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Major Article

Comparing bacterial, fungal, and human cell concentrations with rapid adenosine triphosphate measurements for indicating microbial surface contamination

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Background: The goal of this study was to test for associations between adenosine triphosphate (ATP) content and microbial concentrations on desk surfaces in school classrooms.

Methods: ATP bioluminescence and quantitative polymerase chain reaction (qPCR) techniques were employed to measure total bacterial, fungal, and human cell concentrations on 66 high-traffic desks spread across 9 schools: 3 in Connecticut (CT) and 6 in Oklahoma (OK). In CT, 6 samples were taken from each desk, 1 precleaning and 5 postcleaning (after 30 minutes, 1 day, 3 days, 7 days, and 21 days). In OK, samples were taken immediately before and after cleaning each desk.

Results: Based on simple linear regression analyses, ATP values were good predictors of microbial concentrations ($r = 0.8$, $P = .003$) in both CT school postcleaning samples and OK pre- and postcleaning samples ($r = 0.7$, $P = .00002$). However, biomass reductions measured after cleaning were 1.5-2 times greater when measured by ATP than by qPCR ($P = .007$).

Conclusions: Overall, ATP bioluminescence measurements correlate with qPCR-based surface measurements on school desks but may overestimate the physical removal of bacteria and fungi due to cleaning.

Surface-associated bacteria and fungi present significant pathogen and allergen exposures to humans. Pathways include direct contact with fomites that may result in microbe ingestion or entrance into the body via mucous membranes[1,2] or inhalation or ingestion of microbes that are aerosolized via resuspension from surfaces.[3] These microbial pathogen and allergen exposures are particularly important in high-occupancy environments, including schools and health care facilities, where humans are strong contributors to surface[4] and indoor air microbiomes.[5] Improved surface cleaning interventions and removal of microbial sources have contributed to a reduction of asthma triggers, infectious agents, and incidence of upper respiratory symptoms.[6–8]

Adenosine triphosphate (ATP) measurement is 1 approach for rapidly assessing the effectiveness of cleaning to remove biomass from surfaces in schools and health care facilities. ATP is an intracellular energy storage molecule present in all forms of life and can be quantified via luminescence resulting from the luciferase-catalyzed reaction between ATP and luciferin. ATP concentration on surfaces can be quantitatively measured using commercially available hand-held units that display the ATP content swabbed from a surface as relative light units (RLUs). These commercially available units have been used widely to measure biological cleaning effectiveness on a variety of surfaces[9,10] and as an important marker for surface biocontamination

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in hospitals, schools, and the food industry.[10-13] Important advantages of ATP measurements include rapid results, low cost, and ease of use. However, ATP does not measure cells, which are considered the basic units of most microbial exposures. Thus, there is uncertainty as to how ATP reflects the more biologically relevant measure of cell concentration. Additionally, ATP is present in nonmicrobial biomass, including food residues, human skin cells, plant and animal cells, and other organic residuals. ATP can also fluctuate significantly because of variability within cells.[14,15]

To determine the representativeness of ATP for indicating surface-borne bacterial and fungal contamination, it is necessary to better quantify the relationship between ATP measurements and concentrations of microbial cells. The goal of this study was to test for associations between ATP content on school desk surfaces and concentrations of bacteria and fungi assessed by quantitative polymerase chain reaction (qPCR), which detects live, dead, and unculturable organisms. Side-by-side comparisons of surface cleanliness using ATP measurements in RLUs and qPCR values for total fungal, bacterial, and human cell concentrations in cells per surface area were conducted. Comparisons were performed prior to and after a standard cleaning event on school desks in Connecticut (CT) and Oklahoma (OK).

