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ATP-based assessments of recent cleaning and disinfection for high-touch surfaces in low-resource shared toilets

Graeme Prentice-Mott[1,*], **Lorna Maru**[2], **Alexandra Kossik**[1], **Evelyn Makena Mugambi**[2], **Cynthia Ombok**[2], **Raymond Odinoh**[2], **Florence Mwikali**[3], **Ruthie Rosenberg**[3], **Isaac Ngere**[2,4], **Jennifer Murphy**[1], **David Berendes**[1]

[1]Waterborne Disease Prevention Branch, Division of Foodborne, Waterborne, and Environmental Diseases, Centers for Disease Control and Prevention, Atlanta, GA, USA

[2]Washington State University Global Health Program Kenya, Nairobi, Kenya

[3]Sanergy, Inc.

[4]Paul G. Allen School of Global Health, Washington State University

Abstract

Quality improvements and reduction of disease risk for low-resource shared sanitation facilities require cleanliness assessment approaches that are both rigorous and practical. Using Adenosine Triphosphate (ATP) bioluminescence testing, we assessed contamination on high-touch (HT) surfaces (inner door handles) at 32 shared toilet sites in Kisumu, Kenya. In public toilets, contamination was lowest after cleaning and disinfection (C&D) with 0.5% chlorine solution (adjusted difference in mean $\log_{10}$ Relative Light Units per 100cm^2 (aDiff): −1.61; CI: −2.43, −0.59), followed by C&D with 0.1% chlorine solution (aDiff: −1.16; CI: −1.77, −0.55). ATP levels were not associated with overall observable toilet cleanliness and had poor agreement with visually assessed HT surface cleanliness. Our findings demonstrate the utility of this field-feasible method for detecting the impact of recent C&D in low-resource shared toilets, a novel setting for ATP cleanliness testing, while also highlighting the importance of using effective C&D procedures and addressing HT surfaces within cleaning protocols.

Introduction

In urban areas of sub-Saharan Africa, an estimated 146.8 million people rely on "limited" sanitation services, toilet facilities which, as classified by the Sustainable Development Goal (SDG) target 6.2, are improved but shared with other households [1]. Reliance on shared

*Corresponding author: gprentice-mott@cdc.gov.

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sanitation is associated with increased risk of diarrheal illness and enteric infection [2–4]. Throughout sub-Saharan Africa, both public and residentially shared toilets in low-income neighborhoods commonly exhibit poor cleanliness, a major detriment to the well-being and dignity of users [5–8]. Despite these challenges, high density and limited municipal and housing infrastructure make private (unshared) household sanitation infeasible in many urban areas [9]. In such cases, the World Health Organization (WHO) recommends the promotion of shared sanitation that meets basic standards, including cleanliness/hygiene, as an incremental improvement [10].

Initiatives to promote safe and acceptable shared sanitation – and to identify improvements for unsafe and unacceptable shared sanitation – must distinguish between good versus poor quality services [11]. This entails rigorous characterization of key service aspects, such as cleanliness of facilities or effectiveness of cleaning practices [9,12]. Most commonly-used cleanliness assessment approaches employ observational criteria, including the presence or absence of visible fecal, urine, or solid waste, noticeable odor, visible signs of pests, or alternatively assigning a Likert scale rating of cleanliness [13–17]. These criteria, while critical to service quality and social acceptability, have limited ability to characterize surface contamination relevant to disease transmission. Additionally, these criteria focus on areas typically cleaned when visibly soiled or odorous (e.g., floor, drop hole, or toilet bowl/seat) and neglect surfaces within toilet compartments frequently touched before or after toilet use (e.g., door handles, flush handles, sink taps) that represent significant pathways for the transfer of enteric and respiratory pathogens [18], of particular concern during the COVID-19 pandemic [19]. Previous studies assessing surface contamination in shared toilets in sub-Saharan Africa have sampled and cultured for *E. coli* [20,21]; however, these microbiological methods require laboratory capacity and training, which may present significant barriers in low-resource settings.

Measurement of organic surface contamination via bioluminescence of Adenosine Triphosphate (ATP) is a long-established method for hygiene monitoring in healthcare facilities for prevention of healthcare-acquired infections [22] and in the food industry for prevention of foodborne disease [23]. Studies in healthcare facilities have included sampling from toilets[24,25], as have studies in other notable settings, such as schools[26], libraries[27], and long-term care facilities[28]. ATP testing has also been used to assess cleaning and disinfection in South Africa in a low-resource pediatric hospital [29,30]. Reducing ATP levels is a demonstrated proxy for removal of pathogens and microbial contamination from surfaces [31] even as, in addition to microorganisms, ATP measurements capture non-living material (e.g., skin or plant debris) and so do not correspond directly to measures of microbial contamination [32]. ATP-based cleanliness testing provides near instantaneous results with minimal equipment, comprising surface swabs, positive controls, refrigerated storage, and a luminometer to read samples in the field [33]. Thus, it could potentially provide a comparatively more objective (versus observation alone), field-feasible measure of surface contamination and/or cleaning and disinfection practices for shared toilets in low-resource settings.

While ATP based testing has been used to assess cleanliness in toilets, this application has generally not been the primary focus and studies including toilets have been entirely limited to high-resource settings. The applicability of ATP testing is unknown for low-resource shared toilets in sub-Saharan Africa, which serve as main sanitation access points for communities and differ substantially from public toilets in high-income countries in design, surface materials, and usage patterns. We piloted this methodology to evaluate surface contamination and the efficacy of varying cleaning and disinfection procedures within urban neighborhoods of Kisumu, Kenya, with high usage of shared sanitation [34]. Results will provide insights into use of ATP testing as a practical surface cleanliness evaluation tool, as well as performance of cleaning and disinfection procedures themselves in removing surface contamination, for programs that aim to reduce disease transmission risk in low-resource shared sanitation facilities.

