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ORIGINAL PAPER

# Use of ATP Readings to Predict a Successful Hygiene Intervention in the Workplace to Reduce the Spread of Viruses on Fomites

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**Abstract** The purpose of this study was to validate the use of adenosine triphosphate (ATP) for evaluating hygiene intervention effectiveness in reducing viral dissemination in an office environment. The bacterial virus MS-2 was used to evaluate two scenarios, one where the hand of an individual was contaminated and another where a fomite was contaminated. MS-2 was selected as a model because its shape and size are similar to many human pathogenic viruses. Two separate experiments were conducted, one in which the entrance door push plate was inoculated and the other in which the hand of one selected employee was inoculated. In both scenarios, 54 selected surfaces in the office were tested to assess the dissemination of the virus within the office. Associated surface contamination was also measured employing an ATP meter. More than half of the tested hands and surfaces in the office were contaminated with MS-2 within 4 h. Next, an intervention was conducted, and each scenario was repeated. Half of the participating employees were provided hand sanitizer, facial tissues, and disinfecting wipes, and were instructed in their use. A significant ($p < 0.05$) reduction was observed in the number of surfaces contaminated with virus. This reduction in viral spread was evident from the results of both viral culture and the surface ATP measurements, although there was no direct correlation between ATP measurements with respect to viral concentration. Although ATP does not measure viruses, these results demonstrate that ATP measurements could be useful for evaluating the effectiveness of hygiene interventions aimed at preventing viral spread in the workplace.

**Keywords** Hygiene · Adenosine triphosphate (ATP) · Viral dispersion · Workplace · Intervention · MS-2 virus

## Introduction

Enteric and respiratory illnesses are readily spread among persons working together in an office environment, and can result in significant economic and productivity losses (Bramley et al. 2002; Birbaum et al. 2003; Callan et al. 2005). Although implementation of hygiene interventions may reduce the spread of viral illnesses in the workplace, traditional methods for evaluating their effectiveness are costly and time-consuming. Rapid methods for screening of relative biological loads on surfaces could be helpful in evaluating the efficacy of mitigation efforts. In this study, the measurement of ATP was used to determine its value as a rapid screening method for evaluation of workplace hygiene interventions in reducing the potential for viral spread.

Common illnesses of viral etiology, such as colds and diarrhea, have a significant impact on health care costs and absenteeism among office employees (Bramley et al. 2002). The increasingly globalized economy creates greater opportunity than ever before for viral transmission, as evidenced by the recent and emerging viral pandemics such as those caused by H1N1 (Swine Flu), H7N9 (Avian

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Flu), and norovirus (Cauchemez et al. 2009; Morens et al. 2013; Hall et al. 2013). Even if an employee is not absent as a result of viral infection, the increased cost of significantly reduced productivity can equal or exceed those of absenteeism attributed to illness (Lamb et al. 2006).

In these relatively enclosed environments, commonly touched surfaces, such as break room tables, photocopying machines, door entrances, and restrooms, represent the most likely routes for the spread of enteric and respiratory viruses (Boone and Gerba 2007). Fomites, inanimate objects or surfaces that serve as microbial transmission vehicles, are contaminated by infected individuals through either direct contact or by the settling of aerosols created by sneezing or coughing. The viruses are then transferred to the hands of the individuals touching these surfaces, and are subsequently introduced to the site of infection (i.e., nose, mouth, or eyes). Nicas and Jones (2009) found that adults contact these critical points of microbial entry to the human body approximately 16 times each hour. Because viruses can survive on fomites from a few hours to a month (Boone and Gerba 2007), contaminated surfaces represent an important means of infectious disease transmission.

Bacterial viruses (phages), which do not infect humans, have been used to study the movement of viruses in indoor environments such as day care centers, neonatal nurseries, and home settings (Rheinbaben et al. 2000; Gerhardts et al. 2012; Lopez et al. 2013). The bacterial virus MS-2 was used in this study to represent pathogenic virus spread in the office environment. MS-2 infects the bacterium *Escherichia coli* and is very similar in shape and size (23 nm) to rhinovirus, norovirus (most common cause of adult gastroenteritis), and many other enteric viruses. In addition to studying viral transmission patterns, bacteriophage models are also useful in the validation of preventive measures (Mamane-Gravetz and Linden 2004).