METHODS

Sample collection

This study was conducted on desks in 3 different CT and 6 different Northeast OK public schools. For sampling, surface-associated microbes and ATP were collected from 2.5 × 2.5-cm high touchpoint areas of each desk (1 sample area for each sample time slot) using autoclaved cotton swabs moistened with sterile 0.15 M sodium chloride and 0.1 % Tween 20 solution (Croda, Snaith, United Kingdom) as previously described[16] for qPCR surface-associated microbial sampling and PocketSwab Plus rapid ATP swabs (Charm Sciences, Lawrence, MA) for ATP surface sampling. Ten desks were selected and sampled in the CT schools—each from a different classroom—ranging from grades 7-12. In these schools, desks were cleaned in accordance with school district cleaning protocols, which included spraying with cleaner and wiping dry with a paper towel. Baseline surface samples were collected from each desk prior to cleaning. Once cleaned, 5 subsequent samples were then collected over a 21-day postcleaning period. This approach allowed for samples of variable age and concentration to be collected (Table 1). In OK, 56 desks were selected from 14 different classrooms, ranging from grades 2-9. Each OK desk was cleaned with a more vigorous protocol than in CT, including dry wiping with a disposable shop towel, then spraying with cleaner until visibly wet. After 60 seconds wet, desks were wiped vigorously with pressure in 2 directions with a clean microfiber cloth. OK samples included samples taken just before and immediately after cleaning (Table 2).

All surface samples were collected from desks prior to and directly after cleaning in each selected classroom. For each desk and time point (both CT and OK), 2 adjacent samples were simultaneously collected—1 in which DNA was extracted and quantified for total bacterial, fungal, and human cells via qPCR and 1 for ATP measurement (Tables 1 and 2 present sampling details).

Sample analysis

ATP measurements were conducted using a commercially available novaLUM detection system (Charm Sciences). This simple method converts ATP into a fluorescent light signal and quantifies ATP mass as RLUs per sampled area.

Cotton-tipped swabs used for DNA sample collection were dropped into 2-mL screw-top tubes and DNA extracted using a DNeasy PowerSoil Kit (Qiagen, Germantown, MD) modified to include bead beating for improved cell lysis.[17] Extracted DNA was then quantified via qPCR for total bacterial, fungal, and human cell analysis using bacterial, fungal, and human universal primers as previously described.[5,17-19] PCR inhibition was tested by spiking sample extracts with known target DNA concentrations. No inhibition was observed.

Statistics

Simple linear regression analysis was used to compare mean log ATP and mean log qPCR concentrations for CT and OK desk samples and qPCR bacterial, fungal, and human cell concentrations with each

Table 1
CT school desk sampling summary

School	Number of classrooms (desks sampled per room)	Sample time points (t_1-t_6)	Analysis type
B	3 (1)	t_1, t_2, t_3, t_4, t_5, t_6	ATP and qPCR for bacterial, fungal, and human cells
O	3 (1)	t_1, t_2, t_3, t_4, t_5, t_6	ATP and qPCR for bacterial, fungal, and human cells
H	4 (1)	t_1, t_2, t_3, t_4, t_5, t_6	ATP and qPCR for bacterial, fungal, and human cells

NOTE. Two adjacent samples were collected simultaneously on each of the 10 desks over 6 time points, 1 precleaning (t_1) and 5 postcleaning (t_2: directly postcleaning, t_3: 1 day, t_4: 3 days, t_5: 7 days, t_6: 21 days postcleaning). Schools B, O, and H used glass cleaner, all-purpose cleaner, and antibacterial disinfectant, respectively.
ATP, adenosine triphosphate; *CT*, Connecticut; *qPCR*, quantitative polymerase chain reaction.

Table 2
OK school desk sampling summary

School	Number of classrooms (desks sampled per room)	Sample time points (t_1-t_2)	Analysis type
1	1 (4)	t_1, t_2	ATP and qPCR for bacterial, fungal, and human cells
2	3 (12)	t_1, t_2	ATP and qPCR for bacterial, fungal, and human cells
3	3 (12)	t_1, t_2	ATP and qPCR for bacterial, fungal, and human cells
4	2 (8)	t_1, t_2	ATP and qPCR for bacterial, fungal, and human cells
5	3 (12)	t_1, t_2	ATP and qPCR for bacterial, fungal, and human cells
6	2 (8)	t_1, t_2	ATP and qPCR for bacterial, fungal, and human cells