Methods

Study sites and data collection

The evaluation took place in two densely populated, low-income urban suburbs of Kisumu, Kenya within a convenience sample of high-use shared toilets, totaling 32 sites based on logistical and resource constraints. Half of the sample – 16 sites – comprised toilet sites within a network of a container-based toilets operated by Fresh Life (Sanergy Collaborative) that were participating in a COVID-19 mitigation activity to improve cleaning and management[35]. These 16 toilet sites included all public use toilet sites within the network at commercial and institutional (church, community center) locations and a convenience sample of shared toilet sites within residential compounds with large usership, determined by monthly waste collection volumes. To increase representativeness, another 16 toilet sites were selected from toilets not operated by Sanergy, including all public use (commercial and institutional) toilet sites within the study neighborhoods, and a convenience sample of high use shared toilet sites within residential compounds, determined through canvassing discussions with community organizations.

Data were collected from sites as part of two phases of assessments: an initial assessment and a cleaning and disinfection assessment. The initial assessment visit occurred in September 2021 and included an interview with a site manager or representative to gather data on cleaning practices, combined with observation of all toilets per toilet site (accompanied by the site manager/representative) to gather data on toilet design and sex designation, cleaning practices, reported number of daily toilet users per site, and geolocation. Subsequently, to assess ATP levels, five repeated sampling visits per toilet were conducted ≥2 days apart in all toilets in all sites. For these visits, each toilet was observed for overall toilet cleanliness (visible presence of feces, urine or water puddling, toilet blockages, or rubbish). A swab sample was also taken from a high-touch (HT) surface – the inner door handle or latch used to open the door from within the toilet compartment after toileting and before the opportunity for use of handwashing materials – and, for comparison, a low-touch (LT) surface – an upper wall area within the toilet compartment. Surface swabbing and ATP sampling methods are described below.

Secondly, as part of a cleaning and disinfection assessment, additional repeated sampling visits (2 rounds of 3 visits each, for a total of 6 visits per toilet) were conducted for a convenience sample of 1–2 toilets per site to assess ATP levels on HT surfaces without and immediately following cleaning and/or disinfection (Table 1). To establish reference levels, the first visit of each round included swabbing of HT surfaces without first cleaning or disinfecting. In subsequent visits, swabbing of HT surfaces was done immediately following a cleaning and/or disinfection procedure to assess efficacy of the procedure. For the first round, which took place between November 2021-January 2022, procedures assessed included observed standard practice cleaning and a procedure adapted from recommendations for laboratory cleaning and disinfection [36]. For observed standard practice cleaning, a toilet site representative was asked to clean the toilet as he or she normally would; if the HT surface to be swabbed was not included in the cleaning, the site representative was asked to also clean this area without further instructions for cleaning and/or disinfecting. For the second round, which took place in June 2022, procedures assessed included cleaning and disinfection with detergent and chlorine solutions, each prepared according to local practice, and a procedure adapted from recommendations for cleaning and disinfection in households and healthcare settings [37,38]. Data were also captured on surface composition (e.g., wood, metal, etc.) and whether the surface appeared visibly dirty/dusty (after cleaning and/or disinfection). All environmental swabbing visits were conducted ≥7 days apart within each toilet to minimize artificial carry-over reductions in ATP levels from one visit to the next due to study procedures. During data collection, 2 toilets were closed for use and therefore excluded from cleaning and disinfection analyses.

Environmental swabbing for organic surface contamination

ATP samples were processed on site. Assessment visits were conducted by a team of 2 research enumerators trained on data collection and environmental swabbing. HT and LT surfaces were identified for each toilet, with a mark placed on the surface, to the side of the desired swabbing area, and photographs taken to enable enumerators to swab the same area in each toilet in subsequent visits. For each surface, an area ~100 cm^2 was approximated by holding a ruler or measuring tape near the surface without touching it. The approximated area was swabbed in a crosshatch pattern using a 3M™ Clean-Trace™ Surface ATP Test Swab (3M Co., St. Paul, MN). Immediately after swabbing was complete, the swab was returned to its collection device housing and a reaction initiated by pressing down on the top handle of the swab, subsequently mixing the swab contents with reagents already present in the collection device housing. Following five seconds of side-to-side shaking of the swab to help facilitate mixing and maintain the activated reaction, the collection device housing was placed inside a 3M™ Clean-Trace™ Luminometer LX25 [39–41] and immediately analyzed. ATP readings in relative light units (RLU) were recorded by study enumerators. Surfaces were allowed to fully dry following cleaning and disinfection procedures before swabbing. At the beginning of each day of data collection, 3M Clean-Trace® Surface Positive Controls, vials with a known amount of lyophilized ATP, were used to ensure both standardization of luminometers and to demonstrate proper functionality of the swabs.

