the level of vigor used to scrub hands. Thus, it is possible that a participant who made few errors during handwashing and scrubbed with low vigor or for a short duration had a high percentage of post-handwashing illumination. Similarly, it is possible that a participant who made multiple errors during handwashing but scrubbed with high vigor or for a long duration had a low percentage of post-handwashing illumination.

One limitation of Study 2 is that we did not conduct a complete parametric analysis of best-practice handwashing procedures. First, we chose to assess the variables included in our study based on factors we hypothesized may influence the cleanliness of hands. That is, we manipulated some variables, including duration of scrubbing, amount of vigor, use of soap, and handwashing steps completed, but we did not evaluate those manipulations in conjunction with other variables. For example, we evaluated the effects of handwashing with low vigor using soap for 20 s but not handwashing with low vigor without soap or low vigor for shorter durations. Although individuals may routinely wash their hands using a combination of these manipulated variables, it is unlikely that the combined manipulations of decreased handwashing integrity would produce optimal hand cleanliness. Second, we did not include other handwashing steps that some healthcare agencies suggest in their handwashing procedures. For example, some agencies suggest individuals use a paper towel to turn off the faucet, whereas other agencies suggest alternative drying methods, such as air-drying. Third, we did not assess all combinations of handwashing errors that children made in our previous studies. For example, results of our error analysis showed that multiple children did not wet their hands prior to dispensing soap and some children did not completely rinse their hands of soap. However, we did not evaluate the effects of handwashing with soap dispensed on dry hands or failing to rinse hands of soap. Finally, we did not evaluate the isolated effects of drying hands on post-handwashing illumination. That is, we did not assess varied durations or vigor of drying or the effects of only wiping hands with a paper towel without handwashing. Given the importance of friction for removing pathogens from hands (Hoque, 2003; Luby et al., 2007), it is possible that the friction created by drying hands with a paper towel could remove Glo-Germ™ from hands. Overall, it is possible that these other handwashing steps and factors may affect the cleanliness of hands; thus, future research may wish to extend the current study by conducting a more thorough parametric analysis of handwashing variables manipulated in this study, as well as other handwashing variables we did not manipulate.

Another limitation of this study is that some individual differences of hands may have affected our hands illuminated data. Although we included a hand sensitivity questionnaire for participants to self-disclose potential irritation risks, we did not control for several various individual differences with respect to participants' hands. First, it is possible that Glo-Germ™ is more difficult to wash from dry skin. That is, because the formulation of Glo-Germ™ is similar to lotion, it is possible that some Glo-Germ™ may be absorbed into the skin or wrinkles of dry hands moreso than hydrated skin. Second, individuals with deep nailbeds and long fingernails may have some Glo-Germ™ remaining in the nailbeds and under nails following handwashing. It is possible that Glo-Germ™ is difficult or impossible to remove from deep nailbeds or long fingernails without the use of a nailbrush to scrub those areas. Finally, substances or materials other than Glo-Germ™ could be illuminated by UV light, which may have influenced our hands illuminated data. For example, some nailpolish formulations (e.g., gel polish) or colors (e.g., hot pink) can illuminate brightly under UV light. We did not require participants to remove nailpolish prior to participating in the study; however, future evaluations may wish to exclude participants with painted fingernails to address this limitation.

A final limitation of this study is that some handwashing variables we assessed were difficult to accurately measure through direct observation methods. Some variables, such as use of soap and duration of scrubbing, were easily observable. That is, data collectors could observe a participant dispensing soap and begin a duration measure when a participant began scrubbing. Other variables, such as the amount of vigor used to scrub the hands, were less easily observable and measurable. One way to address this challenge would be to code whether an increase in soap suds was observed during scrubbing to measure vigor. That is, an increase in the amount of soap suds would indicate a high amount of vigor used to scrub the hands. Another solution could include more pre-session training regarding the amount of vigor to use. For example, experimenters could rehearse handwashing using high or low vigor with participants and use additional materials to train this variable prior to the session. For rehearsing low vigor, experimenters could provide participants a piece of high-grit sandpaper and instruct participants to use the least amount of force to make as few scratches as possible on a plastic surface. For rehearsing high vigor, experimenters could instruct participants to use a great amount of force to make as many scratches as possible on a plastic surface. Future research regarding the most effective training and data collection procedures for these types of variables is warranted.

There are several additional areas where research is warranted. First, an additional avenue for research is to evaluate possible post-handwashing contamination of surfaces. Because Glo-

Germ™ remaining on children's hands may transfer to other surfaces (e.g., toys, tabletops, other individuals), researchers could use UV-light to inspect classroom surfaces and hands of other individuals that have been "contaminated" with Glo-Germ™ due to poor handwashing integrity. Additionally, researchers could apply Glo-Germ™ to a classroom surface and evaluate the spread of contamination across children and other surfaces. That is, after covertly applying Glo-Germ™ to a surface and allowing individuals to naturally come into contact with the surface, researchers use UV-light to inspect the hands of children and staff to determine the spread of "contamination" This type of evaluation could be used as a training procedure to show children and teachers how quickly contamination across materials and individuals can spread in a classroom. If used in conjuction with a handwashing training procedure, this evaluation could also determine the effects of frequent and effective handwashing on potential contamination.

Second, future evaluations regarding outcomes of different image analysis procedures and software are warranted. Researchers may be interested in not only measuring the surface area of illuminated areas, but also the brightness of illuminated areas. Brightly illuminated areas may suggest areas that were not scrubbed during handwashing, whereas dimly illuminated areas may suggest areas of the hands that were scrubbed with low vigor or without soap. This type of measure could allow researchers to identify and measure areas of the hands that were scrubbed inadequately during handwashing. This measure could also be used to provide visual feedback to individuals regarding the difference between appropriate and inadequate handwashing. Additionally, because some surfaces of the hand (e.g., under fingernails, between fingers, between wrinkles or crevices in skin) are not easily visible in a two-dimensional image, there may be some areas of the hands where the software can not adequately measure illumination. It is possible that other image analysis software (e.g., Adobe Photoshop) may allow for more indepth analyses of illumination of not easily visible areas in images of Glo-Germ™-illuminated hands.

References

Aiello, A. E., Coulborn, R. M., Perez, V., & Larson, E. L. (2008). Effect of hand hygiene on infectious disease risk in the community setting: A meta-analysis. *American Journal of Public Health*, *98*(8), 1372–1381. https://doi.org/10.2105/ajph.2007.124610

Alberto, P. A., Taber, T. A., & Fredrick, L. D. (1999). Use of self-operated auditory prompts to decrease aberrant behaviors in students with moderate mental retardation. *Research in Developmental Disabilities*, *20*(6), 429–439. https://doi.org/10.1016/s0891-4222(99)00023-2

Aronson, S. S. & Shope, T. R. (Eds.). (2019). *Managing infectious diseases in child care and schools (*5th ed.). American Academy of Pediatrics.

Au, W. H., Suen, L. K. P., & Kwok, Y. L. (2009). Handwashing programme in kindergarten: A pilot study. *Health Education, 110*, 5-16. https://doi.org/10.1108/09654281011008717

Burton, M., Cobb, E., Donachie, P., Judah, G., Curtis, V., & Schmidt, W.-P. (2011). The effect of handwashing with water or soap on bacterial contamination of hands. *International Journal of Environmental Research and Public Health*, *8*(1), 97–104. https://doi.org/10.3390/ijerph8010097

Carabin, H., Gyorkos, T. W., Soto, J. C., Joseph, L., Payment, P., & Collet, J. P. (1999). Effectiveness of a training program in reducing infections in toddlers attending day care centers. *Epidemiology*, *10*, 219-227.

Carey, R. G. & Bucher, B. (1981). Identifying the educative and suppressive effects of positive practice and restitutional overcorrection. *Journal of Applied Behavior Analysis*, *14*, 71-80. https://doi.org/10.1901/jaba.1981.14-71

Centers for Disease Control and Prevention. (2015). *Show me the science- how to wash your hands*. Retrieved from https://www.cdc.gov/handwashing/show-me-the-science-handwashing.html

Centers for Disease Control and Prevention. (2020, April 4). *Coronavirus disease 2019 basics*. https://www.cdc.gov/coronavirus/2019-ncov/faq.html

Charbonneau, D. L., Ponte, J. M., & Kochanowski, B. A. (2000). A method of assessing the efficacy of hand sanitizers: Use of real soil encountered in the food service industry. *Journal of Food Protection, 63*(4), 495-501. https://doi.org/10.4315/0362-028X-63.4.495

Cooper, J. O., Heron, T. E., & Heward, W. L. (2007). *Applied behavior analysis* (2nd ed.). Pearson.

Cruz, N. J., Wilder, D. A., Phillabaum, C., Thomas, R., Cusick, M., & Gravina, N. (2019). Further evaluation of the performance diagnostic checklist-safety (PDC-Safety). *Journal of Organizational Behavior Management*, *39*(3-4), 266–279. https://doi.org/10.1080/01608061.2019.1666777

Deochand, N., & Deochand, M. E. (2016). Brief report on hand-hygiene monitoring systems: A pilot study of a computer-assisted image analysis technique. *Journal of Environmental Health*, *78*(10), 14–20.

Deochand, N., Hughes, H. C., & Fuqua, R. W. (2019). Evaluating visual feedback on the handwashing behavior of students with emotional and developmental disabilities. *Behavior Analysis: Research and Practice, 19*(3), 232–240. https://doi.org/10.1037/bar0000154

Dib, N. E., & Sturmey, P. (2007). The effects of verbal instruction, modeling, rehearsal, and feedback on correct posture during flute playing. *Behavior Modification, 31*(4), 382-388. https://doi.org/10.1177/0145445506296798

Dingman, D., Wu, J., & Murphy, H. M. (2020). School-based, blacklight handwashing program can improve handwashing quality and knowledge among pre-school aged children. *Evaluation and Program Planning*, *78*, 1–6. https://doi.org/10.1016/j.evalprogplan.2019.101731

Early, E., Battle, K., Cantwell, E., English, J., Lavin, J. E., & Larson, E. (1998). Effect of several interventions on the frequency of handwashing among elementary public school children. *American Journal of Infection Control*, *26*, 263-169. https://doi.org/10.1016/S0196-6553(98)80011-4

Fishbein, A. B., Tellez, I., Lin, H., Sullivan, C., & Groll, M. E. (2011). Glow gel hand washing in the waiting room: A novel approach to improving hand hygiene education. *Infection Control & Hospital Epidemiology*, *32*(7), 661–666. https://doi.org/10.1086/660359

Franklin, R. D., Allison, D. B., & Gorman, B. S. (1996). *Design and analysis of single-case research.* Psychology Press.