NOTE. Two adjacent samples were collected simultaneously on each of the 56 desks pre- (t_1) and immediately postcleaning (t_2). All OK school desks were cleaned with Ramsey FreQuency 256 disinfectant.
ATP, adenosine triphosphate; *OK*, Oklahoma; *qPCR*, quantitative polymerase chain reaction.

other for both CT and OK schools. ATP and qPCR measurements from each CT classroom and each OK classroom were averaged together to accommodate the need for replicate analyses in ATP samples.[20] A 2-tailed paired t test was used to compare the removal efficiency between ATP and qPCR sum measurements. For all tests, a statistical difference was defined as $P < .05$. Statistical analyses were performed using GraphPad Prism 7 (GraphPad Software, La Jolla, CA).

RESULTS

ATP associations with microbial concentrations on desks

Linear regression analyses were conducted to assess relationships between qPCR and ATP concentrations and to see if the qPCR-measured bacterial, fungal, and human cell measurements were independent variables. For all CT postcleaning samples, ATP values were good predictors of bacterial concentration ($r = 0.8$, $P = .003$), human cells ($r = 0.6$, $P = .05$), and qPCR sum ($r = 0.8$, $P = .003$) but not for fungi ($r = 0.5$, $P = .06$) (Fig 1). The slope of the log values for qPCR sum was 0.8 log qPCR/log ATP. An additional regression compared ATP with qPCR values precleaning and postcleaning for 56 desks in OK schools. ATP was a good predictor of bacteria ($r = 0.7$, $P < .00001$), fungi ($r = 0.5$, $P = .01$), human cells ($r = 0.5$, $P = .05$), and qPCR sum ($r = 0.7$, $P = .00002$) for all samples (Fig 2). The slope of the log values for qPCR sum was 0.68 log qPCR/log ATP. Linear regression on qPCR measures from CT schools revealed correlations between bacterial and fungal ($r = 0.6$, $P = .01$) and bacterial and human cells ($r = 0.7$. $P = .01$) but not between fungal and human cells ($r = 0.3$, $P = .3$). Corresponding linear regression analysis of OK qPCR measures showed correlations between bacterial and fungal ($r = 0.7$, $P = .0002$) and bacterial and human cells ($r = 0.6$, $P = .003$) but not between fungal and human cells ($r = 0.4$, $P = .07$).

Effectiveness of cleaning protocols

Figure 3 demonstrates microbial reductions after the cleaning of CT and OK desks. The average removal percentages for CT desks were 46% ± 9.6%, 42% ± 22%, 46% ± 14%, and 46% ± 11% for bacterial, fungal, and human cells and qPCR sum, respectively. ATP indicated a greater biomass removal than measured microbial biomass (removal average 89% ± 5%) ($P = .009$, t test for the difference between qPCR sum and ATP).

The average removal percentages for OK school desks were 54% ± 27%, 25% ± 43%, 16% ± 58%, and 52% ± 29% for bacterial, fungal, and human cells and qPCR sum, respectively. ATP removal values again indicated a greater biomass removal than measured microbial