Availability and use of hygiene interventions have been shown effective in the disruption of both direct contact and surface-mediated microbial transmission in school and hospital environments (Liu et al. 2009; Bright et al. 2010; Bloomfield et al. 2016). Preventive measures such as hand washing (Ryan et al. 2001;Curtis and Cairncross 2003) and use of hand sanitizers (Sandora et al. 2005), as well as antimicrobial wipes and cleaners (Kochar et al. 2009), have been demonstrated to reduce gastrointestinal and respiratory infectious disease incidence. In addition, proper uses of disposable surgical masks and facial tissues have been used to reduce transmission of respiratory infections through aerosolization. Although these measures have been shown effective when properly implemented, there is not currently a rapid, cost-effective means to routinely monitor their sustained effectiveness.

Adenosine triphosphate (ATP), the universal energy molecule found in all animal, plant, bacterial, yeast, and mold cells, produces bioluminescence that has been suggested as a potential alternative for rapid confirmation of hygiene intervention effectiveness. ATP detection methods have been validated for assessment of relative microbial presence in aquatic environments (Hammes et al. 2010) and intravenous fluids (Anderson et al. 1986), as well as on inanimate surfaces in hospitals (Moore et al. 2010; Aikin et al. 2011) and food preparation areas (Aycicek et al. 2006). ATP is thus used as a general indicator of cleanliness of a surface. Simple, hand held, ATP meters are now available that allow for testing of fomites with results available in 15 s (Hygienia 2016). This creates an opportunity for rapid screening of fomites with minimum cost and time.

This study describes the effectiveness of a workplace hygiene intervention in preventing the spread of a bacterial virus in an office environment as a model for removal of a pathogenic virus. ATP measurements were then compared to viral load after the implementation of the hygiene intervention to determine if the general cleanliness of a surface could be correlated with the removal of viral pathogens. This could provide an alternative monitoring tool for the simple and rapid confirmation of the effectiveness of a hygiene intervention on reducing the spread of viruses in the workplace.

## Materials and Methods

### Study Approach

MS-2 was used to evaluate two scenarios, one where the hand of one selected employee was contaminated and another where a fomite, the entrance door push plate, was contaminated. Samples from 54 selected surfaces were sampled in the office setting at the beginning of the day and at 4 and 7 h after inoculation to assess the dissemination of the virus within the office. The samples were subsequently tested using traditional viral culture and ATP detection methods to assess the spread of the virus through the office environment from each of the two starting points. Each experiment was repeated after the employees were instructed on the use of provided hygiene interventions including facial tissues, disinfecting wipes, and hand sanitizer, as well as hand-washing techniques using basic (not labeled as anti-microbial) hand soap.

### Office Description

This study was conducted in an office setting with approximately 80 full-time employees. The office is located on the second floor of a three-story building, with three stairway accesses and one elevator access to the floor. The

main entry door to the floor is located near the elevator, such that persons exiting the elevator enter through the main door. The entire office shares a common kitchen area (break room) equipped with a microwave oven, a sink area, a coffee machine, and a refrigerator. Other features include several individual offices with doors located along the perimeter of the floor, a central area divided into cubicles, and seven locations containing shared photocopy machines. A floor plan of the office can be seen in Fig. 1.

### MS-2 Virus

MS-2 (ATCC 15597-B1) was obtained from the American Type Culture Collection (ATCC Manassas, VA). MS-2 virus was prepared as previously described with minor modifications (Rusin et al. 2002). The agar overlay technique was used to isolate and enumerate phage MS-2. Dilutions of sample suspension (1 mL) followed by log-phase host culture (1 mL) were added to melted top agar tubes. The inoculated top agar tubes were mixed and poured over a TSA plate, and then the solidified agar overlay was inverted and incubated at 37 °C for 24 h. Phage plaques were counted, and the concentration of phage isolated per 100 $cm^2$ was calculated for each fomite sampled.

### ATP Detection

ATP bioluminescence was measured in relative light units (RLU) using a SystemSURE$^{TM}$ ATP meter (Hygiena, Camarillio, CA) according to the manufacturer's recommended protocol. An adjacent 100 $cm^2$ to where the phage sample was collected was swabbed with the swabs provided by the manufacturer for use with the device.