Franks, A. H., Harmsen, H. J., Raangs, G. C., Jansen, G. J., Schut, F., & Welling, G. W. (1998). Variations of bacterial populations in human feces measured by fluorescent in situ hybridization with group-specific 16S rRNA-targeted oligonucleotide probes. *Applied and Environmental Microbiology*, *64*(9), 3336–3345. https://doi.org/10.1128/AEM.64.9.3336-3345.1998

Fuls, J. L., Rodgers, N. D., Fischler, G. E., Howard, J. M., Patel, M., Weidner, P. L., & Duran, M. H. (2008). Alternative hand contamination technique to compare the activities of antimicrobial and nonantimicrobial soaps under different test conditions. *Applied and Environmental Microbiology*, *74*(12), 3739–3744. https://doi.org/10.1128/aem.02405-07

Geller, E. S., Eason, S. L., Phillips, J. A., & Pierson, M. D. (1980). Interventions to improve sanitation during food preparation. *Journal of Organizational Behavior Management*, *2*(3), 229–240. https://doi.org/10.1300/j075v02n03_08

Gould, D. J., Chudleigh, J., Drey, N. S., & Moralejo, D. (2007). Measuring handwashing performance in health service audits and research studies. *Journal of Hospital Infection*, *66*(2), 109–115. https://doi.org/10.1016/j.jhin.2007.02.009

Gresham, F. M., Gansle, K. A., Noell, G. H., Cohen, S., & Rosenblum, S. (1993). Treatment integrity of school-based behavioral intervention studies: 1980–1990. *School Psychology Review*, *22*(2), 254-272. https://doi.org/10.1080/02796015.1993.12085651

Grover, E., Hossain, M. K., Uddin, S., Venkatesh, M., Ram, P. K., & Dreibelbis, R. (2018). Comparing the behavioural impact of a nudge-based handwashing intervention to high-intensity hygiene education: A cluster-randomised trial in rural Bangladesh. *Tropical Medicine & International Health*, *23*(1), 10–25. https://doi.org/10.1111/tmi.12999

Guinan, M., McGuckin, M., & Ali, Y. (2002). The effect of a comprehensive handwashing program on absenteeism in elementary schools. *American Journal of Infection Control*, *30*, 217-220. https://doi.org/10.1067/mic.2002.120366

Guinan, M. E., McGuckin-Guinan, M., & Sevareid, A. (1997). Who washes hands after using the bathroom? *American Journal of Infection Control*, *25*(5), 424–425. https://doi.org/10.1016/s0196-6553(97)90092-4

Gustafson, D. R., Vetter, E. A., Larson, D. R., Ilstrup, D. M., Maker, M. D., Thompson, R. L., & Cockerill, F. R. (2000). Effects of 4 hand-drying methods for removing bacteria from washed hands: A randomized trial. *Mayo Clinic Proceedings*, *75*(7), 705–708. https://doi.org/10.1016/s0025-6196(11)64617-x

Haas, J. P., & Larson, E. L. (2007). Measurement of compliance with hand hygiene. *Journal of Hospital Infection*, *66*(1), 6–14. https://doi.org/10.1016/j.jhin.2006.11.013

Hansen, T. B., & Knochel, S. (2003). Image analysis method for evaluation of specific and non-specific hand contamination. *Journal of Applied Microbiology*, *94*(3), 483–494. https://doi.org/10.1046/j.1365-2672.2003.01855.x

Harrison, J. (2012). Teaching children to wash their hands—Wash your hands, Georgia! Handwashing education initiative. *Food Protection Plans*, *32*, 116-123.

Hautemaniere, A., Hunter, P. R., Diguio, N., Albuisson, E., & Hartemann, P. (2009). A prospective study of the impact of colonization following hospital admission by glycopeptide-resistant Enterococci on mortality during a hospital outbreak. *American Journal of Infection Control*, *37*(9), 746–752. https://doi.org/10.1016/j.ajic.2009.02.007

Hays, T., & Romani, P. W. (2021). Use of the performance diagnostic checklist-human services to assess hand hygiene compliance in a hospital. *Behavior Analysis in Practice*, *14*(1), 51–57. https://doi.org/10.1007/s40617-020-00461-8

Hoque, B. A. (2003). Handwashing practices and challenges in Bangladesh. *International Journal of Environmental Health Research*, *13*(sup1). https://doi.org/10.1080/0960312031000102831

Ingvarsson, E. T. & Hollobaugh, T. (2010). Acquisition of interverbal behavior: Teaching children with autism to mand for answers to questions. *Journal of Applied Behavior Analysis*, *43*, 1–17. https://doi.org/10.1901/jaba.2010.43-1

Jensen, D. A., Danyluk, M. D., Harris, L. J., & Schaffner, D. W. (2015). Quantifying the effect of hand wash duration, soap use, ground beef debris, and drying methods on the removal of enterobacter aerogenes on hands. *Journal of Food Protection*, *78*(4), 685–690. https://doi.org/10.4315/0362-028x.jfp-14-245

Jess, R. L., Dozier, C. L., & Foley, E. A. (2019). Effects of a handwashing intervention package on handwashing in preschool children. *Behavioral Interventions*, *34*(4), 475–486. https://doi.org/10.1002/bin.1684

Johnson, D. A. (2013). A component analysis of the impact of evaluative and objective feedback on performance. *Journal of Organizational Behavior Management*, *33*(2), 89-103. https://doi.org/10.1080/01608061.2013.785879

Kissel, R. C., Whitman, T. L., & Reid, D. H. (1983). An institutional staff training and self-management program for developing multiple self-care skills in severely/profoundly retarded individuals. *Journal of Applied Behavior Analysis, 18,* 395-415. https://doi.org/10.1901/jaba.1983.16-395

Kramer, S. A. (1978). The effects of music as a cue in maintaining handwashing in preschool children. *Journal of Music Therapy*, *15*(3), 136–144. https://doi.org/10.1093/jmt/15.3.136

Lamdin, D. J. (2010). Evidence of student attendance as an independent variable in education production functions. *The Journal of Educational Research, 89,* 155-162. https://doi.org/10.1080/00220671.1996.9941321

Larson, E. (1988). A causal link between handwashing and risk of infection? Examination of the evidence. *Infection Control Hospital Epidemiology, 9*(1), 28-36. https://doi.org/10.1086/645729

Lau, C. H., Springston, E. E., Sohn, M.-W., Mason, I., Gadola, E., Damitz, M., & Gupta, R. S. (2012). Hand hygiene instruction decreases illness-related absenteeism in elementary schools: a prospective cohort study. *BMC Pediatrics*, *12*(1). https://doi.org/10.1186/1471-2431-12-52

Lee, R. L. T., & Lee, P. H. (2014). To evaluate the effects of a simplified hand washing improvement program in schoolchildren with mild intellectual disability: A pilot study. *Research in Developmental Disabilities*, *35*(11), 3014–3025. https://doi.org/10.1016/j.ridd.2014.07.016

Lee, R. L., Leung, C., Tong, W. K., Chen, H., & Lee, P. H. (2015). Comparative efficacy of a simplified handwashing program for improvement in hand hygiene and reduction of school absenteeism among children with intellectual disability. *American Journal of Infection Control*, *43*(9), 907-912. https://doi.org/10.1016/j.ajic.2015.03.023

Lerman, D. C., Dittlinger, L. H., Fentress, G., & Lanagan, T. (2011). A comparison of methods for collecting data on performance during discrete trial teaching. *Behavior Analysis in Practice*, *4*(1), 53–62. https://doi.org/10.1007/BF03391775

Lin, C. N., Wu, F. O., Kim, H. G., Doyle, M. P., Michaels, B. S., & Williams, L. I. (2003). A comparison of hand washing techniques to remove escherichia coli and caliciviruses under natural or artificial fingernails. *Journal of Food Protection*, *66*(12), 2296–2301. https://doi.org/10.4315/0362-028x-66.12.2296

Luby, S. P., Agboatwalla, M., Billhimer, W., & Hoekstra, R. M. (2007). Field trial of a low cost method to evaluate hand cleanliness. *Tropical Medicine & International Health*, *12*(6), 765–771. https://doi.org/10.1111/j.1365-3156.2007.01847.x

Luby, S. P., Agboatwalla, M., Painter, J., Altaf, A., Billhimer, W. L., & Hoekstra, R. M. (2004). Effect of intensive handwashing promotion on childhood diarrhea in high-risk communities in Pakistan. *JAMA*, *291*(21), 2547. https://doi.org/10.1001/jama.291.21.2547

Luby, S. P., Agboatwalla, M., Feikin, D. R., Painter, J., Billhimer, W., Altaf, A., & Hoekstra, R. M. (2005). Effect of handwashing on child health: a randomised controlled trial. *The Lancet*, *366*(9481), 225–233. https://doi.org/10.1016/s0140-6736(05)66912-7

Luby, S. P., Halder, A. K., Huda, T., Unicomb, L., & Johnston, R. B. (2011). The effect of handwashing at recommended times with water alone and with soap on child diarrhea in rural Bangladesh: An observational study. *PLoS Medicine*, *8*(6). https://doi.org/10.1371/journal.pmed.1001052

Luke, M. M., & Alavosius, M. (2011). Adherence with universal precautions after immediate, personalized performance feedback. *Journal of Applied Behavior Analysis*, *44*(4), 967–971. https://doi.org/10.1901/jaba.2011.44-967

Mangiapanello, K. A., & Hemmes, N. S. (2015). An analysis of feedback from a behavior analytic perspective. *The Behavior Analyst*, *38*(1), 51–75. https://doi.org/10.1007/s40614-014-0026-x

Miltenberger, R. G., (2008). Teaching safety skills to children: Prevention of firearm injury as an exemplar of best practice in assessment, training, and generalization of safety skills. *Journal of Applied Behavior Analysis*, *1*(1), 30-36. https://doi.org/10.1007/BF03391718

Miltenberger, R. G. (2016). Behavior modification: Principles and procedures (6th ed.). Cengage Learning.

Mizumoto, K. & Chowell, G. (2020). Estimating risk for death from 2019 novel coronavirus disease, China, January–February 2020. *Emerging Infectious Diseases*, *26*(6). https://doi.org/10.3201/eid2606.200233

Nandrup-Bus, I. (2009). Mandatory handwashing in elementary schools reduces absenteeism due to infectious illness among pupils: a pilot intervention study. *American Journal of Infection Control, 37*, 820-826.

National Association for the Education of Young Children. (2019). *NAEYC Early Learning Program Accreditation Standards and Assessment Items.* https://www.naeyc.org/sites/default/files/globally-shared/downloads/PDFs/accreditation/early-learning/standards_assessment_2019.pdf

National Institutes of Health. (2020). Aerosol and surface stability of SARS-CoV-2 as compared with SARS-CoV-1. *The New England Journal of Medicine.* https://doi.org/10.1056/NEJMc2004973

Neuzil, K. M., Hohlbein, C., Zhu, Y. (2002). Illness among schoolchildren during influenza season: effect on school absenteeism, parental absenteeism from work, and secondary illness in families. *Archives of Pediatrics and Adolescent Medicine, 156*(10), 986–991. https://doi.org/10.1001/archpedi.156.10.986

Niffenegger, J. P. (1997). Proper handwashing promotes wellness in child care. *Journal of Pediatric Health Care*, *11*, 11-16. https://doi.org/10.1016/S0891-5245(97)90141-3

Oncu, E., Vayisoglu, S. K., Lafci, D., Yurtsever, D., Bulut, E. R., & Peker, E. (2018). Comparison of interactive education versus fluorescent concretization on hand hygiene compliance among primary school students: A randomized controlled trial. *The Journal of School Nursing*, *35*(5), 337–347. https://doi.org/10.1177/1059840518785447

Pickering, L. K. (1986). The day care center diarrhea dilemma. American Journal of Public Health, 76, 623-624. https://doi.org/10.2105/AJPH.76.6.623

Pickering, A. J., Davis, J., Scalmanini, J., Okoth, G., Ram, P. K., Blum, A. G., … Oyier, B. (2013). Access to waterless hand sanitizer improves student hand hygiene behavior in primary schools in Nairobi, Kenya. *The American Journal of Tropical Medicine and Hygiene*, *89*(3), 411–418. https://doi.org/10.4269/ajtmh.13-0008

Poche, C., Brouwer, R., & Swearingen, M. (1981). Teaching self-protection to young children. *Journal of Applied Behavior Analysis*, *14*, 169-176. https://doi.org/10.1901/jaba.1981.14-169

Ponka, A., Poussa, T., & Laosmaa, M. (2004). The effect of enhanced hygiene practices on absences due to infectious diseases among children in day care centers in Helsinki. Infection, 32, 2-7. https://doi.org/10.1007/s15010-004-3036-x

Rabie, T., & Curtis, V. (2006). Handwashing and risk of respiratory infections: a quantitative systematic review. *Tropical Medicine and International Health*, *11*(3), 258–267. https://doi.org/10.1111/j.1365-3156.2006.01568.x

Randle, J., Metcalfe, J., Webb, H., Luckett, J. C., Nerlich, B., Vaughan, N., Segal, J. I., & Hardie, K. R. (2013). Impact of an educational intervention upon the hand hygiene compliance of children. *Journal of Hospital Infection*, *85*(3), 220–225. https://doi.org/10.1016/j.jhin.2013.07.013

Roberts, L., Jorm, L., Patel, M., Smith, W., Douglas, R. M., & McGilchrist, C. (2000). Effect of infection control measures on the frequency of diarrheal episodes in child care: a randomized, controlled trial. *Pediatrics, 105*, 743-746.