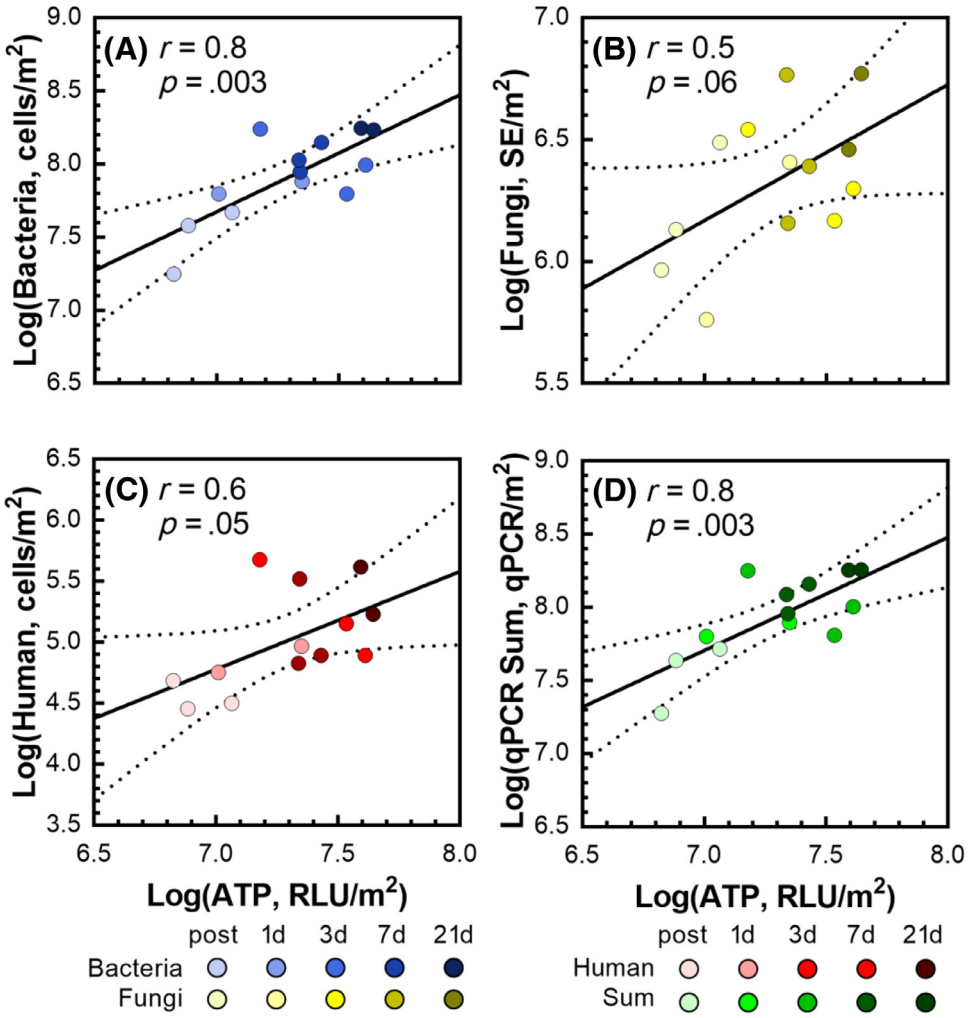

Fig 1. Concentrations of ATP (RLU/m^2) versus (A) bacteria (cells/m^2), (B) fungi (SE/m^2), (C) human cells (cells/m^2), and (D) qPCR sum (sum/m^2) in CT schools, with qPCR concentrations for desk surfaces at time points directly postcleaning: 1 day, 3 days, 7 days, and 21 days postcleaning. Solid lines represent simple linear regression models, and dotted lines represent 95% confidence intervals. *ATP*, adenosine triphosphate; *CT*, Connecticut; *d*, day (s); *qPCR*, quantitative polymerase chain reaction; *RLU*, relative light unit; SE, spore equivalents.