Statistical Analysis

Surface contamination (RLU/100cm^2) was modeled using multivariable log-linear regression with generalized estimating equations given log-normal distribution of RLU concentrations and to account for repeat sampling at a toilet. Estimates are presented as adjusted means, back-transformed to the original nonlogarithmic scale. When comparing adjusted means, $\log_{10}$ differences are presented. In the initial assessment, factors hypothesized to affect ATP variability (e.g., toilet design, sex designation) were assessed jointly in a single model. Subsequently, covariates identified to be associated with RLU concentrations in the initial assessment were controlled for in the model estimating differences in RLU concentrations for the cleaning and disinfection assessment, with time of day and surface composition also included *a priori* as covariates. The frequency of HT surfaces that either "passed" or "failed" according to the manufacturer recommended cleanliness threshold of <250 RLU/100cm^2 [33] was examined – as well as thresholds from previously published studies of <500 RLU/100cm^2 (used in a study of a variety of fomites throughout long-term care facilities that included bathroom door handles) [42] and <1,000 RLU/100cm^2 (used specifically for bathroom door handles and cutting boards in a study of these and other dining hall surfaces on a US university campus) [43]. Interrater agreement versus visual assessment of surface cleanliness (dirty vs. not dirty) was also tested. Lastly, new pass/caution/fail limits were calculated using adjusted means and upper 99.7% confidence limits (i.e. 3 standard deviations), based on methodology to calculate limits for specific sampling sites, whereby measurements below the lower limit are considered "pass", within the lower and upper limit are considered "caution", and above the upper limit are considered "fail" [44]. Statistical analysis was carried out in R version 4.2.1 [45] with regression modeling carried out using the 'geepack' package [46] and mapping carried out using the 'sf' package[47].

Ethics

Informed consent was obtained from all participants, and protocols for data collection were reviewed by institutional review boards at the Kenya Medical Research Institute (Protocol No. SERU 4323), an ethics reliance from Washington State University Institutional Review Board (WSU IRB) and at CDC, which determined data collection to be non-research (CDC project id: 0900f3eb81e62919).

Results

Initial assessment

A total of 73 toilets in 12 residential, 9 public/commercial, and 11 institutional sites were assessed in the neighborhoods of Nyalenda A and B, southeast of Kisumu city center (Figure 1, Supplementary table 1). Flush or pour-flush toilets were the most common type (64%), followed by container-based toilets (26%), and pit latrines (10%). Most site representatives reported that water for cleaning was available on site (72%) and toilets were cleaned at least once daily (72%), although more than half public/commercial site representatives reported more frequent cleanings of ≥2 times daily (56%). Individuals with primary cleaning duties varied by type of site and most commonly were attendants (at public/commercial sites: 56%), residents (at residential sites: 58%), and attendants and volunteers (at institutional sites: 45% and 36%, respectively).

At initial assessment, ATP levels on HT surfaces ranged from 1,536 adjusted mean RLU per 100 cm^2 (aMRLU) (95% confidence interval (CI): 847–2,787) to 3,302 aMRLU (CI: 1,917–5,691) by toilet site type (Table 2). ATP levels on HT surfaces in public/commercial toilets were greater than in residential toilets (adjusted difference in mean $\log_{10}$ RLU per 100 cm^2 (aDiff): 0.33; CI: 0.02, 0.64). There was no difference in ATP levels between institutional and residential toilets, nor any difference by toilet type, sex designation, or the time of day when environmental sampling occurred. ATP levels on HT surfaces were lower in toilets where rubbish was present (aDiff: −0.15; CI: −0.26, −0.03); however, there were no differences by presence/absence of all other observation-based metrics of toilet cleanliness. ATP levels were consistently greater on HT surfaces than LT surfaces ($\log_{10}$ RLU per 100cm^2 differences ranging from 0.66 (CI: 0.35, 0.97) in public/commercial toilets to 0.83 (CI: 0.40, 1.26) in residential toilets Supplementary Table 2).

Cleaning and disinfection assessment

A subset of 33 toilets were each swabbed according to cleaning and disinfection procedures in Table 1 for a total of 198 surface swabs (Supplementary Table 3). Given ATP levels were notably higher for public/commercial toilets in the initial assessment – as well as factors that influence cleaning intervention approaches, such as existing cleaning practices and personnel – we elected to analyze cleaning and disinfection efficacy by toilet site type (public/commercial vs. residential/institutional). Additionally, time of day and surface composition (bare wood, painted wood, metal) were controlled for in all cleaning and disinfection estimates. In public/commercial toilet sites, lower ATP levels on HT surfaces were observed following all cleaning and/or disinfection procedures compared to reference levels taken when no procedures had occurred (Figure 2). These reductions were greatest following cleaning plus disinfection with 0.5% chlorine solution (aDiff: −1.61; CI: −2.43, −0.79) and cleaning plus disinfection with 0.1% chlorine solution (aDiff: −1.16; CI: −1.77, −0.55), but smaller following cleaning plus disinfection with a 0.02% chlorine solution (aDiff: −0.87; CI: −1.39, −0.35) and minimal following observed standard practice cleanings (−aDiff: −0.56: CI: −0.84, −0.28). In residential/institutional toilet sites, we observed reductions following cleaning plus disinfection with 0.5% chlorine solution (aDiff: −0.99; CI: −1.37, −0.60) and cleaning plus disinfection with a 0.1% chlorine solution (aDiff: −0.93; CI: −1.27, −0.59). However, in residential/institutional sites, reductions were not as large following cleaning plus disinfection with a 0.02% chlorine solution (aDiff: −0.50; CI: −0.96, −0.05) and there was no reduction following observed standard practice cleanings. Observed standard practice cleanings featured no or improper application of disinfectant (i.e. mixed in soapy solution) (Supplementary table 4).