Rosen, L., Manor, O., Englehard, D., Brody, D., Rosen, B., Peleg, H. Meir, M., & Zucker, D. (2006). Can a handwashing intervention make a difference? Results from a randomized controlled trial in Jerusalem preschools. *Preventive Medicine, 42*, 27-32. https://doi.org/10.1016/j.ypmed.2005.09.012

Rosen, L., Zucker, D., Brody, D., Engelhard, D., Meir, M., & Manor, O. (2011). Enabling hygienic behavior among preschoolers: Improving environmental conditions through a multifaceted intervention. *American Journal of Health Promotion, 25*(4), 248-256. https://doi.org/10.4278/ajhp.081104-QUAN-265

Rosenberg, N. E., Schwartz, I. S., & Davis, C. A. (2010). Evaluating the utility of commercial videotapes for teaching hand washing to children with autism. *Education and Treatment of Children*, *33*, 443-455. https://doi.org/10.1353/etc.0.0098

Rotter, M. L., Sattar, S. A., & Suchomel, M. (2017). Methods to evaluate the antimicrobial efficacy of hand hygiene agents. In D. Pittet, J. M. Boyce, & B. Allegranzi (Eds.), *Hand hygiene: A handbook for medical professionals* (pp. 58–69). John Wiley & Sons, Ltd.

Saboori, S., Greene, L. E., Moe, C. L., Freeman, M. C., Caruso, B. A., Akoko, D., & Rheingans, R. D. (2013). Impact of regular soap provision to primary schools on hand washing and e. coli hand contamination among pupils in Nyanza Province, Kenya: A cluster-randomized trial. *The American Journal of Tropical Medicine and Hygiene*, *89*(4), 698–708. https://doi.org/10.4269/ajtmh.12-0387

Sacri, A. S., De Serres, G., Quach, C., Boulianne, N., Valiquette, L., & Skowronski, D. M. (2014). Transmission of acute gastroenteritis and respiratory illness from children to parents. *The Pediatric Infectious Disease Journal, 33*(6), 583-538. https://doi.org/10.1097/INF.0000000000000220

Schreibman, L., O'Neill, R. E., & Koegel, R. L. (1983). Behavioral training for siblings of autistic children. *Journal of Applied Behavior Analysis*, *16*, 129-138. https://doi.org/10.1901/jaba.1983.16-129

Shi, T., Balsells, E., Wastnedge, E., Singleton, R., Rasmussen, Z. A., Zar, H. J., Rath, B. A., Madhi, S. A., Campbell, S., Vaccari, L. C., Bulkow, L. R., Thomas, E. D., Barnett, W., Hoppe, C., Campbell, H., & Nair, H. (2015). Risk factors for respiratory syncytial virus associated with acute lower respiratory infection in children under five years: Systematic review and meta-analysis. *Journal of global health*, *5*(2), 020416. https://doi.org/10.7189/jogh.05.020416

Sigurdsson, S. O., & Austin, J. (2008). Using real-time visual feedback to improve posture at computer workstations. *Journal of Applied Behavior Analysis*, *41*(3), 365–375. https://doi.org/10.1901/jaba.2008.41-365

Smith, W. M., McCrary, J. W., & Smith, K. U. (1960). Delayed visual feedback and behavior. *Science*, *132*(3433), 1013–1014. https://doi.org/10.1126/science.132.3433.1013

Snow, M., White, G. L., & Kim, H. S. (2008). Inexpensive and time-efficient hand hygiene interventions increase elementary school children's hand hygiene rates. *Journal of School Health*, *78*(4), 230–233. https://doi.org/10.1111/j.1746-1561.2008.00291.x

Staniford, L.J. & Schmidtke, K.A. (2020). A systematic review of hand-hygiene and environmental-disinfection interventions in settings with children. *BMC Public Health, 20*(195), 1-11. https://doi.org/10.1186/s12889-020-8301-0

Steere, A. C. & Mallison, G. F. (1975). Handwashing practices for the prevention of nosocomial infections. Annals of Internal Medicine, 83, 683-690. https://doi.org/10.7326/0003-4819-83-5-683

Todd, E. C., Michaels, B. S., Holah, J., Smith, D., Greig, J. D., & Bartleson, C. A. (2010). Outbreaks where food workers have been implicated in the spread of foodborne disease. Part 10. Alcohol-based antiseptics for hand disinfection and a comparison of their effectiveness with soaps. *Journal of Food Protection*, *73*(11), 2128–2140. https://doi.org/10.4315/0362-028x-73.11.2128

Todd, E. C., Michaels, B. S., Smith, D., Grieg, J. D., & Bartleson, C. A. (2010). Outbreaks where food workers have been implicated in the spread of foodborne disease. Part 9. Washing and drying of hands to reduce microbial contamination. *Journal of Food Protection*, *73*(10), 1937–1955. https://doi.org/10.4315/0362-028x-73.10.1937

University of Chicago Consortium on School Research. (2013). *Preschool attendance in Chicago Public Schools: Relationships with learning outcomes and reasons for absences: Research summary*. Chicago, IL: Elrich, S. B., Gwynne, J. A., Pareja, A. S., & Allensworth, E. M.

Walmsley, C., Mahoney, A., Durgin, A., & Poling, A. (2013). Fostering hand washing before lunch by students attending a special needs young adult program. *Research in Developmental Disabilities*, *34*, 95–101. https://doi.org/10.1016/j.ridd.2012.08.002

Wang, Z., Lapinski, M., Quilliam, E., Jaykus, L.-A., & Fraser, A. (2017). The effect of hand-hygiene interventions on infectious disease-associated absenteeism in elementary schools: A systematic literature review. *American Journal of Infection Control*. https://doi.org/10.1016/j.ajic.2017.01.018

Whitby, M., Pessoa-Silva, C. L., McLaws, M. L., Allegranzi, B., Sax, H., Larson, E., Seto, W. H., Donaldson, L., & Pittet, D. (2007). Behavioural considerations for hand hygiene practices: The basic building blocks. *The Journal of Hospital Infection*, *65*(1), 1–8. https://doi.org/10.1016/j.jhin.2006.09.026

Wilder, D. A., Allison, J., Nicholson, K., Abellon, O. E., & Saulnier, R. (2010). Further evaluation of antecedent interventions on compliance: The effects of rationales to increase compliance among preschoolers. *Journal of Applied Behavior Analysis*, *43*(4), 601–613. https://doi.org/10.1901/jaba.2010.43-601

World Health Organization. (2009). *WHO guidelines on hand hygiene in health care.* World Health Organization. https://apps.who.int/iris/handle/10665/44102

World Health Organization. (2020, March 9). *Questions and answers on coronaviruses (COVID-19)*. https://www.who.int/news-room/q-a-detail/q-a-coronaviruses

World Health Organization. (2020). *Hand hygiene for all initiative: Improving access and behaviour in health care facilities.* World Health Organization. https://apps.who.int/iris/rest/bitstreams/1311838/retrieve

Worsdell, A. S., Iwata, B. A., Dozier, C. L., Johnson, A. D., Neidert, P. L., & Thomason, J. L. (2005). Analysis of response repetition as an error-correction strategy during sight-word reading. *Journal of Applied Behavior Analysis*, *38*(4), 511-527. https://doi.org/10.1901/jaba.2005.115-04

Figure 1

Percentage of Sessions Across Participants with Handwashing Errors (Study 1)

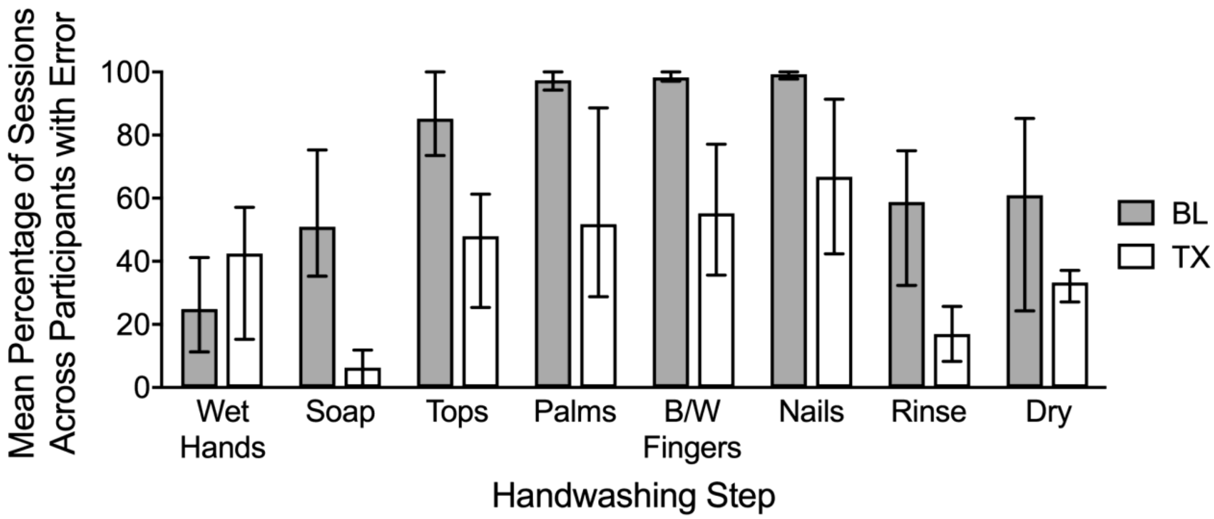


Figure 2

Percentage of Sessions Across Participants Across Studies with Handwashing Errors (Study 1)