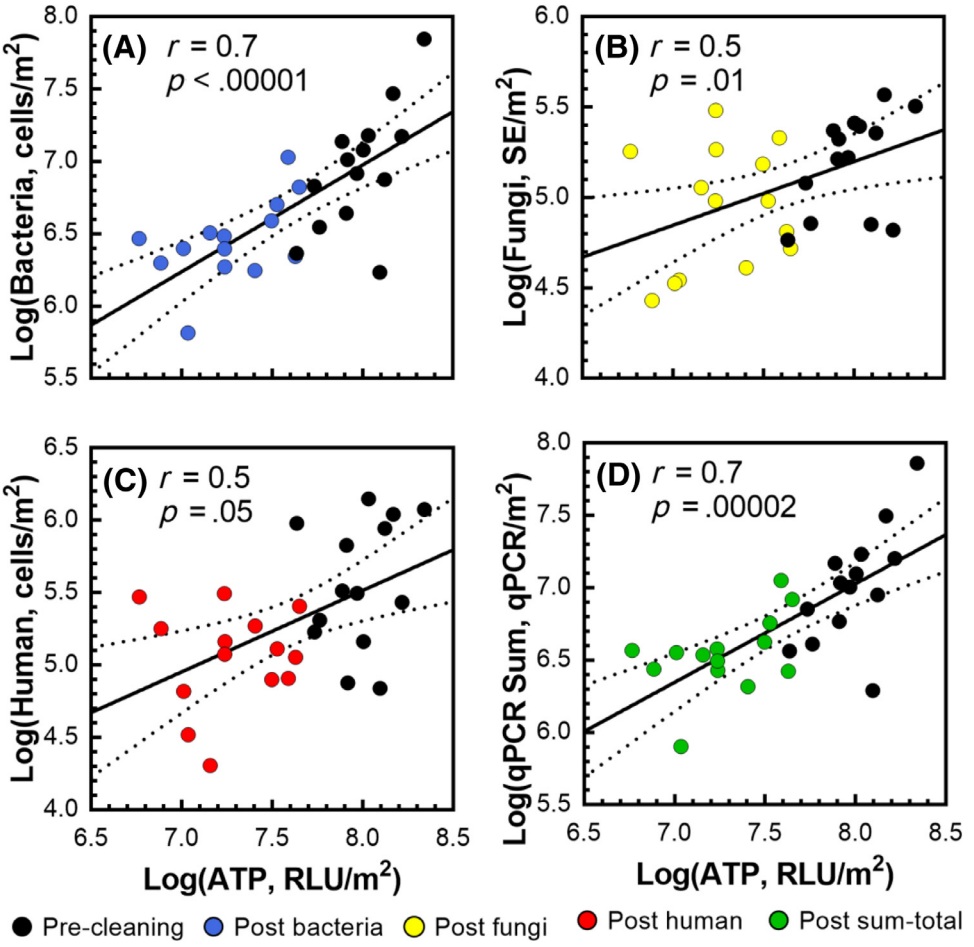

Fig 2. OK school ATP (RLU/m^2) concentrations versus (A) bacteria (cells/m^2), (B) fungi (SE/m^2), (C) human cells (cells/m^2), and (D) qPCR sum (sum/m^2) for desk surfaces pre- (closed circles) and postcleaning (open-colored circles). Solid lines represent simple linear regression models, and dotted lines represent 95% confidence intervals. *ATP*, adenosine triphosphate; *OK*, Oklahoma; *qPCR*, quantitative polymerase chain reaction; *RLU*, relative light unit.

biomass (removal average 72% ± 16%) ($P = .05$, t test for the difference between qPCR sum and ATP). No differences were observed in cleaning effectiveness between CT and OK schools ($P > .4$ for bacterial, fungal, and human cells and qPCR sum and $P = .09$ for ATP).

DISCUSSION

ATP is an indicator of biomass concentration, but because all forms of life have ATP, including food, plant matter, and human cells, there

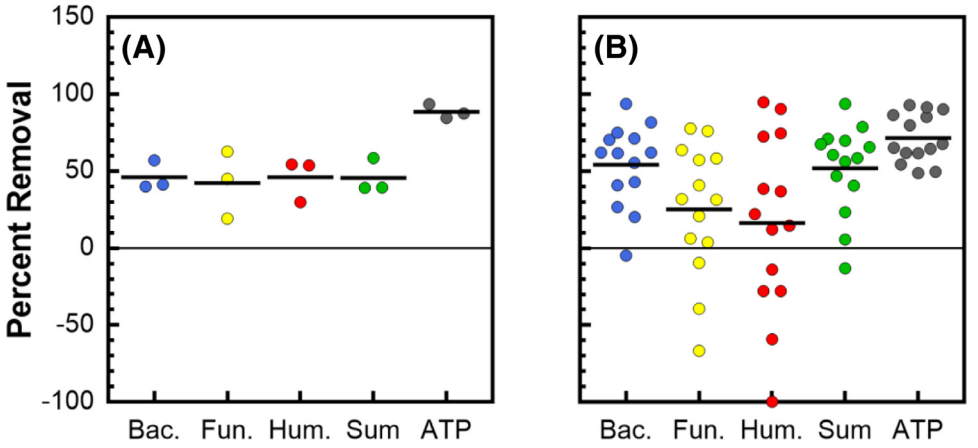

Fig 3. Percent removal of deposited biomass after surface cleaning in (A) CT schools and (B) OK schools for bacteria, fungi, human cells, qPCR sum, and ATP. Horizontal lines denote the sample means for each microbial category. *ATP*, adenosine triphosphate; *Bac.*, bacteria; *CT*, Connecticut; *Fun.*, fungi; *Hum.*, human; *OK*, Oklahoma; *qPCR*, quantitative polymerase chain reaction.