ATP levels were lowest following cleaning plus disinfection with 0.5% chlorine solution for public/commercial toilet sites (aMRLU: 324; CI: 98–1,070) despite reference levels for this round being the highest assessed during this study (aMRLU: 13,243; CI: 5,420–32,358) (Figure 3). In residential/institutional toilets, ATP levels were similarly lowest following cleaning plus disinfection with 0.5% chlorine solution (aMRLU: 483; CI: 227–1,030). Applying a threshold of <1,000 RLU/100cm^2 for determining cleanliness “passing”, following cleaning plus disinfection, 73% (0.5% chlorine solution) and 58% (0.1% chlorine solution) of HT surfaces, respectively, would have been classified as “passing”, while only 3% of HT surfaces would have been classified as “passing” when no procedures had occurred (Supplementary Table 8). When applying pass/fail thresholds of <500 or <250 RLU/100cm^2, fewer post- cleaning and disinfection HT surfaces at either 0.1% or 0.5%

chlorine solution were classified as "passing" (30–58%). When comparing the number of HT surfaces determined to be visibly dirty via observational assessment to the number of surfaces "passing" at the most liberal threshold of <1,000 RLU/100cm^2, there was poor agreement both without and following cleaning and disinfection procedures, with a Kappa value ranging from −0.16 to 0.04 (Table 3). Lower and upper pass/caution/fail limits calculated from data in this study ranged from 520–635 and 1,530–2,540 RLU/100cm^2, respectively (Supplementary table 9).

Discussion

Recommended cleaning and disinfection procedures for high-touch surfaces (interior door handles) in publicly and residentially shared toilets yielded consistent and substantial reductions in organic surface contamination assessed via ATP testing. However, results were mixed for standard practice cleanings, with only minimal or no reductions when disinfection did not accompany cleaning. Additionally, we observed notable discordance between levels of organic surface contamination on high-touch surfaces and observation-based metrics of surface and overall toilet cleanliness. Taken together, these findings suggest ATP testing may be a useful complement to traditional observation-based WASH assessment metrics for its ability to characterize recent cleaning and disinfection. However, even after use of rigorous cleaning and disinfection procedures, most surfaces did not meet the manufacturer-recommended pass/fail threshold, suggesting a different, yet-to-be-determined benchmark may be appropriate for low-resource shared toilets. Background levels of organic surface contamination were higher in public toilets than shared residential toilets, suggesting toilets near commercial activities should be prioritized for cleaning and disinfection interventions and may also yield the most detectable pre- and post-disinfection differences.

Microbiological contamination of surfaces in shared toilets in sub-Saharan Africa has been characterized previously, largely via testing for *E. coli* in studies in Mozambique, Tanzania, and Ghana [12,20,21], as well as via recovery of pathogens from surfaces, such as SARS-COV-2 [48]. However, to our knowledge, this study is the first to present data on ATP testing for this setting. Such rapid environmental assessment tools that require relatively less technical training are typically easier to use than laboratory-based culture methods [49]. ATP testing has the capacity to detect surface contamination of any biological origin, both living and non-living. In shared toilets, this may include bacterial contamination that largely traces back to human contact [50] but also may include cell material from other organisms, such as human skin itself or material from plants [32], all of which are relevant to surface cleanliness. This analysis also presents evidence assessing cleaning and disinfection procedures already in practice at toilet sites that offer an important comparison to cleaning and disinfection procedures recommended in the literature [36,37].

Observed reductions in surface contamination align with findings presented in a systematic review of several studies of ATP testing in healthcare facilities reporting lower surface contamination after disinfection despite a wide variety of surfaces, study sites, cleaning procedures, testing equipment, and cleanliness thresholds used throughout these studies [39]. In a study in a Brazilian hospital, toilet door handles were one of few tested surfaces where cleaning and disinfection with a quaternary ammonium-based disinfectant resulted in reduced surface contamination, measuring at a median 137 RLU/100cm^2 post-disinfection compared to 358/100cm^2 RLU pre-disinfection [24]. Conversely, in the Researching Effective Approaches to Cleaning in Hospitals (REACH) trial, ATP-based cleanliness improvements were observed for patient rooms but not bathroom surfaces; however, this study assessed the relative impact of a cleaning intervention on post-cleaning surfaces exclusively, with a high proportion of bathroom surface cleanings that met the cleanliness threshold before the training intervention took place [25].

While no widely-recognized, non-healthcare protocols exist for cleaning and disinfecting in shared toilets, recommendations for low-resource healthcare settings – either cleaning then use of 0.5% chlorine solution given biohazardous spillages or excessive organic matter or cleaning then use of 0.1% chlorine solution to address potential pathogenic contamination on most non-excessively dirty surfaces [51] – may be appropriate for shared toilets in managing contamination due to soiling or deposition from hands. Our observation of reduced surface contamination following these procedures, but less consistent reductions when disinfecting using lower chlorine concentrations (~0.02%), supports the relatively better performance of chlorine solutions in these ranges. Additionally, little-to-no impact on surface contamination for observed standard practices supports recommendations that a two-stage process – cleaning then application of an appropriate disinfectant – is required for effectiveness [51]. The underlying mechanism may involve a combination of additional wiping [52] and degradation of ATP. Interrogation of the isolated contribution of each step may be informative, although disinfection alone should not substitute the cleaning step given its reduced efficacy on dirty surfaces [51]. Disinfectants applied directly to testing swabs may also interfere with ATP testing reactions [53]; however, for recommended procedures in this study, disinfectants were wiped off surfaces before ATP swabbing using a clean paper towel. Of importance to note, ATP testing alone cannot determine the efficacy of disinfection to inactivate microorganisms; indeed, 18% of HT surface swabs in this study included areas of bare wood that are porous and for which complete disinfection may not be achieved [52]. Where possible, high-touch surfaces should be made from non-porous materials to allow for effective cleaning and disinfection [51]; otherwise, effective sanitizing products should be used if controlling contamination on porous high-touch surfaces is needed.