### Surface Inoculation

An area of approximately 50 $cm^2$ on an office entrance door push plate and the hand of one of employee were inoculated with $6 \times 10^9/cm^2$ plaque forming units (PFU) of MS-2 bacterial virus. This was done before the arrival of any of the employees to ensure they were unaware of the inoculation. This level of phage was used to make it easily traceable through the facility and not necessarily to reflect an actual viral load from an infected person. However, this level is not out of reality as persons infected with adenovirus, rotavirus, and norovirus may have $10^{11}$–$10^{12}$ virus particles per gram of feces (Maier et al. 2009). Thus, $10^9$ would represent only 0.01–0.001 grams of fecal material.

### Sample Collection

Samples from 54 selected fomites and the hands of 42 participating employees were collected for viral assays using sterile cotton transport swabs containing a buffer to neutralize any residual disinfectant used on the surface or hand (3 M Corporation, St. Paul, MN). Fomites sampled included desk tops, table tops, refrigerator door handles, microwave oven door handles, coffee pot handles, and vending machine buttons. (Reynolds et al. 2016). Fomite samples for ATP detection were also collected from adjacent surface areas using swabs recommended by the manufacturer for use with the SystemSURE$^{TM}$ ATP meter (Hygiena, Camarillio, CA). Each swab was aseptically removed from its transport container, swabbed over an area of approximately 100 $cm^2$ for each surface, and carefully returned to its transport container.

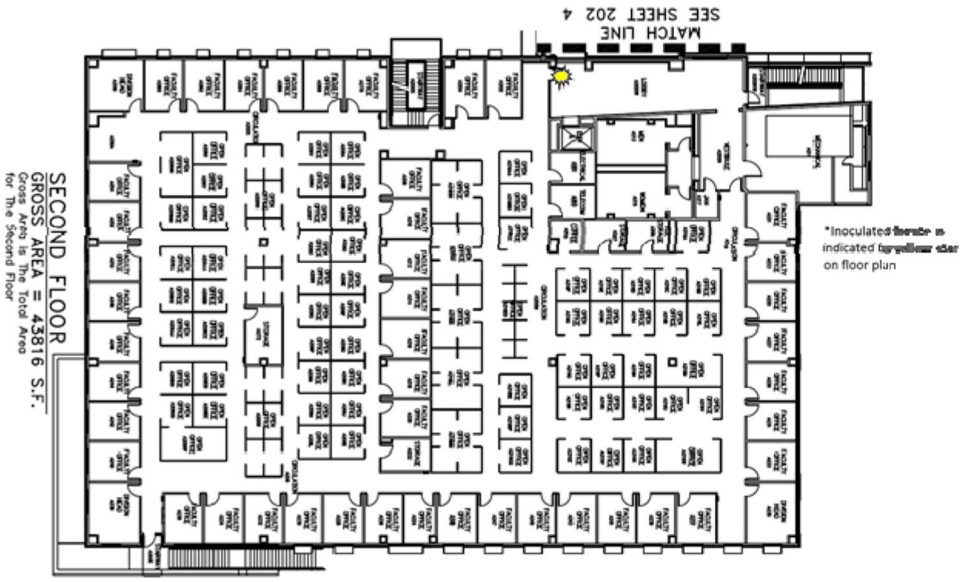


**Fig. 1** Office floor plan

### The Healthy Workplace Project Intervention

Individual offices and communal areas were provided with a series of intervention products intended to reduce the spread of virus. The hygiene intervention was evaluated by conducting the same inoculation and sampling protocol before and after its implementation. The following products were provided for each individual office of these individuals who agreed to participate in the intervention: a bactericidal hand sanitizer (KIMTECH Moisturizing Instant Hand Sanitizer, Kimberly-Clark Professional, Roswell, GA), virucidal and bactericidal surface disinfecting wipes (SCOTT® Disinfectant Wipes, Kimberly-Clark Professional, Roswell, GA), and facial tissue (KLEENEX® Tissue, Kimberly-Clark Corporation, Neenah, WI). Of the 80 persons in the office, 42 agreed to take part in the intervention study.