is concern that it does not provide an effective assay for measuring the exposure to or removal of biomass relevant to the transmission of infectious and allergic disease from bacteria and fungi. These data demonstrate that on school desks, qPCR-based bacterial and fungal surface concentrations were correlated with ATP-based surface concentrations.

Association between ATP content and microbial loading

Prior studies have investigated the validity of ATP as an indicator of surface biocontamination through comparisons with the more traditional hygiene swabbing method, in which a surface is sampled with a sterile cotton-tipped swab and colonies counted via culture-based analyses of total aerobic bacteria.[21] In general, ATP and hygiene swabbing have demonstrated a statistically significant agreement when applied to surfaces used in food preparation.[22-24] Results are more ambiguous for hard, dry surfaces, where concentrations are lower and deposition, rather than growth, is a major contributor to microbial loading. ATP versus swabbing comparisons on dry hospital surfaces have not yielded strong associations between ATP levels and culture-based bacterial concentrations.[25] No comparative information is available for school surfaces.

The results presented here represent 2 important extensions to our current understanding of how ATP is associated with microbial loading on surfaces. First, ATP comparisons were extended to school surfaces. Second, ATP was compared with qPCR-based measurements of bacterial, fungal, and human cells. ATP indicates biomass and not viability loss due to disinfection. Thus, qPCR better reflects microbial concentrations, regardless of viability state. Because of the well-documented enrichment of unculturable taxa in environmental microbial communities,[26] molecular methods such as qPCR are favored over culturing for indicating the full microbial surface load.

Although only specific agents are usually associated with disease, the overall presence of total bacteria and fungi has important implications for health. Atopic asthma severity is associated with exposure to allergenic fungi, and nonatopic asthma has been associated with exposure to total fungal concentrations.[27] Recent ecology studies have demonstrated that approximately 25% of environmental fungi are known allergens.[17] Total bacteria also contribute to endotoxin exposure, which is associated with severity of respiratory disease[28] and may confer a protective effect on asthma and allergies in early life.[29] Previous studies focused on the ecology of bacteria on school desks demonstrated a dominance (~80% of total bacteria) of human skin, oral cavity, and gut microbes.[30] Each of these sources is known to harbor human pathogens.[31-33]

ATP for indicating cleaning effectiveness

Our results provide evidence that ATP indicates a greater biomass reduction in cleaning than that revealed by qPCR values. In both CT and OK schools, cleaning reductions were on average 32% higher and significantly greater for ATP than for qPCR sum. One potential explanation is that the age of the surface microbial community is a significant variable in the correlation of these different biomass assays. In particular, older (precleaning) microbial community samples may include significant amounts of extracellular ATP from dead and lysed cells as well as intracellular ATP. This would result in high precleaning concentrations of ATP and thus a greater removal after cleaning if cleaning was effective at removing the unbound ATP. Results also demonstrate that no difference in cleaning effectiveness was observed between OK and CT schools, even though the OK schools used a more rigorous cleaning approach. Prior studies in hospitals have noted that many microbes on dry surfaces are contained in a biofilm, which confers some protection from cleaning.[34] The cleaning protocols common in schools, regardless of rigor, may not adequately remove these dry surface biofilms.

Limitations

Although the above research has extended our understanding of the use of ATP to indicate biomass concentration on dry surfaces, the study has important limitations. Only 1 type of surface was considered. To limit confounding and provide maximum statistical power for comparisons, other types of classroom surfaces were not considered. We also observed correlations between bacterial and fungal and bacterial and human cells, thus indicating that bacterial, fungal, and human cells were not independent variables. This lack of independence was expected because of the similar source (humans) for many of the bacterial and fungal cells on desks.[30] Thus, because of their association dependencies on each other, although it is certain that the sum of qPCR concentrations is associated with ATP, we cannot unambiguously state that values such as bacterial, fungal, and human cells are uniquely associated with ATP.