The high discordance between visually assessed toilet cleanliness and ATP-based measures is consistent with a review presenting findings from several studies in healthcare settings that compared visual assessment to ATP-based cleanliness testing, as well as to other cleanliness assessment methods (involving removal of fluorescent markers or culture of aerobic colony counts) [22]. Unreliability of visual cleanliness assessment has also been noted in studies involving bathroom surfaces in university libraries and hotels [27,54]. Therefore, the previously-described metrics of toilet cleanliness, which are broadly used and rely primarily on visual assessment [13–16], may only be sufficient for identifying highly soiled surfaces in toilets. Even so, observation-based cleanliness monitoring, which makes use of these common metrics, provides essential feedback to inform effective strategies to improve overall toilet cleanliness [55,56] and targets aspects of toilet cleanliness imperative to user acceptability, dignity, and alleviation of sanitation-related stress [11,8]. While more sensitive at characterizing surface contamination, routine ATP testing involves a cost burden that, even if comparatively lower than some microbiological methods, would be unrealistic given the already considerable operation costs of public toilets [57] or for the limited pooled financial contributions used for residential toilet maintenance [55,58]. Therefore, routine ATP-based cleanliness monitoring of shared toilets might only be prioritized for specialized contexts, such as in areas of high transmission risk in disease outbreak settings [59]. More broadly, ATP-based cleanliness assessments may be well-suited to evaluate whether staff trained on cleaning interventions are able to practice them effectively [25], aid in the development of or training on effective cleaning procedures by identifying surfaces with persistent contamination [42], or assess the validity of widely-deployable cleaning practice monitoring tools or approaches (e.g. cleaning protocol checklists) that may be candidates for inclusion in standards or criteria of quality shared sanitation services.

Even after carrying out procedures recommended for effective surface cleaning and disinfection, most post-disinfection levels recorded in this study fell above the manufacturer's recommended pass/fail threshold of <250 RLU/100cm^2, suggesting it may not be achievable for low-resource shared toilets. ATP measurements can vary by surface material type and age, which affect ease of cleaning and surface topography [33]; shared toilets in low-resource settings are often constructed from a variety of low-cost materials, which may age before replacement due to barriers to regular maintenance [13]. Furthermore, previous research has identified the importance of using benchmarks specified for a given setting [27]. The pass/caution/fail limits reported in this study may provide practical initial values for comparison by other cleanliness evaluations of high-touch surfaces in low-resource shared toilets. The same methodology may also be used to set limits for additional surfaces and cleaning protocols [44]. However, it is crucial that threshold values represent meaningful reduction in disease transmission risk. To this end, standardized healthcare ATP cleanliness benchmarks have been established through assessing the removal of pathogens of concern for healthcare-acquired infections, such as *Staphylococcus aureus* [31]. In shared toilets, disease transmission concerns include a wide variety of enteric and respiratory pathogens – including norovirus, *Salmonella*, Hepatitis A, *Shigella*, SARS-CoV-2, and others – all of which have been associated with public toilets via outbreak investigation or recovery from a variety of surfaces, including toilet seats, flush handles, sink taps, and door handles [18]. Consequently, evaluating ATP testing levels alongside markers for fecal and potential enteric pathogen contamination on surfaces would strengthen the validity of cleanliness monitoring benchmarks in shared toilets [60,61]. Without such evidence, comparison of pre- and post-cleaning ATP levels may be more meaningful than absolute ATP levels, because it most clearly demonstrates relative cleanliness improvements for a variety of surface types.

Higher reference levels of organic surface contamination on door handles in public toilets are likely related to more frequent use. A study of public toilets in Kathmandu, Nepal, found a linear correlation between usership estimated through structured observations and bacterial surface contamination [62]. In our study, site representatives were surveyed on the estimated number of daily toilet users; however, reported estimates may have limited accuracy, especially for sites without toilet attendants. Higher levels of contamination at public toilet sites are consistent with other findings that overall quality is poorer and user satisfaction lower for open-use public versus shared residential toilets or when the latter is shared among more households [34,63,64]. Given substantial use and population mixing, promoting regular cleaning and disinfection of high-touch surfaces, which are a significant pathway for the transfer of pathogens [18,19], is critical for shared toilets, especially public toilets. This may be achieved through cleaning practice improvement interventions that establish cleaning protocols and employ participatory approaches for designing behavior change and organizational strategies that address contextual constraints [13,56,62].

This study has several limitations. Toilet sites were selected as a convenience sample, targeting facilities with high use, and therefore may not be representative of shared toilets outside of the neighborhoods where the study took place. The greatest variability in ATP sampling measures is known to occur between individuals taking samples [33], and so where possible adjacent paired swabs should be taken from target surfaces. However, in this study, most swabbing was conducted on inner door handles, which did not have enough surface area for two paired 100 cm^2 swabs to be taken by different research enumerators. Additionally, door handle swabbing surfaces were not flat, and so approximations of ~100cm^2 may have varied. Given repeated sampling of the same surfaces, post-disinfection reductions may in part reflect study procedures from previous visits; however, all cleaning and disinfection assessment visits were conducted at least 7 days apart to minimize artificial impacts of study procedures. Due to travel restrictions, trainings of research enumerators for the initial assessment were conducted virtually whereas trainings for the cleaning and disinfection assessment were carried out in-person in the field. This may have led to differences in swabbing technique and ATP measurements between the two assessments, as ATP levels were lower in the initial assessment compared to reference levels in the cleaning and disinfection assessment. These differences were consistent across the whole sample and for the subset of toilets in the cleaning and disinfection assessment, and, as visits occurred within the same season, there was no apparent rainy versus dry seasonality.

Findings from this study demonstrate that cleaning and disinfection practices in low-resource shared toilets can be assessed using ATP testing, while also highlighting the need to use effective cleaning and disinfection procedures and robust assessment methods that target surface contamination that is indiscernible by visual assessment. ATP testing may be a useful complement to observational methods for studies that assess cleanliness when testing objectives align with the method's non-specific target material (i.e., all living and non-living biologic material) and given careful consideration of potential barriers to application (e.g., challenges properly identifying surfaces for testing) [22]. Further evaluation of ATP testing against standard microbiologic surface testing methods should be undertaken to identify ATP levels that indicate adequate removal of fecal and/or pathogenic contamination to establish standardized cleanliness benchmarks for this setting.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data Availability:

All datasets generated and analyzed during the current study are included in this published article and its supplementary materials.