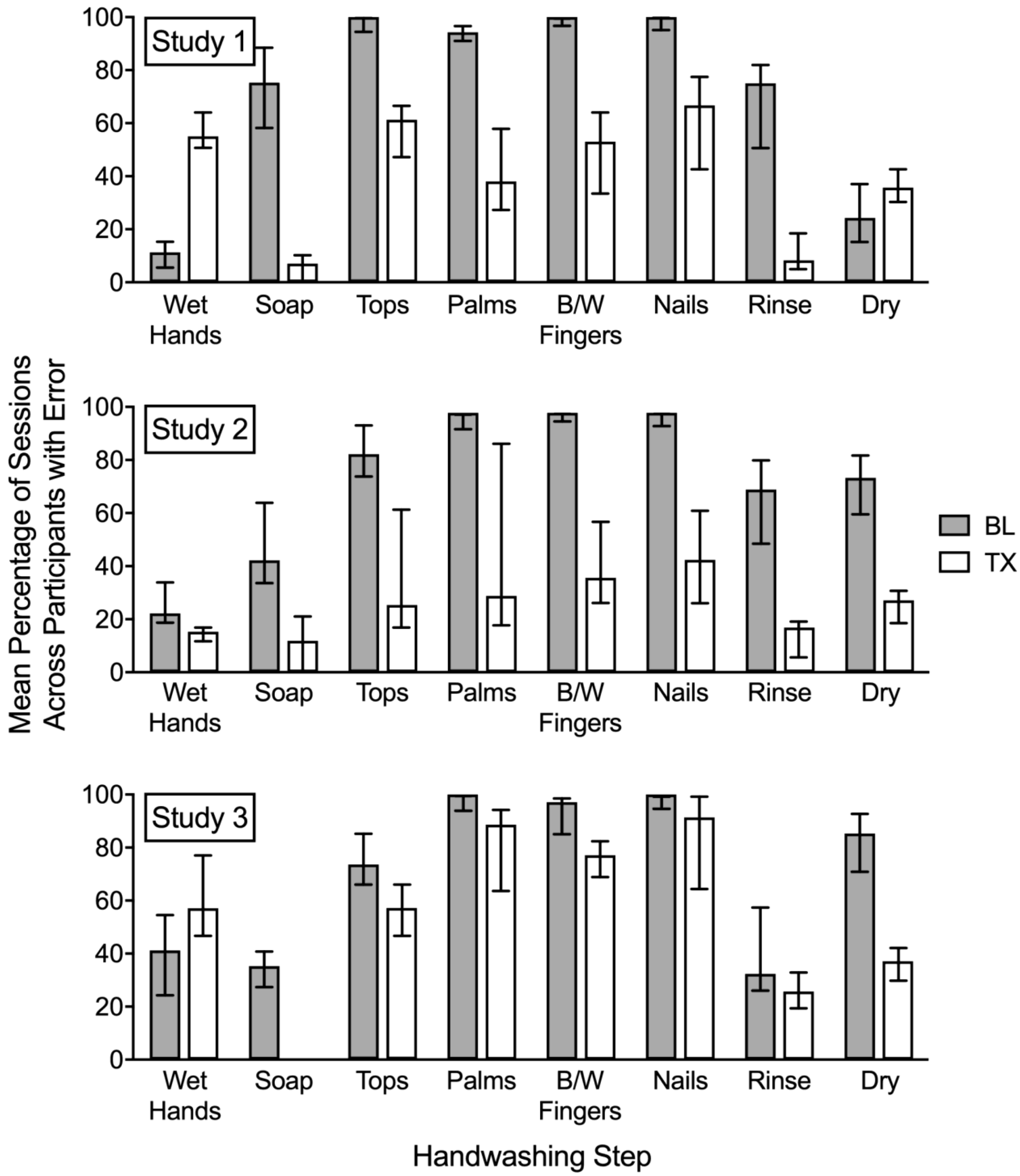


Figure 3

Percentage of Participants Across Studies with Handwashing Errors in Last Session (Study 1)

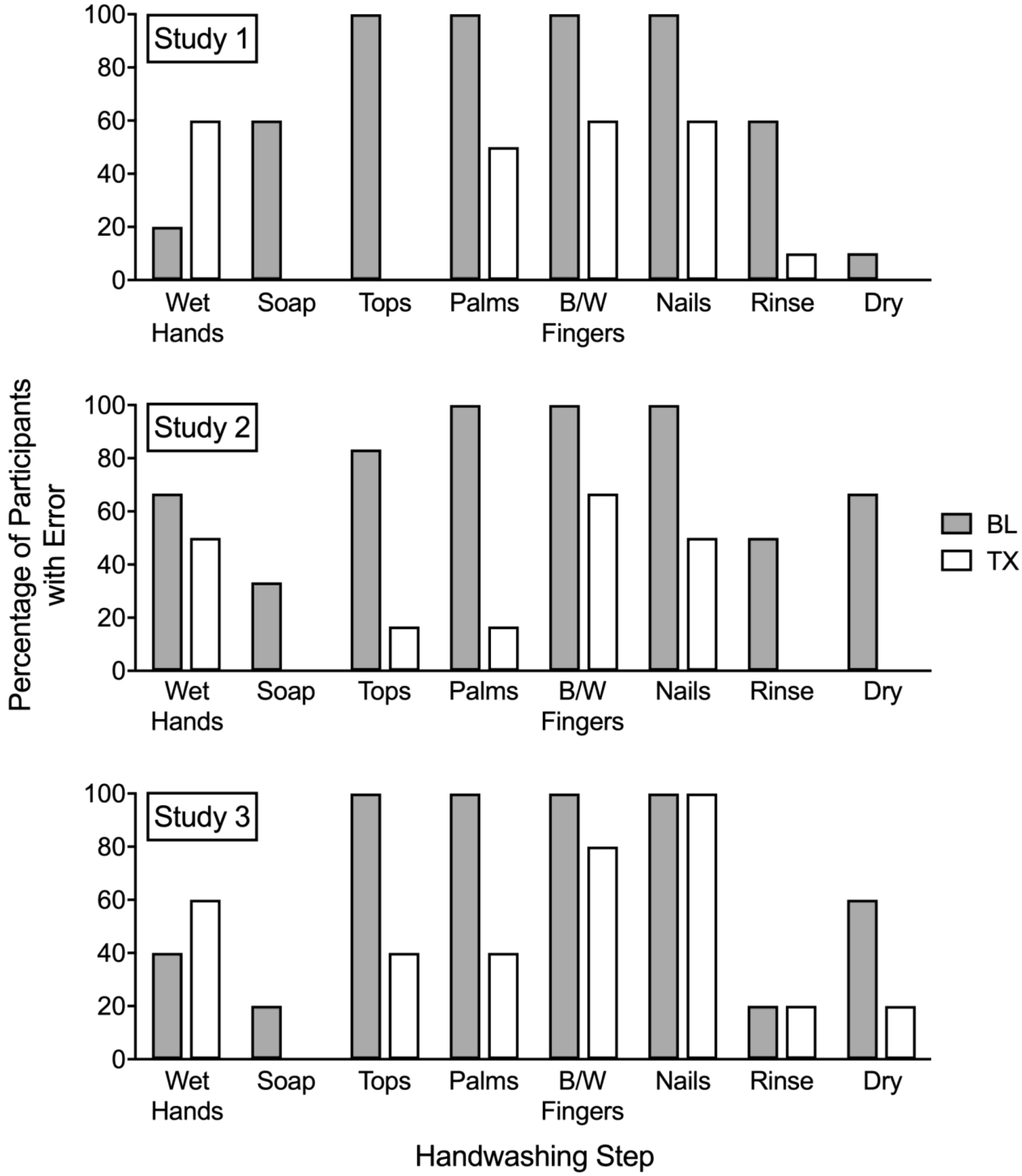


Figure 4

Mean Percentage of Hands Illuminated Across Participants (Study 1)

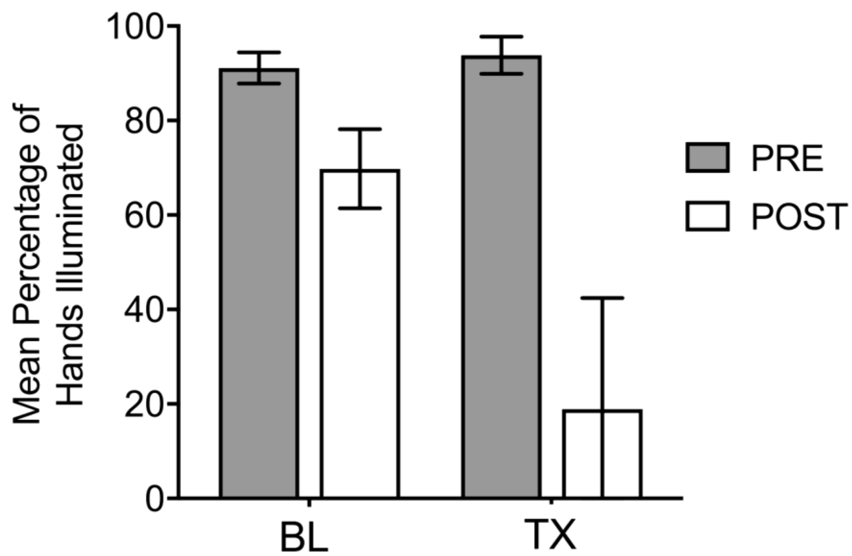


Figure 5

Mean Percentage of Hands Illuminated for Participants Across Studies (Study 1)

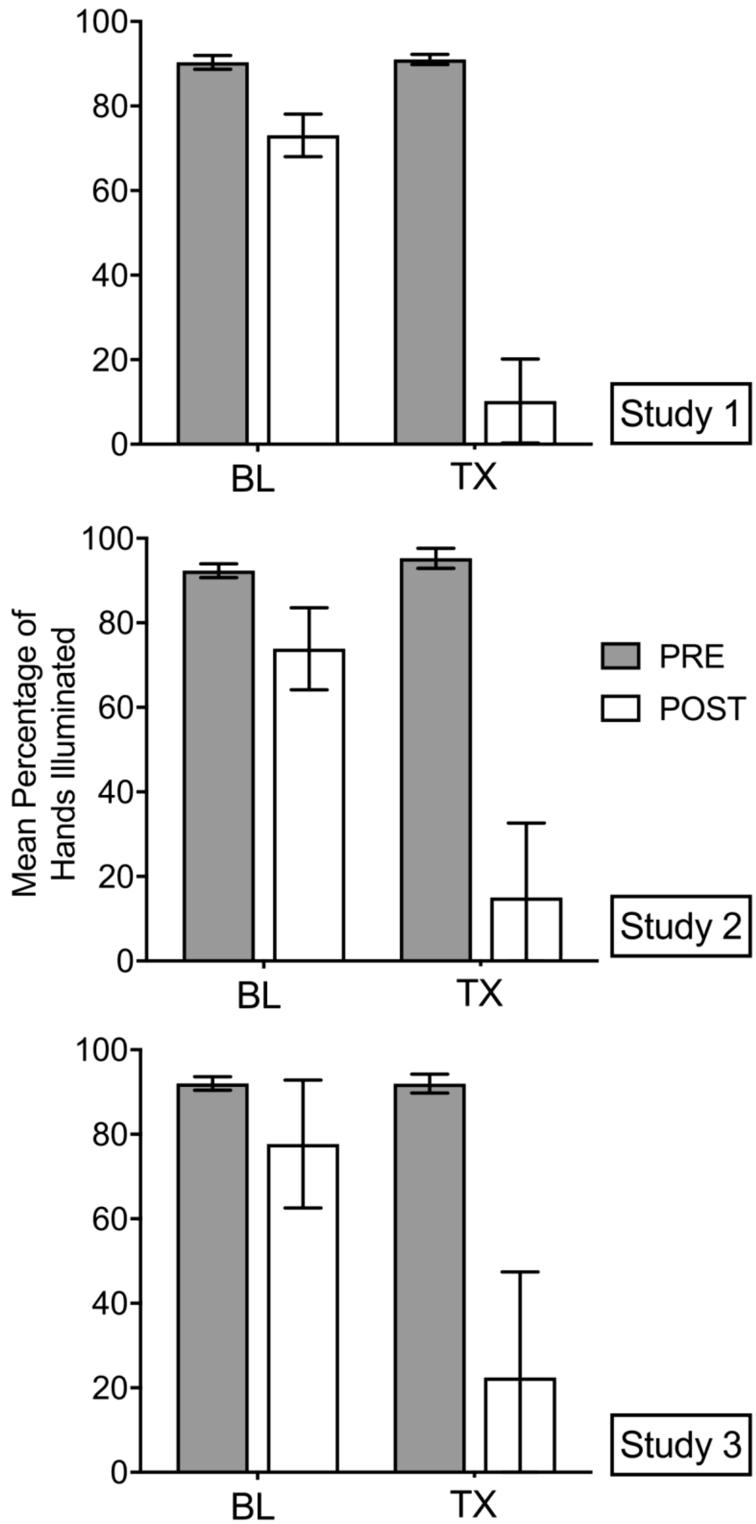


Figure 6

Mean Percentage of Hands Illuminated for Participants Across Studies in Last Session (Study 1)