CONCLUSIONS

ATP bioluminescence measurements correlate with qPCR-based surface concentration analysis of bacterial, fungal, and human cell concentrations when used to assess biological surface cleanliness on school desks. However, ATP measurements of cleaning efficacy are on average 1.5-2 times higher when compared with qPCR-based estimates. Nonetheless, with the advantages of low cost, ease of use, and rapid results, ATP bioluminescence is a viable technique for assessing total bacterial and fungal removal effectiveness on school desks.

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References

1. Li S, Eisenberg JN, Spicknall IH, Koopman JS. Dynamics and control of infections transmitted from person to person through the environment. Am J Epidemiol 2009;170:257-65.
2. Lippmann M, Schlesinger RB. Interspecies comparisons of particle deposition and mucociliary clearance in tracheobronchial airways. J Toxicol Environ Health 1984;13:441-69.
3. Qian J, Ferro AR, Fowler KR. Estimating the resuspension rate and residence time of indoor particles. J Air Waste Manag Assoc 2008;58:502-16.
4. Lax S, Sangwan N, Smith D, Larsen P, Handley KM, Richardson M, et al. Bacterial colonization and succession in a newly opened hospital. Sci Transl Med 2017;9:391-402.
5. Hospodsky D, Qian J, Nazaroff WW, Yamamoto N, Bibby K, Rismani-Yazdi H, et al. Human occupancy as a source of indoor airborne bacteria. PLoS One 2012;7:e34867.
6. Bright KR, Boone SA, Gerba CP. Occurrence of bacteria and viruses on elementary classroom surfaces and the potential role of classroom hygiene in the spread of infectious diseases. J Sch Nurs 2010;26:33-41.
7. Krieger J, Takaro TK, Allen C, Song L, Weaver M, Chai S, et al. The Seattle-King County healthy homes project: implementation of a comprehensive approach to improving indoor environmental quality for low-income children with asthma. Environ Health Perspect 2002;110:311-22.
8. Wälinder R, Norbäck D, Wieslander G, Smedje G, Erwall C, Venge P. Nasal patency and lavage biomarkers in relation to settled dust and cleaning routines in schools. Scand J Work Environ Health 1999;25:137-43.
9. Lewis T, Griffith C, Gallo M, Weinbren M. A modified ATP benchmark for evaluating the cleaning of some hospital environmental surfaces. J Hosp Infect 2008;69:156-63.