References

1. WHO & UNICEF. Joint Monitoring Programme: Data - Household sanitation. https://washdata.org/data/household#!/.
2. Fuller JA, Clasen T, Heijnen M & Eisenberg JNS Shared sanitation and the prevalence of diarrhea in young children: Evidence from 51 countries, 2001–2011. American Journal of Tropical Medicine and Hygiene 91, 173–180 (2014). [PubMed: 24865679]
3. Heijnen M et al. Shared sanitation versus individual household latrines: a systematic review of health outcomes. PLoS One 9, e93300 (2014). [PubMed: 24743336]
4. Baker KK et al. Sanitation and Hygiene-Specific Risk Factors for Moderate-to-Severe Diarrhea in Young Children in the Global Enteric Multicenter Study, 2007–2011: Case-Control Study. 13, e1002010 (2016).
5. Peprah D et al. Public toilets and their customers in low-income Accra, Ghana. Environ Urban 27, 589–604 (2015).
6. Kwiringira J, Atekyereza P, Niwagaba C & Günther I Descending the sanitation ladder in urban Uganda: Evidence from Kampala Slums. BMC Public Health 14, 1–10 (2014). [PubMed: 24383435]
7. Shiras T et al. Shared latrines in Maputo, Mozambique: Exploring emotional well-being and psychosocial stress. BMC Int Health Hum Rights 18, 1–12 (2018). [PubMed: 29325549]
8. Sclar GD et al. Exploring the relationship between sanitation and mental and social well-being: A systematic review and qualitative synthesis. Soc Sci Med 217, 121–134 (2018). [PubMed: 30316053]
9. Tidwell JB et al. Where Shared Sanitation is the Only Immediate Option: A Research Agenda for Shared Sanitation in Densely Populated Low-Income Urban Settings. Am J Trop Med Hyg 104, 429 (2021).
10. WHO. Guidelines On Sanitation and Health. (World Health Organization, 2018).
11. Schelbert V et al. When is shared sanitation acceptable in low-income urban settlements? A user perspective on shared sanitation quality in Kumasi, Kisumu and Dhaka. Journal of Water, Sanitation and Hygiene for Development 10, 959–968 (2020).
12. Exley JLR, Liseka B, Cumming O & Ensink JHJ The sanitation ladder, what constitutes an improved form of sanitation? Environ Sci Technol 49, 1086–1094 (2015). [PubMed: 25513885]
13. Alam MU et al. Behaviour change intervention to improve shared toilet maintenance and cleanliness in urban slums of Dhaka: a cluster-randomised controlled trial. Trop Med Int Health 22, 1000–1011 (2017). [PubMed: 28556458]
14. Heijnen M, Routray P, Torondel B & Clasen T Shared sanitation versus individual household latrines in urban slums: A cross-sectional study in orissa, India. American Journal of Tropical Medicine and Hygiene 93, 263–268 (2015). [PubMed: 26123953]
15. Antwi-Agyei P, Monney I, Adjei KA, Kweyu R & Simiyu S Shared but Clean Household Toilets: What Makes This Possible? Evidence from Ghana and Kenya. Int J Environ Res Public Health 19, 4271 (2022). [PubMed: 35409952]
16. Duijster D et al. Improving Toilet Usability and Cleanliness in Public Schools in the Philippines Using a Packaged Operation and Maintenance Intervention. Int J Environ Res Public Health 19, 10059 (2022). [PubMed: 36011699]