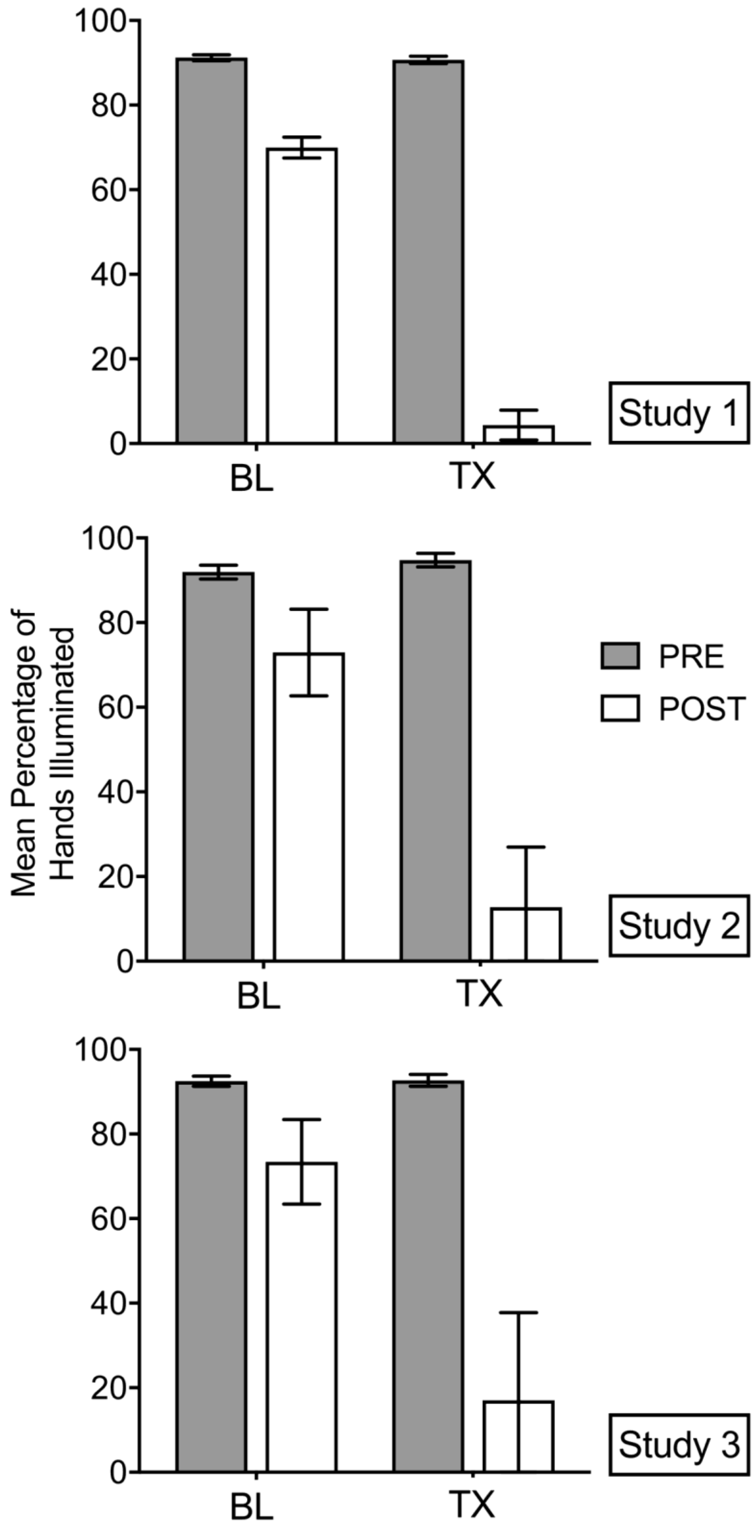


Figure 7

Handwashing Errors and Percentage of Hands Illuminated – No Scrubbing Errors (Study 1)

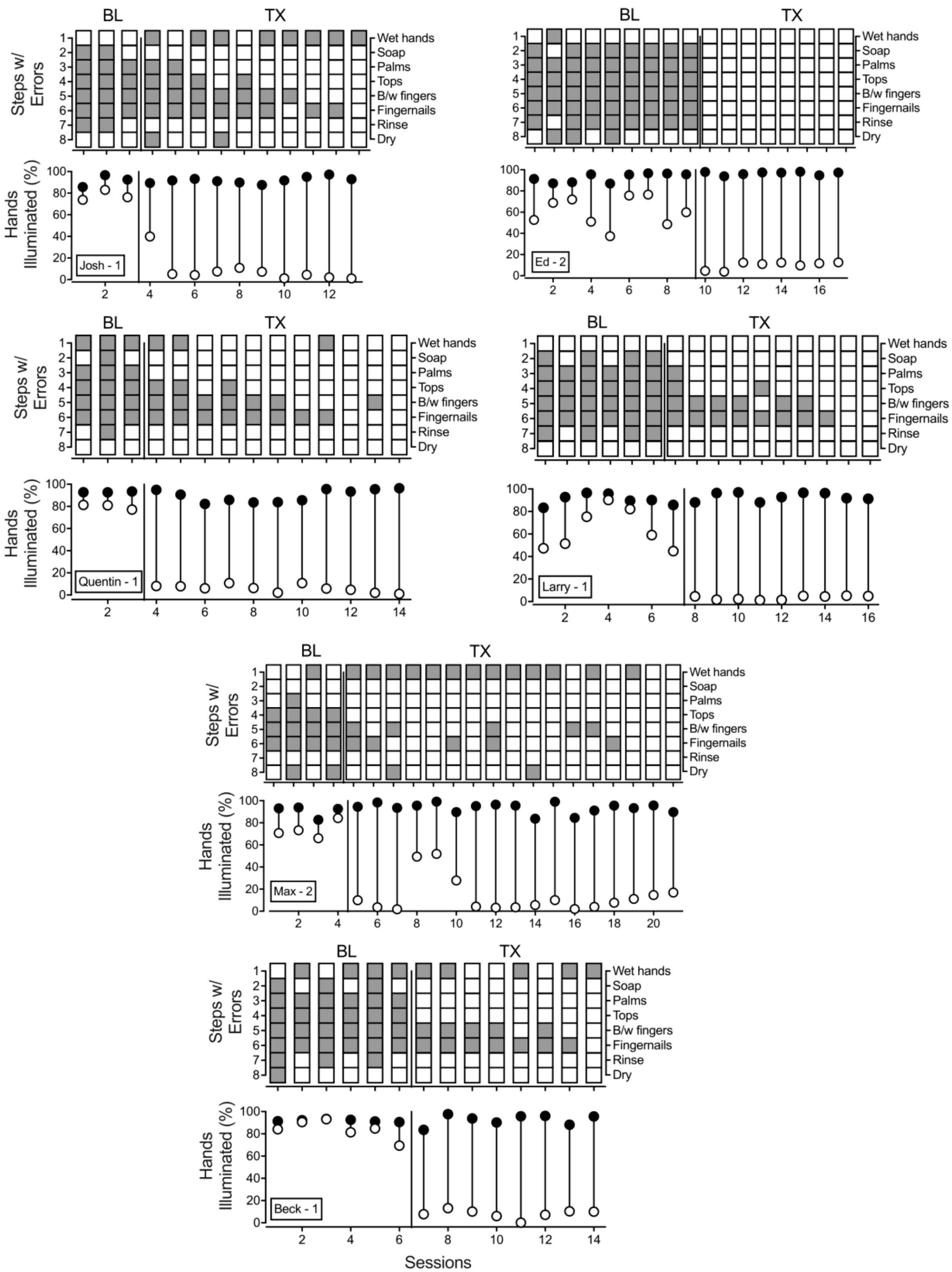


Figure 8

Handwashing Errors and Percentage of Hands Illuminated – Some Scrubbing Errors (Study 1)

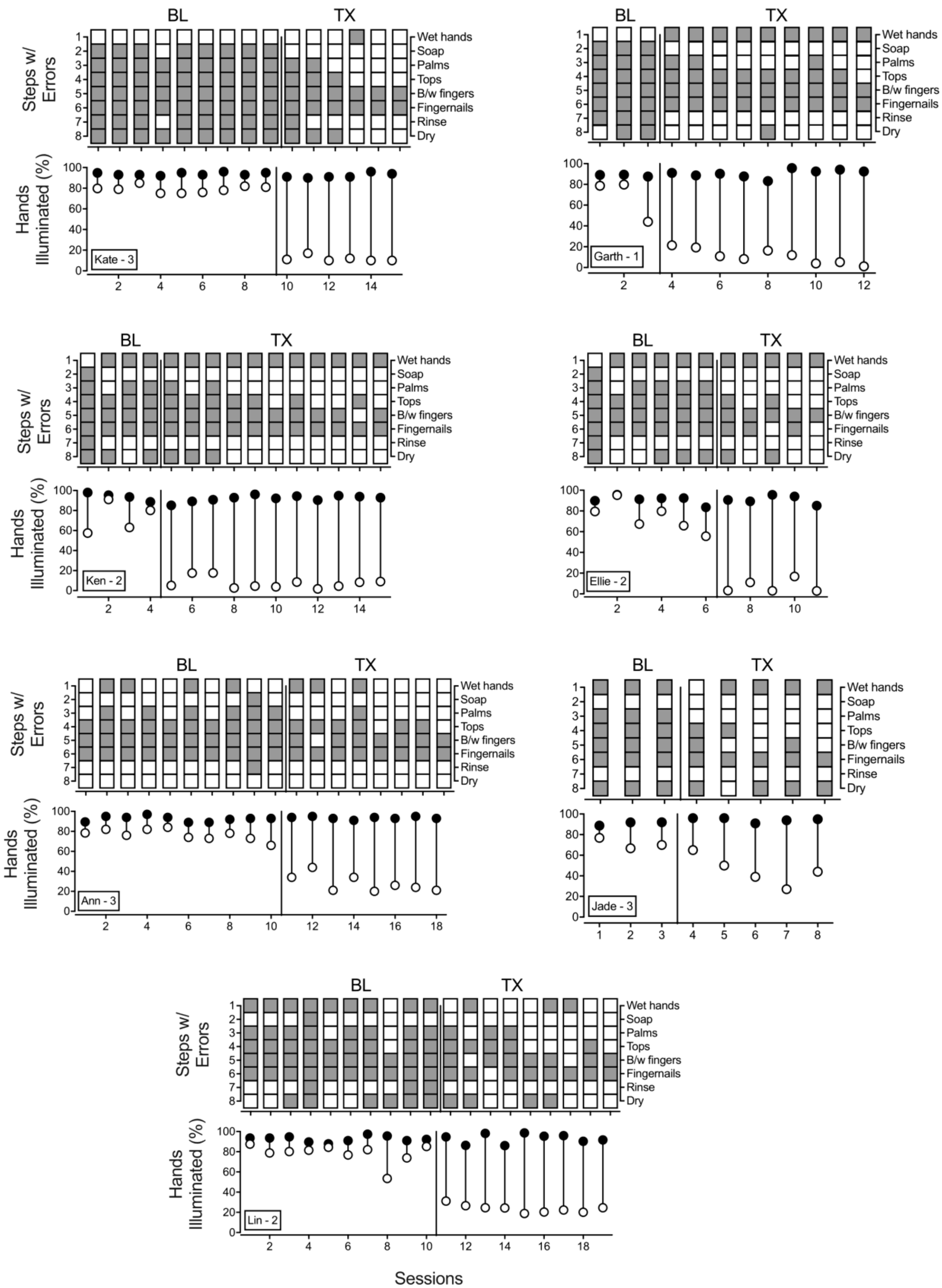


Figure 9

Handwashing Errors and Percentage of Hands Illuminated – Multiple Scrubbing Errors (Study 1)