Restrooms were stocked with basic hand soap (not antibacterial) and paper towels. The communal areas were provided with a bactericidal hand sanitizer with stands/dispensers (KLEENEX® Moisturizing Instant Hand Sanitizer, Kimberly-Clark Global Sales, Roswell, GA). The break room was provided with food safe bactericidal sanitizing wipes (KIMTECH PREP* Surface Sanitizer Wipes, Kimberly-Clark Professional, Roswell, GA), and the conference rooms were equipped with a bactericidal hand sanitizer (KIMTECH* Moisturizing Instant Hand Sanitizer, Kimberly-Clark Professional, Roswell, GA), virucidal and bactericidal surface disinfecting wipes (SCOTT® Disinfectant Wipes, Kimberly-Clark Professional, Roswell, GA), and facial tissue (KLEENEX® Tissue, Kimberly-Clark, Neenah, WI).

Employees were instructed on the implementation of the Healthy Workplace Project. They were advised to sanitize hands when entering and leaving the office, as well as before and after shaking hands, after touching communal surfaces, and after touching the nose or face. In addition, they were provided with tissues to wipe or blow their noses, and instructed to wash their hands for 15 s with soap and dry with a clean paper towel after using the restroom and before eating food. Employees were advised to use disinfecting wipes to clean desk areas (keyboard, mouse, and phone) at the beginning of each day and to wipe down the conference room table before starting a meeting; and to use sanitizing wipes to clean frequently touched items in the break room such as refrigerator handles, microwave handles and buttons, coffee pot handles, vending machine buttons, and tables.

#### *Statistical Analysis*

Adenosine triphosphate (ATP) measurements taken before and after the intervention were compared with results from the viral culture analysis to identify any statistical correlations. The statistical analysis was performed using Microsoft® Excel® 2010 (Microsoft Corporation, Redmond, WA). The data were plotted to determine whether it followed a normal distribution model. The log-transformed data ($\log_{10}$) were normally distributed, which allowed us to perform the Analysis of Variance (ANOVA) with the assumption of normal distribution. In order to answer specific questions, multiple ANOVA tests were conducted on the data to test different hypotheses. Completely randomized designs were used to perform the ANOVA, with a rejection region of 5 % using the $F$ distribution.

## Results

In order to evaluate the effectiveness of a hygiene intervention for the reduction of MS-2 in an office setting, 54 samples were collected from commonly touched surfaces for two baseline experiments and two interventions. Correlations between the viral data and ATP measurements were used to evaluate ATP as a rapid indicator of the success of the intervention. No bacterial viruses were detected on the fomites before the beginning of the study (i.e., no background virus was recovered). MS-2 was observed to spread throughout the office setting rapidly, within 4 h, to a variety of fomites. Before implementation of The Healthy Workplace Project, MS-2 virus had spread to approximately 56 % of the fomites sampled within 4 h after inoculation of one employee's hand (Fig. 2), and increased to 63 % at 7 h after inoculation.

Implementation of The Healthy Workplace Project resulted in a reduction of MS-2 on fomites. The virus was detected on 9 % of the fomites after 4 h and 30 % after 7 h (Fig. 2). After 7 h, the number of contaminated fomites and hands increased, but was still half as much that was observed on the surfaces before the intervention (Fig. 3). When the door push plate was contaminated, there were