17. Tidwell JB, Chipungu J, Chilengi R & Aunger R Assessing peri-urban sanitation quality using a theoretically derived composite measure in Lusaka, Zambia. Journal of Water, Sanitation and Hygiene for Development 8, 668–678 (2018).
18. Abney SE, Bright KR, McKinney J, Ijaz MK & Gerba CP Toilet hygiene—review and research needs. Journal of Applied Microbiology vol. 131 2705–2714 (2021). [PubMed: 33899991]
19. Vardoulakis S, Espinoza Oyarce DA & Donner E Transmission of COVID-19 and other infectious diseases in public washrooms: A systematic review. Sci Total Environ 803, (2022).
20. Capone D et al. A localized sanitation status index as a proxy for fecal contamination in urban Maputo, Mozambique. PLoS One 14, e0224333 (2019). [PubMed: 31652287]
21. Raj SJ et al. The SaniPath Exposure Assessment Tool: A quantitative approach for assessing exposure to fecal contamination through multiple pathways in low resource urban settlements. PLoS One 15, e0234364 (2020). [PubMed: 32530933]
22. Han JH et al. Cleaning Hospital Room Surfaces to Prevent Health Care–Associated Infections. 10.7326/M15-1192 163, 598–607 (2015).
23. Hewage SN, Makawita P, Gibson KE, Lee JA & Fraser AM Relationship between ATP Bioluminescence Measurements and Microbial Assessments in Studies Conducted in Food Establishments: A Systematic Literature Review and Meta-Analysis. J Food Prot 85, 1855–1864 (2022). [PubMed: 36173898]
24. Santos-Junior AG et al. Effectiveness of Surface Cleaning and Disinfection in a Brazilian Healthcare Facility. Open Nurs J 12, 36 (2018). [PubMed: 29643951]
25. Mitchell BG et al. Evaluating bio-burden of frequently touched surfaces using Adenosine Triphosphate bioluminescence (ATP): Results from the Researching Effective Approaches to Cleaning in Hospitals (REACH) trial. 25, 168–174 (2020).
26. 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 10, 336 (2013). [PubMed: 23611158]
27. Fisher JJ & Rundels JJ How clean is the library? Using ATP bioluminescence technology to identify surface contamination. The Journal of Academic Librarianship 49, 102706 (2023).
28. Cannon JL et al. Hygienic monitoring in long-term care facilities using ATP, crAssphage, and human noroviruses to direct environmental surface cleaning. Am J Infect Control 50, 289–294 (2022). [PubMed: 35184878]
29. Dramowski A et al. NeoCLEAN: a multimodal strategy to enhance environmental cleaning in a resource-limited neonatal unit. Antimicrob Resist Infect Control 10, 1–9 (2021). [PubMed: 33407871]
30. Dramowski A, Whitelaw A & Cotton MF Assessment of terminal cleaning in pediatric isolation rooms: Options for low-resource settings. Am J Infect Control 44, 1558–1564 (2016). [PubMed: 27561433]
31. Lewis T, Griffith C, Gallo M & Weinbren M A modified ATP benchmark for evaluating the cleaning of some hospital environmental surfaces. Journal of Hospital Infection 69, 156–163 (2008). [PubMed: 18468725]
32. van Arkel A, Willemsen I & Kluytmans J The correlation between ATP measurement and microbial contamination of inanimate surfaces. Antimicrob Resist Infect Control 10, 116 (2021). [PubMed: 34362450]
33. 3M. Hygiene Management Guide for Environmental Surfaces: 3MTM Clean-TraceTM Hygiene Management System. https://multimedia.3m.com/mws/media/1482795O/hygiene-management-guide-for-environmental-surfaces.pdf (2016)
34. Simiyu S, Swilling M, Cairncross S & Rheingans R Determinants of quality of shared sanitation facilities in informal settlements: Case study of Kisumu, Kenya. BMC Public Health 17, 1–13 (2017). [PubMed: 28049454]
35. Berendes D et al. Improving water, sanitation, and hygiene (WASH), with a focus on hand hygiene, globally for community mitigation of COVID-19. PLOS Water 1, e0000027 (2022).
36. World Health Organization. Laboratory biosafety manual, 3rd edition. World Health Organization 1–178 (2004) doi:10.1007/SpringerReference_61629.

37. CDC. Chemical Disinfectants: Guideline for Disinfection and Sterilization in Healthcare Facilities. https://www.cdc.gov/infectioncontrol/guidelines/disinfection/disinfection-methods/chemical.html (2016).
38. CDC. Cleaning and Disinfecting With Bleach. https://www.cdc.gov/hygiene/cleaning/disinfecting-bleach.html (2022).
39. Amodio E & Dino C Use of ATP bioluminescence for assessing the cleanliness of hospital surfaces: A review of the published literature (1990–2012). J Infect Public Health 7, 92–98 (2014). [PubMed: 24231159]
40. Nante N, Ceriale E, Messina G, Lenzi D & Manzi P Effectiveness of ATP bioluminescence to assess hospital cleaning: a review. J Prev Med Hyg 58, E177 (2017). [PubMed: 28900359]
41. Moore G, Smyth D, Singleton J & Wilson P The use of adenosine triphosphate bioluminescence to assess the efficacy of a modified cleaning program implemented within an intensive care setting. 38, 617–622 (2010).
42. Cannon JL et al. Hygienic monitoring in long-term care facilities using ATP, crAssphage, and human noroviruses to direct environmental surface cleaning. Am J Infect Control 50, 289–294 (2022). [PubMed: 35184878]
43. Cunningham AE, Rajagopal R, Lauer J & Allwood P Assessment of hygienic quality of surfaces in retail food service establishments based on microbial counts and real-time detection of ATP. J Food Prot 74, 686–690 (2011). [PubMed: 21477489]
44. Bakke M A Comprehensive Analysis of ATP Tests: Practical Use and Recent Progress in the Total Adenylate Test for the Effective Monitoring of Hygiene. J Food Prot 85, 1079–1095 (2022). [PubMed: 35503956]
45. R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/. (2021).
46. Halekoh U, Højsgaard S & Yan J The R Package geepack for Generalized Estimating Equations. J Stat Softw 15, 1–11 (2006).
47. Pebesma E & Bivand R Spatial Data Science: With Applications in R. Spatial Data Science: With Applications in R 1–300 (2023) doi:10.1201/9780429459016/SPATIAL-DATA-SCIENCE-EDZER-PEBESMA-ROGER-BIVAND.
48. Amoah ID et al. Detection of SARS-CoV-2 RNA on contact surfaces within shared sanitation facilities. Int J Hyg Environ Health 236, 113807 (2021). [PubMed: 34265632]
49. Ghuh A, Carling P & Environmental Evaluation Workgroup. Options for Evaluating Environmental Cleaning. (2010).
50. Flores GE et al. Microbial Biogeography of Public Restroom Surfaces. PLoS One 6, e28132 (2011). [PubMed: 22132229]
51. CDC & ICAN. Best Practices for Environmental Cleaning in Healthcare Facilities in Resource Limited Settings. https://www.cdc.gov/hai/prevent/resource-limited/index.html (2019).
52. Rutala WA & Weber DJ Monitoring and improving the effectiveness of surface cleaning and disinfection. Am J Infect Control 44, e69–e76 (2016). [PubMed: 27131138]
53. Omidbakhsh N, Ahmadpour F & Kenny N How Reliable Are ATP Bioluminescence Meters in Assessing Decontamination of Environmental Surfaces in Healthcare Settings? PLoS One 9, (2014).
54. Almanza BA et al. How Clean Are Hotel Rooms? Part I: Visual Observations vs. Microbiological Contamination. J Environ Health 78, 8–13 (2015).
55. Simiyu SN, Kweyu RM, Antwi-Agyei P & Adjei KA Barriers and opportunities for cleanliness of shared sanitation facilities in low-income settlements in Kenya. BMC Public Health 20, (2020).
56. Simiyu S, Antwi-Agyei P, Adjei K & Kweyu R Developing and Testing Strategies for Improving Cleanliness of Shared Sanitation in Low-Income Settlements of Kisumu, Kenya. Am J Trop Med Hyg 105, 1816–1825 (2021). [PubMed: 34695798]
57. Ssekamatte T et al. Opportunities and barriers to effective operation and maintenance of public toilets in informal settlements: perspectives from toilet operators in Kampala. 10.1080/09603123.2018.1544610 29, 359–370 (2018).