10. Shaughnessy RJ, Cole EC, Moschandreas D, Haverinen-Shaughnessy U. ATP as a marker for surface contamination of biological origin in schools and as a potential approach to the measurement of cleaning effectiveness. J Occup Environ Hyg 2013;10:336-46.
11. Haverinen-Shaughnessy U, Shaughnessy RJ, Cole EC, Toyinbo O, Moschandreas DJ. An assessment of indoor environmental quality in schools and its association with health and performance. Indoor Air 2015;91:35-40.
12. ISSA. Clean standard K-12 schools 1014-2013. Available from: https://www.issa.com/certification-standards/issa-clean-standards/clean-standard-k-12.html. Accessed December 9, 2018.
13. Sherlock O, O'Connell N, Creamer E, Humphreys H. Is it really clean? An evaluation of the efficacy of four methods for determining hospital cleanliness. J Hosp Infect 2009;72:140-6.
14. Fairbanks BC, Woods LE, Bryant RJ, Elliott ET, Cole CV, Coleman DC. Limitations of ATP estimates of microbial biomass. Soil Biol Biochem 1984;16:549-58.
15. Murray HD, Schneider DA, Gourse RL. Control of rRNA expression by small molecules is dynamic and nonredundant. Mol Cell 2003;12:125-34.
16. Fierer N, Hamady M, Lauber CL, Knight R. The influence of sex, handedness, and washing on the diversity of hand surface bacteria. Proc Natl Acad Sci U S A 2008;105:17994-9.
17. Yamamoto N, Bibby K, Qian J, Hospodsky D, Rismani-Yazdi H, Nazaroff WW, et al. Particle-size distributions and seasonal diversity of allergenic and pathogenic fungi in outdoor air. ISME J 2012;6:1801-11.
18. Dannemiller KC, Mendell MJ, Macher JM, Kumagai K, Bradman A, Holland N, et al. Next-generation DNA sequencing reveals that low fungal diversity in house dust is associated with childhood asthma development. Indoor Air 2014;24:236-47.
19. Qian J, Hospodsky D, Yamamoto N, Nazaroff WW, Peccia J. Size-resolved emission rates of airborne bacteria and fungi in an occupied classroom. Indoor Air 2012;22:339-51.
20. Sciortino CV, Kemper M, Parthasarathy L, Lay J. Surveillance of methicillin-resistant *Staphylococcus aureus* mupirocin resistance in a veterans affairs hospital. Infect Control Hosp Epidemiol 2015;36:235-6.
21. Moore G, Griffith C. Problems associated with traditional hygiene swabbing: the need for in-house standardization. J Appl Microbiol 2007;103:1090-103.
22. Aycicek H, Oguz U, Karci K. Comparison of results of ATP bioluminescence and traditional hygiene swabbing methods for the determination of surface cleanliness at a hospital kitchen. Int J Hyg Environ Health 2006;209:203-6.
23. Chen FC, Godwin SL. Comparison of a rapid ATP bioluminescence assay and standard plate count methods for assessing microbial contamination of consumers' refrigerators. J Food Prot 2006;69:2534-8.
24. Davidson CA, Griffith CJ, Peters AC, Fielding LM. Evaluation of two methods for monitoring surface cleanliness—ATP bioluminescence and traditional hygiene swabbing. Luminescence 1999;14:33-8.
25. Shama G, Malik DJ. The uses and abuses of rapid bioluminescence–based ATP assays. Int J Hyg Environ Health 2013;216:115-25.
26. Amann RI, Ludwig W, Schleifer KH, Amann RI, Ludwig W. Phylogenetic identification and in situ detection of individual microbial cells without cultivation. Microb Rev 1995;59:143-69.
27. Dannemiller KC, Gent JF, Leaderer BP, Peccia J. Indoor microbial communities: influence on asthma severity in atopic and nonatopic children. J Allergy Clin Immunol 2016;138:76-83.
28. Michel O, Kips J, Duchateau J, Vertongen F, Robert L, Collet H, et al. Severity of asthma is related to endotoxin in house dust. Am J Respir Crit Care Med 2016;154:1641-6.
29. Braun-Fahrländer C, Riedler J, Herz U, Eder W, Waser M, Grize L, et al. Environmental exposure to endotoxin and its relation to asthma in school-age children. N Engl J Med 2002;347:869-77.
30. Kwan SE, Shaughnessy RJ, Hegarty B, Haverinen-Shaughnessy U, Peccia J. The reestablishment of microbial communities after surface cleaning in schools. J Appl Microbiol 2018;125:897-906.
31. Aly R. Microbial infections of skin and nails. In: Baron S, ed. Medical microbiology. Galveston (TX): University of Texas Medical Branch at Galveston; 1996: Chapter 98.
32. Mégraud F, Broutet N. The faecal-oral route—a critical look. In: Hunt RH, Tytgat GN, eds. *Helicobacter pylori*: basic mechanisms to clinical cure. Dordrecht (The Netherlands): Springer; 1998:51-7.
33. Slots J, Slots H. Bacterial and viral pathogens in saliva: disease relationship and infectious risk. Periodontol 2000 2011;55:48-69.
34. Almatroudi A, Gosbell IB, Hu H, Jensen SO, Espedido BA, Tahir S, et al. *Staphylococcus aureus* dry-surface biofilms are not killed by sodium hypochlorite: implications for infection control. J Hosp Infect 2016;93:263-70.