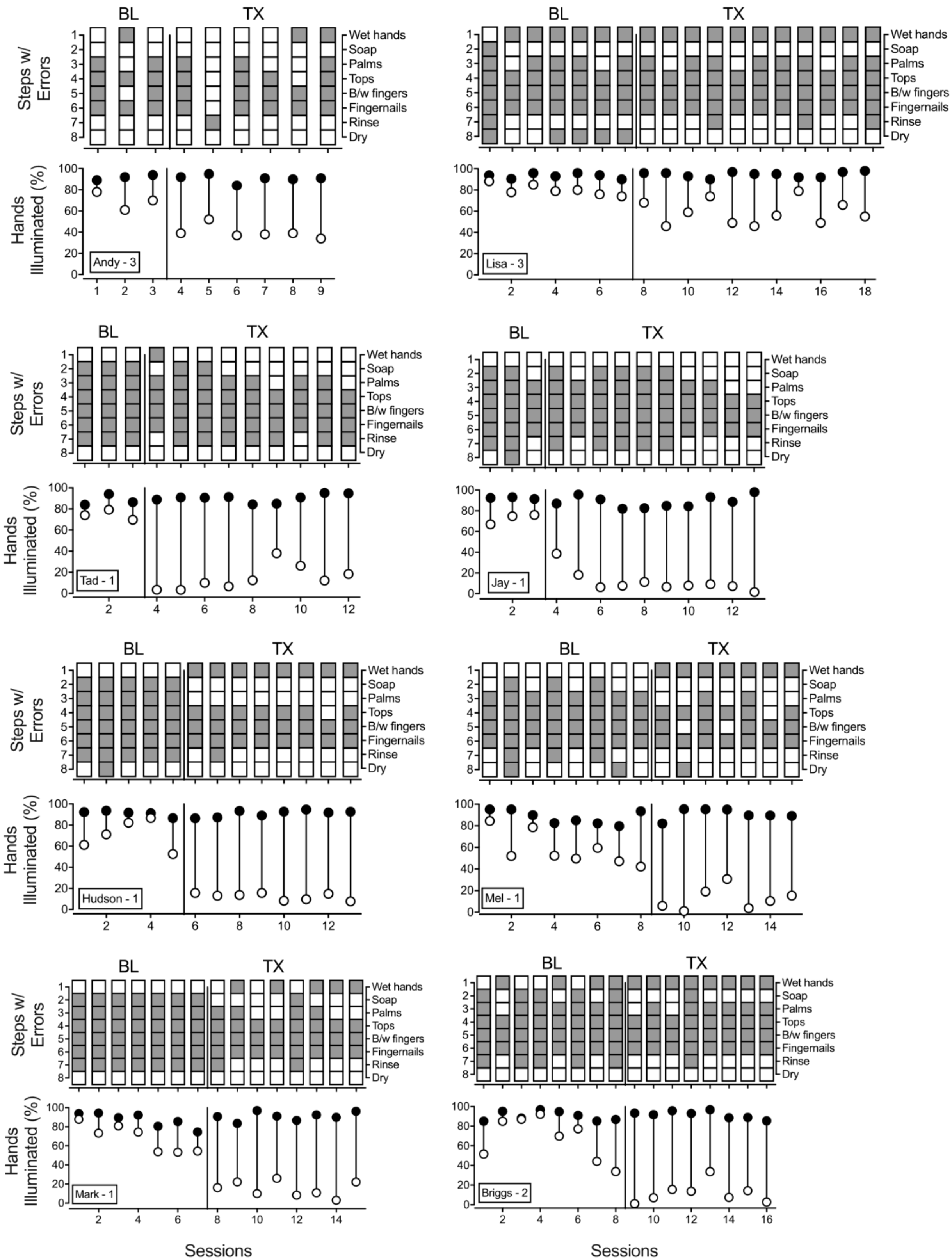


Figure 10

Percentage of Hands Illuminated Across Conditions (Study 2)

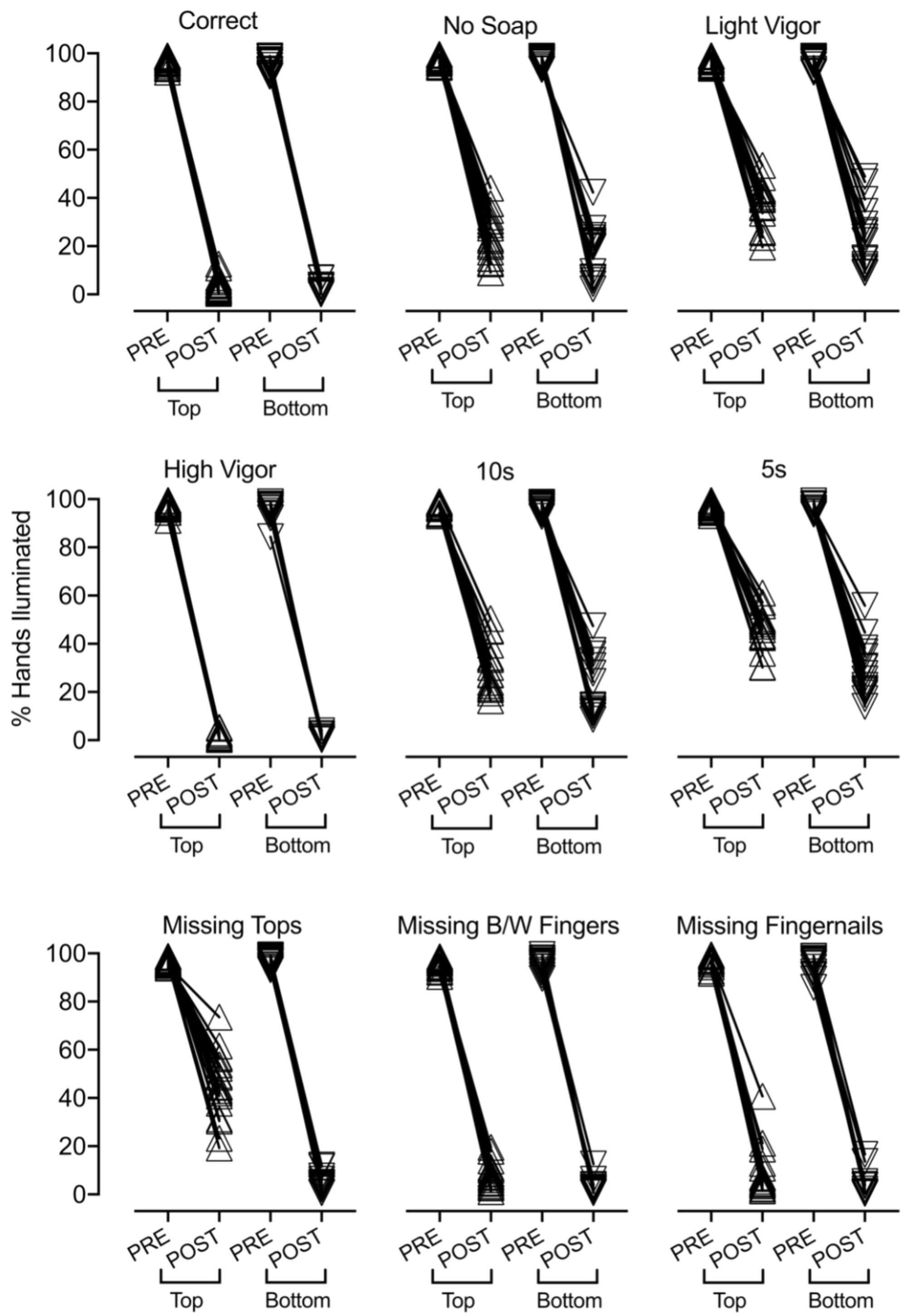
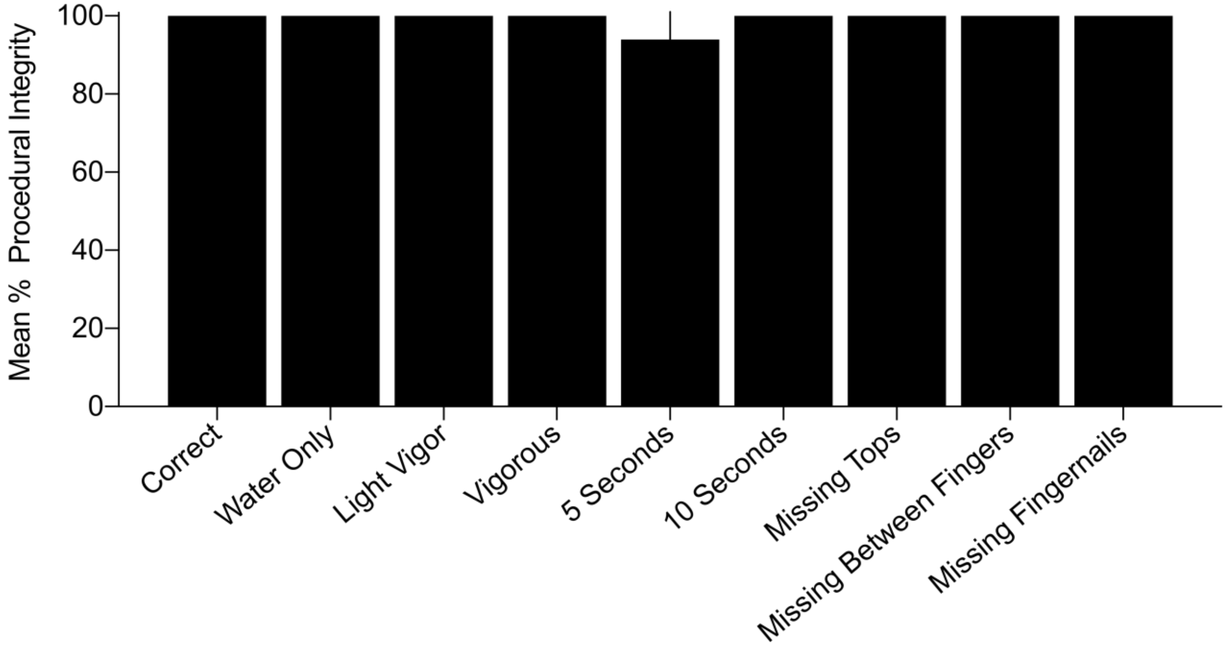


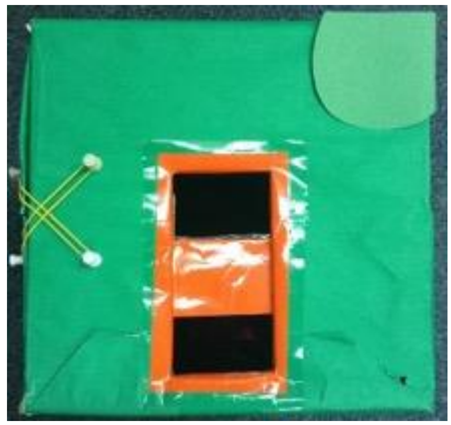
Figure 11

Procedural Integrity (Study 2)



Appendix A

Study 1 Materials



Side of UV light box

Top of UV light box

Appendix B

Study 1 Handwashing Checklist

Handwashing Steps	Correct?		
1. Turns on water and wets hands under water BEFORE soap Turns on water and places hands under water such that hands (from wrist to fingertip, bottom and top) are wet	L	R	N
2. Dispenses soap Places hand under soap dispenser or depresses pump on soap bottle such that at least one hand has soap.	Y		N
3. Lathers bottoms of hands Rubs hands together such that at least 75% of both palms of hands have visible soap suds.	L	R	N
4. Lathers tops of hands Rubs hands together such that at least 75% of both tops of hands have visible soap suds.	L	R	N
5. Lathers between fingers Rubs hands together such that the webs of each finger of each hand have visible soap suds.	L	R	N
6. Lathers fingernails Scrubs nails of hands such that each fingernail of each hand has visible soap suds.	L	R	N
7. Rinses hands clean of soap Places hands underneath stream of water and rinses hands clean such that no suds are visible on the hands.	L	R	N
8. Dries hands Uses paper towel to dry tops and bottoms of hand. Water should not drip off hands following drying.	L	R	N
	# steps correct: / 8		

Appendix C

Example Images of ImageJ Procedures

Post-handwashing picture.

Post-handwashing picture with threshold adjusted.

Appendix D

Handwashing Video Script

The Handwashing Video shows a familiar adult guiding the participants through every step of the Handwashing Checklist. The adult acts out the steps described in the video.