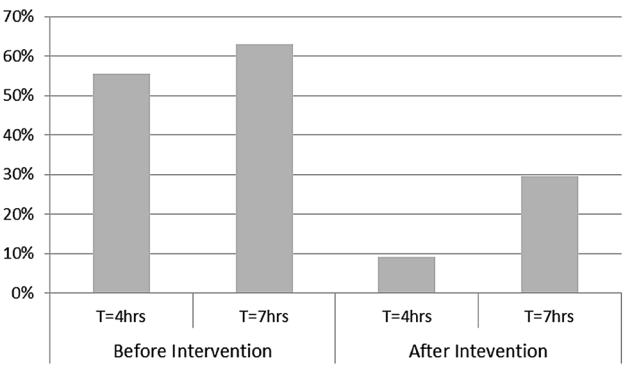


**Fig. 2** Percent positive of MS-2 occurrence on surfaces before and after intervention

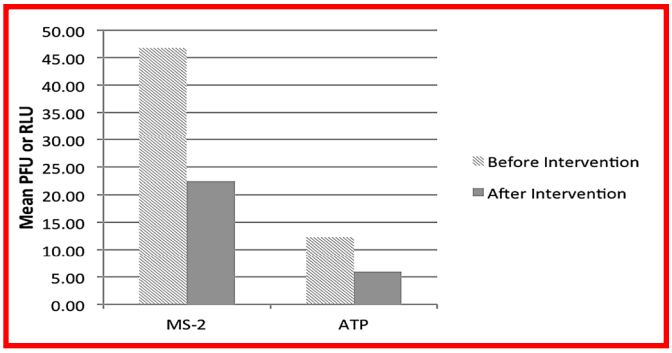


**Fig. 3** Virus concentrations per 100 $cm^2$ on surfaces ($n = 54$) and ATP relative light units (RLU) before and after intervention

70 % fewer fomites contaminated after 4 h during the intervention portion of the study. Thus, there was a significant overall reduction in virus occurrence on both hand and fomite surfaces after implementation of The Healthy Workplace Project.

A statistically significant difference was observed between MS-2 concentrations with no intervention and intervention experiments on fomites at the 4 h sampling time ($p < 0.005$). Using the ANOVA test with a 5 % rejection region, the differences between pre-intervention concentrations of MS-2 and those observed after a 7-h workday remained marginally significant ($p = 0$ 054). Combined data from both the 4 and 7 h time points demonstrated that the impact of the intervention was highly significant for reducing the exposure to MS-2 (Fig. 2). The number of sites with an ATP reading of greater than 2 RLU was 45 % before the intervention and 15 % after the intervention. This difference was shown to be statistically significant ($p < 0.005$). Thus, ATP readings were useful in determining the success of the hygiene intervention as they demonstrated a significant reduction of virus on the sampled fomites after the intervention.

## Discussion and Conclusions

ATP bioluminescence is a general measurement of biological contamination. It does not directly monitor viruses, but indicates a mixture of biological forms, such as human cellular materials, bacteria, and plant and fungal cells. Materials like epithelium from the upper respiratory tract mucus membranes, saliva, and associated material from the coughs and sneezes of persons with viral or bacterial infections can also contribute to ATP measurements (Shaughnessy et al. 2013).While there are limits to the use of ATP measurements in assessing the impact of cleaning practices (Green et al. 1999), recent studies have demonstrated its potential usefulness for validating the effectiveness of cleaning practices in schools (Shaughnessy et al. 2013) and hospitals (Boyce et al. 2009a, b).

The rapid spread of virus throughout the office after a high-touch surface (office door bar) or the hand of one employee was inoculated illustrates how a contaminated surface can result in viral transmission in the workplace. Shared facilities (e.g., break room, copy machine), where transfer of viruses from infected to uninfected persons is most likely to occur, were readily contaminated. The viral culture results show statistically significant reductions in the spread of virus (MS-2) from either a contaminated hand or fomite throughout an office environment after implementation of hygiene interventions. This was accomplished with only 52 % of the occupants of the building participating in the intervention, demonstrating that a significant reduction in virus spread can occur without everyone in the office following the intervention protocol. In addition, this improvement occurred after the intervention protocol was in place for only 3 days before the virus was seeded onto the hands/push plate. The seeding of the door handle or hand of an employee made no significant difference in the spread of the virus throughout the facility or the success of the intervention (Reynolds et al. 2016). ATP measurements correlated significantly with reduced viral recovery and showed statistically significant differences in measurements taken before and after an intervention. This suggests that although ATP readings do not specifically predict the occurrence or degree of reduction in microbial contamination, the method can be useful for monitoring the success of health interventions in the workplace in terms of the reduction in the spread of a virus.

Despite the described limitations of ATP measurement as a method for monitoring microbial contamination, the results of this study clearly demonstrate that workplace hygiene interventions can result in a significant reduction of viral contamination, and ATP can be used to monitor performance rapidly. It also illustrates that a general measure of cleanliness with a quantitative tool can be related to the spread of viruses in indoor environments and can be used as an aid to assess of potential interventions .

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