Narrator: "Hi, everyone! I am here to teach you about washing your hands. We use our hands every day to do things like eat food and play with our friends! Everybody, look down at your hands."

video shows a pair of hands covered in dirt and marker

Narrator: "We can get germs on our hands from sharing toys, playing outside, and sneezing and coughing. If those germs get inside our body, we can get really sick. What should we do to get rid of the germs on these hands?"

video pauses for children to respond chorally: experimenter prompts

Narrator: "That's right! We should wash them! Let's go over to the sink and wash our hands."

video shows a pair of hands following the steps of handwashing

Narrator: "First, we need to turn on the water. Everyone pretend to turn on the water with me! Now, we are going to get our hands wet in the sink. Let me see everybody get their hands wet! Let's get some soap on our hands. Now that we have soap, we need to scrub, scrub scrub! Don't rinse the soap until you are all done washing. Sing the handwashing song while you scrub your hands: 'Top and bottom, top and bottom. In between, in between. Scrub under your nails, scrub under your nails. Now again, now again. Top and bottom, top and bottom. In between, in between. Scrub under your nails, scrub under your nails. Now you're clean, squeaky clean.' Now that our hands are washed, we can rinse off our suds. Put your hands back in the water like this, and make sure all of the soap is gone. Way to go! Now let's turn off the water and dry off. Get some paper towels and get your hands dry- make sure to get the tops and bottoms of your hands dry! You guys did such a good job! Let's see if we got the germs off."

video shows the pair of hands without dirt or marker

Narrator: "After we washed our hands, there aren't any more germs left. There's no more germs on the tops or bottoms of our hands! Scrubbing our hands with soap gets all of the germs off our hands. Now YOU get to practice it! Your teacher will let you go wash your hands. Until next time… see you later!"

Appendix E

Study 2 Informed Consent Form

Consent to Participate in:
Validation of Computer-Assisted Image Analysis as a Measurement Tool for Hand Cleanliness
INFORMED CONSENT

The Department of Applied Behavioral Science at the University of Kansas supports the practice of protection for human subjects participating in research. The following information is provided for you to decide whether you wish to participate in the present study. You may refuse to sign this form and not participate in this study. You should be aware that even if you agree to participate, you are free to withdraw at any time. If you do withdraw from this study, it will not affect your relationship with this unit, the services it may provide to you, or the University of Kansas.

Key Information

- This project is studying the degree to which various handwashing components result in hand cleanliness.
- Your participation in this research project is completely voluntary.
- Your participation will take 20 minutes per day over a total of 9 days.
- You will be asked to do the following procedures: apply UV-sensitive lotion to hands, wash hands following specific steps, and place hands under UV light for no more than 10 seconds. More detailed information on the procedures can be found below.
- Possible risks include dry skin on the hands as a result of frequent handwashing.
- Your participation will benefit society by providing information about the handwashing components necessary and sufficient for optimal hand cleanliness.
- Your alternative to participating in this research study is not to participate.

Purpose
The purpose of this study is to (a) evaluate the degree to which the Centers for Disease Control and Prevention (CDC) handwashing procedure results in hand cleanliness using ultra-violet (UV) light-illuminated lotion (Glo-germ) on participants' hands and (b) determine the handwashing components necessary and sufficient for optimal hand cleanliness.

Procedures
By participating in this study, you will be asked to wash your hands following specific steps. We will measure your hand cleanliness using Glo-Germ, a non-hazardous cosmetic gel that illuminates under UV light. Your time commitment for each session will be no longer than 20 minutes. However, you will be asked to complete at least 9 research sessions (total time of study is no more than 3 hours). All sessions will be video recorded. Your data and video files will be stored on a password-protected computer saved to a secure server in a locked office. You may ask to have the taping stopped at any time and choose not to participate in the study. Trained graduate and undergraduate research assistants will score the video tapes. The recordings will be erased after five years from the date of recording. Your video will not be used in any other manner without signed permission from you.

Risks
There are minimal risks associated with participation in this study. You may experience dry skin on the hands as a result of frequent handwashing.

KU Lawrence IRB # STUDY00146417 | Approval Period 11/24/2020 – 11/4/2021

Benefits
Your participation in this study will benefit society by providing information about the effects of handwashing on hand cleanliness. Additionally, data collected from this study may identify components necessary and sufficient for optimal hand cleanliness.

COVID-19 Procedures
The University of Kansas recognizes that the COVID-19 pandemic has changed the level of risk to you regarding your participation in this research. The university is following Centers for Disease Control & Prevention (CDC), state, and institutional guidelines and best practices and is requiring additional precautions and procedures for this project in light of this.

Please be advised that although the researchers will take precautions to maintain your health and safety, the nature of COVID-19 prevents the researchers from guaranteeing protection from the virus. The researchers would like to remind you to follow the CDC's recommended guidelines for protecting yourself and others from exposure to the virus. If you are at risk for contracting COVID-19, or if you do not feel comfortable participating due to the risk of COVID-19, you are encouraged not to participate.

COVID-19 Symptoms
As is currently in place, upon arrival at the Child Development Center, you will be asked to take a self-assessment of symptoms and to withdraw from participating in study sessions for at least 14 days if you have symptoms, if you have recently traveled to a high-risk area, or if you have come into contact with someone experiencing symptoms or who has tested positive for the virus. If you develop symptoms, test positive or discover you have been in contact with someone who has tested positive after a research session, we ask that you notify us immediately so we can inform others who might have been exposed during your visit.

COVID-19 Safety Plan
Specific steps have been taken to minimize the risk of contracting or spreading COVID-19. Specifically, screening will occur for researchers and participants (i.e., completion of CVKey app symptom checker) prior to being allowed entry to the building. If any researcher or participants report or exhibit symptoms of COVID-19, they will be instructed to go/stay home and follow instructions provided on the by Lawrence-Douglas County Health Department, Douglas County COVID Hotline, or Watkins Health Center. Screening and health statuses will be documented on the study-specific procedural integrity checklist. While in the research area, researchers and participants will socially distance from each other (e.g., 6 ft apart) and will wear cloth or surgical face masks for the duration of all sessions. Prior to all sessions, researchers will use an Environmental Protection Agency (EPA) approved odorless sanitizer (Member's Mark sanitizing solution) to disinfect all surfaces of items or fixtures that participants may come into contact with. Following all sessions, researchers and participants will wash their hands and researchers will disinfect all surfaces of items or fixtures that participants touched.

Payment to participants
No payment will be made to you.

Confidentiality

All research related records and information from this study will be kept confidential. Research results will only be presented to others using participant number or alias. Be assured that your name will not be associated with the research findings in any way. By signing this form, you give permission for the use and disclosure of your information, excluding your name, for purposes of this study at any time in the future.

Video observations of sessions conducted with you will be kept on a locked computer that can only be accessible via password by the research team. Any other use of videos (e.g., for educational or conference presentation purposes) will not occur without signed permission from you.

Private Information (Data)
Your identifiable information will be removed from the data collected during this project, and the de-identified data may be used for future research without additional consent from you.

Consent refusal and withdrawal of consent
You may withdraw your consent to participate at any point in time for this study. You also have the right to cancel your permission to use and disclose information collected about you, in writing, at any time, by sending your written request to Rachel Jess or Claudia Dozier (see address below). If you cancel permission to use your information, the researchers will stop collecting additional information about you. However, the research team may use and disclose information that was gathered before they received your cancellation, as described above.

Questions
I have read the information in this form. I know if I have any more questions after signing this from, I should contact Rachel Jess at (936) 522-7027 or Claudia Dozier at (785) 864-0526. If I have any questions about my rights as a research participant, I may call (785) 864-7429 or write the Human Research Protection Program, University of Kansas, Youngberg Hall, 2385 Irving Hill Road, Lawrence, Kansas 66045.

Consent
The investigators gave me information about what will be done in this research study. They also told me how it will be done, what I will have to do, and how long the research will take. The investigators told me about any inconvenience, discomfort, or risks I might experience due to this research. I am aware that I may quit or refuse any part of the research study at any time. I know that if I have any more questions after signing this form, I may contact the investigator directly or the Human Research Protection Program listed above.

Rachel L. Jess, M.A.
Principal Investigator
Applied Behavioral Science
University of Kansas
4001 Dole Building
Lawrence, KS 66045
(785) 864-4840

Claudia L. Dozier, Ph.D.
Faculty Supervisor
Applied Behavioral Science
University of Kansas
4001 Dole Building
Lawrence, KS 66045
(785) 864-0256

Appendix F

Handwashing Study Recruitment Questionnaire

Have you experienced any of the following sensitivities in one or both hands (including fingernails) in the last 2 weeks?

Yes	No	
		Sensitivity to UV light
		Cracked skin
		Pain
		Itching
		Burning
		Tingling
		Skin tightness
		Blisters
		Redness
		Swelling
		Scabs
		Flaking skin
		Eczema
		Rash
		Dermatitis
		Rosacea

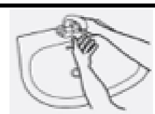
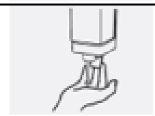
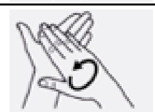
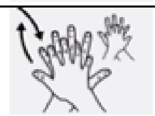
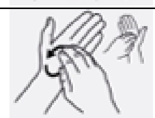
Appendix G

COVID-19 Treatment Integrity Checklist

Safety Procedure	Date	Person Completing the Form	Implementation Status 2 = fully implemented 1 = partially implemented 0 = not in place	Comments
PRIOR TO SESSION				
Sanitize faucet				
Sanitize soap dispenser				
Sanitize paper towel dispenser				
Sanitize countertop				
Sanitize UV light box				
Experimenter PPE				
Participant PPE				
Experimenter health screen				
Participant health screen				
DURING SESSION				
Social distancing in place				
FOLLOWING SESSION				
Sanitize faucet				
Sanitize soap dispenser				
Sanitize paper towel dispenser				
Sanitize countertop				
Sanitize UV light box				
Provide participant hand sanitizer				
			%	

*If below 80%, safety procedures will be re-addressed

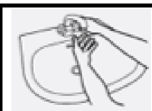
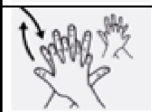
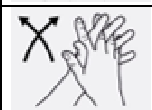
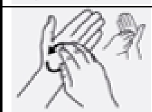
Appendix H

	Correct Handwashing - Handwashing Steps			Response
SOAP	1. Turn on water and wet hands		Turn on water and place hands under water such that hands (from wrist to fingertip, bottom and top) are wet	C C/FB I I/FB
	2. Dispense soap		Place hand under soap dispenser and depresses pump on dispenser such that at least one hand has soap	C C/FB I I/FB
20 seconds	3. Scrub **palms** of hands		Rub palms of both hands together	C C/FB I I/FB
	4. Scrub **tops** of hands		Rub top of each hand with palm of opposite hand	C C/FB I I/FB
	5. Scrub **between fingers**		Rub hands together such that the webs of each finger of each hand have visible soap suds	C C/FB I I/FB
	6. Scrub **fingernails**		Scrub nails of hands such that each fingernail of each hand has visible soap suds	C C/FB I I/FB
Moderate Vigor	7. Rinse hands		Place hands underneath stream of water and rinse both hands clean such that no suds are visible on the hands	C C/FB I I/FB
	8. Dry hands		Use paper towel to dry tops and bottoms of hand. Water should not drip off hands following drying	C C/FB I I/FB

9. Duration	10. Vigor
________	LOW MODERATE HIGH

KEY:
C: Correct C/FB: Correct after FB
I: Incorrect I/FB: Incorrect after FB

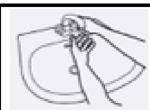
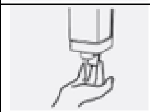
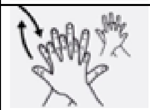
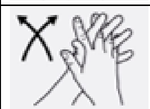
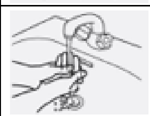
Appendix I

	NO SOAP - Handwashing Steps			Response
NO SOAP	1. Turn on water and wet hands		Turn on water and place hands under water such that hands (from wrist to fingertip, bottom and top) are wet	C C/FB I I/FB
	[redacted]			
20 seconds	3. Scrub **palms** of hands		Rub palms of both hands together	C C/FB I I/FB
	4. Scrub **tops** of hands		Rub top of each hand with palm of opposite hand	C C/FB I I/FB
	5. Scrub **between fingers**		Rub hands together such that the webs of each finger of each hand have visible soap suds	C C/FB I I/FB
	6. Scrub **fingernails**		Scrub nails of hands such that each fingernail of each hand has visible soap suds	C C/FB I I/FB
Moderate Vigor	7. Rinse hands		Place hands underneath stream of water and rinse both hands clean such that no suds are visible on the hands	C C/FB I I/FB
	8. Dry hands		Use paper towel to dry tops and bottoms of hand. Water should not drip off hands following drying	C C/FB I I/FB

9. Duration	10. Vigor
________	LOW MODERATE HIGH

KEY:
C: Correct C/FB: Correct after FB
I: Incorrect I/FB: Incorrect after FB

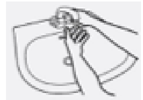
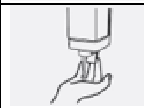
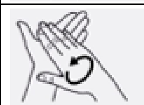
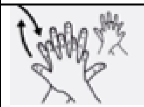
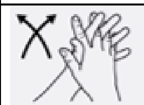
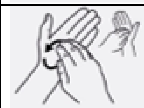
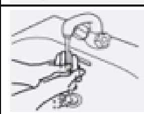
Appendix J

	LOW VIGOR - Handwashing Steps			Response
SOAP	1. Turn on water and wet hands		Turn on water and place hands under water such that hands (from wrist to fingertip, bottom and top) are wet	C C/FB I I/FB
	2. Dispense soap		Place hand under soap dispenser and depresses pump on dispenser such that at least one hand has soap	C C/FB I I/FB
20 seconds	3. Scrub **palms** of hands		Rub palms of both hands together	C C/FB I I/FB
	4. Scrub **tops** of hands		Rub top of each hand with palm of opposite hand	C C/FB I I/FB
	5. Scrub **between fingers**		Rub hands together such that the webs of each finger of each hand have visible soap suds	C C/FB I I/FB
	6. Scrub **fingernails**		Scrub nails of hands such that each fingernail of each hand has visible soap suds	C C/FB I I/FB
Low Vigor	7. Rinse hands		Place hands underneath stream of water and rinse both hands clean such that no suds are visible on the hands	C C/FB I I/FB
	8. Dry hands		Use paper towel to dry tops and bottoms of hand. Water should not drip off hands following drying	C C/FB I I/FB

9. Duration	10. Vigor
________	LOW MODERATE HIGH

KEY:
C: Correct C/FB: Correct after FB
I: Incorrect I/FB: Incorrect after FB

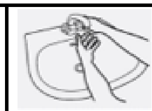
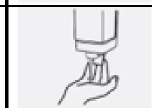
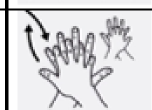
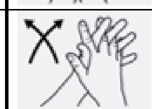
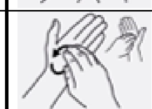
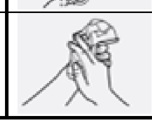
Appendix K

	HIGH VIGOR - Handwashing Steps			Response
SOAP	1. Turn on water and wet hands		Turn on water and place hands under water such that hands (from wrist to fingertip, bottom and top) are wet	C C/FB I I/FB
	2. Dispense soap		Place hand under soap dispenser and depresses pump on dispenser such that at least one hand has soap	C C/FB I I/FB
20 seconds	3. Scrub **palms** of hands		Rub palms of both hands together	C C/FB I I/FB
	4. Scrub **tops** of hands		Rub top of each hand with palm of opposite hand	C C/FB I I/FB
	5. Scrub **between fingers**		Rub hands together such that the webs of each finger of each hand have visible soap suds	C C/FB I I/FB
	6. Scrub **fingernails**		Scrub nails of hands such that each fingernail of each hand has visible soap suds	C C/FB I I/FB
High Vigor	7. Rinse hands		Place hands underneath stream of water and rinse both hands clean such that no suds are visible on the hands	C C/FB I I/FB
	8. Dry hands		Use paper towel to dry tops and bottoms of hand. Water should not drip off hands following drying	C C/FB I I/FB

9. Duration	10. Vigor
________	LOW MODERATE HIGH

KEY:
C: Correct C/FB: Correct after FB
I: Incorrect I/FB: Incorrect after FB

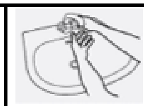
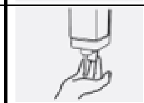
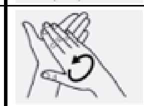
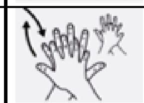
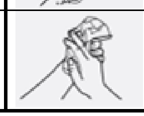
Appendix L

	10 SECONDS - Handwashing Steps			Response
SOAP	1. Turn on water and wet hands		Turn on water and place hands under water such that hands (from wrist to fingertip, bottom and top) are wet	C C/FB I I/FB
	2. Dispense soap		Place hand under soap dispenser and depresses pump on dispenser such that at least one hand has soap	C C/FB I I/FB
10 seconds	3. Scrub **palms** of hands		Rub palms of both hands together	C C/FB I I/FB
	4. Scrub **tops** of hands		Rub top of each hand with palm of opposite hand	C C/FB I I/FB
	5. Scrub **between fingers**		Rub hands together such that the webs of each finger of each hand have visible soap suds	C C/FB I I/FB
	6. Scrub **fingernails**		Scrub nails of hands such that each fingernail of each hand has visible soap suds	C C/FB I I/FB
Moderate Vigor	7. Rinse hands		Place hands underneath stream of water and rinse both hands clean such that no suds are visible on the hands	C C/FB I I/FB
	8. Dry hands		Use paper towel to dry tops and bottoms of hand. Water should not drip off hands following drying	C C/FB I I/FB

9. Duration	10. Vigor
________	LOW MODERATE HIGH

KEY:
C: Correct C/FB: Correct after FB
I: Incorrect I/FB: Incorrect after FB

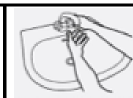
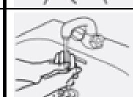
Appendix M

	5 SECONDS - Handwashing Steps			Response
SOAP	1. Turn on water and wet hands		Turn on water and place hands under water such that hands (from wrist to fingertip, bottom and top) are wet	C C/FB I I/FB
	2. Dispense soap		Place hand under soap dispenser and depresses pump on dispenser such that at least one hand has soap	C C/FB I I/FB
5 seconds	3. Scrub **palms** of hands		Rub palms of both hands together	C C/FB I I/FB
	4. Scrub **tops** of hands		Rub top of each hand with palm of opposite hand	C C/FB I I/FB
	5. Scrub **between fingers**		Rub hands together such that the webs of each finger of each hand have visible soap suds	C C/FB I I/FB
	6. Scrub **fingernails**		Scrub nails of hands such that each fingernail of each hand has visible soap suds	C C/FB I I/FB
Moderate Vigor	7. Rinse hands		Place hands underneath stream of water and rinse both hands clean such that no suds are visible on the hands	C C/FB I I/FB
	8. Dry hands		Use paper towel to dry tops and bottoms of hand. Water should not drip off hands following drying	C C/FB I I/FB

9. Duration	10. Vigor
________	LOW MODERATE HIGH

KEY:
C: Correct C/FB: Correct after FB
I: Incorrect I/FB: Incorrect after FB

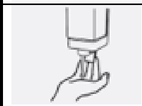
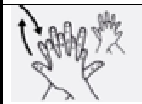
Appendix N

	MISSING TOPS- Handwashing Steps			Response
SOAP	1. Turn on water and wet hands		Turn on water and place hands under water such that hands (from wrist to fingertip, bottom and top) are wet	C C/FB I I/FB
	2. Dispense soap		Place hand under soap dispenser and depresses pump on dispenser such that at least one hand has soap	C C/FB I I/FB
20 seconds	3. Scrub **palms** of hands		Rub palms of both hands together	C C/FB I I/FB
	[redacted]			
	5. Scrub **between fingers**		Rub hands together such that the webs of each finger of each hand have visible soap suds	C C/FB I I/FB
	6. Scrub **fingernails**		Scrub nails of hands such that each fingernail of each hand has visible soap suds	C C/FB I I/FB
Moderate Vigor	7. Rinse hands		Place hands underneath stream of water and rinse both hands clean such that no suds are visible on the hands	C C/FB I I/FB
	8. Dry hands		Use paper towel to dry tops and bottoms of hand. Water should not drip off hands following drying	C C/FB I I/FB

9. Duration	10. Vigor
________	LOW MODERATE HIGH

KEY:
C: Correct C/FB: Correct after FB
I: Incorrect I/FB: Incorrect after FB

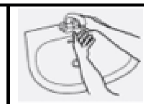
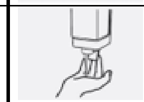
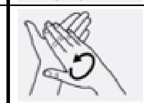
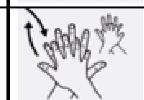
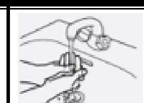
Appendix O

	MISSING BETWEEN FINGERS- Handwashing Steps			Response
SOAP	1. Turn on water and wet hands		Turn on water and place hands under water such that hands (from wrist to fingertip, bottom and top) are wet	C C/FB I I/FB
	2. Dispense soap		Place hand under soap dispenser and depresses pump on dispenser such that at least one hand has soap	C C/FB I I/FB
20 seconds	3. Scrub **palms** of hands		Rub palms of both hands together	C C/FB I I/FB
	4. Scrub **tops** of hands		Rub top of each hand with palm of opposite hand	C C/FB I I/FB
	[redacted]			
	6. Scrub **fingernails**		Scrub nails of hands such that each fingernail of each hand has visible soap suds	C C/FB I I/FB
Moderate Vigor	7. Rinse hands		Place hands underneath stream of water and rinse both hands clean such that no suds are visible on the hands	C C/FB I I/FB
	8. Dry hands		Use paper towel to dry tops and bottoms of hand. Water should not drip off hands following drying	C C/FB I I/FB

9. Duration	10. Vigor
________	LOW MODERATE HIGH

KEY:
C: Correct C/FB: Correct after FB
I: Incorrect I/FB: Incorrect after FB

Appendix P

	MISSING FINGERNAILS - Handwashing Steps			Response
SOAP	1. Turn on water and wet hands		Turn on water and place hands under water such that hands (from wrist to fingertip, bottom and top) are wet	C C/FB I I/FB
	2. Dispense soap		Place hand under soap dispenser and depresses pump on dispenser such that at least one hand has soap	C C/FB I I/FB
20 seconds	3. Scrub **palms** of hands		Rub palms of both hands together	C C/FB I I/FB
	4. Scrub **tops** of hands		Rub top of each hand with palm of opposite hand	C C/FB I I/FB
	5. Scrub **between fingers**		Rub hands together such that the webs of each finger of each hand have visible soap suds	C C/FB I I/FB
	[redacted]			
Moderate Vigor	7. Rinse hands		Place hands underneath stream of water and rinse both hands clean such that no suds are visible on the hands	C C/FB I I/FB
	8. Dry hands		Use paper towel to dry tops and bottoms of hand. Water should not drip off hands following drying	C C/FB I I/FB

9. Duration	10. Vigor
________	LOW MODERATE HIGH

KEY:
C: Correct C/FB: Correct after FB
I: Incorrect I/FB: Incorrect after FB

Appendix Q

Study 2 Materials

UV-Light Box

Glo Germ™

UV Light