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58. Shiras T et al. Shared Sanitation Management and the Role of Social Capital: Findings from an Urban Sanitation Intervention in Maputo, Mozambique. Int J Environ Res Public Health 15, (2018).
59. Nakiire L et al. Population Movement Patterns Among the Democratic Republic of the Congo, Rwanda, and Uganda During an Outbreak of Ebola Virus Disease: Results from Community Engagement in Two Districts - Uganda, March 2019. MMWR Morb Mortal Wkly Rep 69, 10–13 (2020). [PubMed: 31917781]
60. Kolus RC, Zahrah M, String G & Lantagne DS Culturable E. coli as Surrogate for Culturable V. cholerae in Surface Disinfection Testing with Chlorine. Journal of Environmental Engineering 147, 06021002 (2021).
61. Park GW et al. CrAssphage as a Novel Tool to Detect Human Fecal Contamination on Environmental Surfaces and Hands. Emerg Infect Dis 26, 1731 (2020). [PubMed: 32511090]
62. Mraz AL et al. Impact of usership on bacterial contamination of public latrine surfaces in Kathmandu, Nepal. PLOS Water 2, e0000091 (2023).
63. Tumwebaze IK & Mosler HJ Shared toilet users' collective cleaning and determinant factors in Kampala slums, Uganda. BMC Public Health 14, 1260 (2014). [PubMed: 25494556]
64. Heijnen M, Routray P, Torondel B & Clasen T Neighbour-shared versus communal latrines in urban slums: a cross-sectional study in Orissa, India exploring household demographics, accessibility, privacy, use and cleanliness. Trans R Soc Trop Med Hyg 109, 690–699 (2015). [PubMed: 26464231]

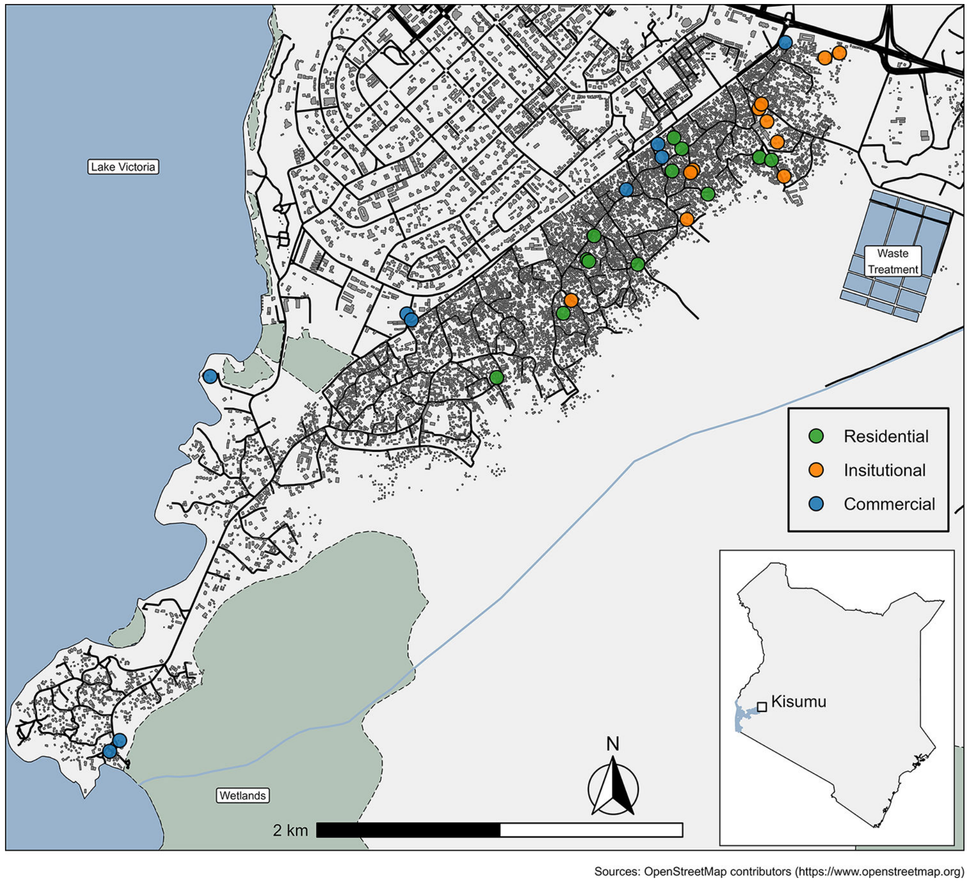

Figure 1. Map of shared toilet sites, Kisumu, Kenya

Locations of toilet sites are shown by dots, with colors by type of site. Streets, buildings, and water features retrieved from https://www.openstreetmap.org. Map created using R version 4.2.1 and the ‘sf’ package.

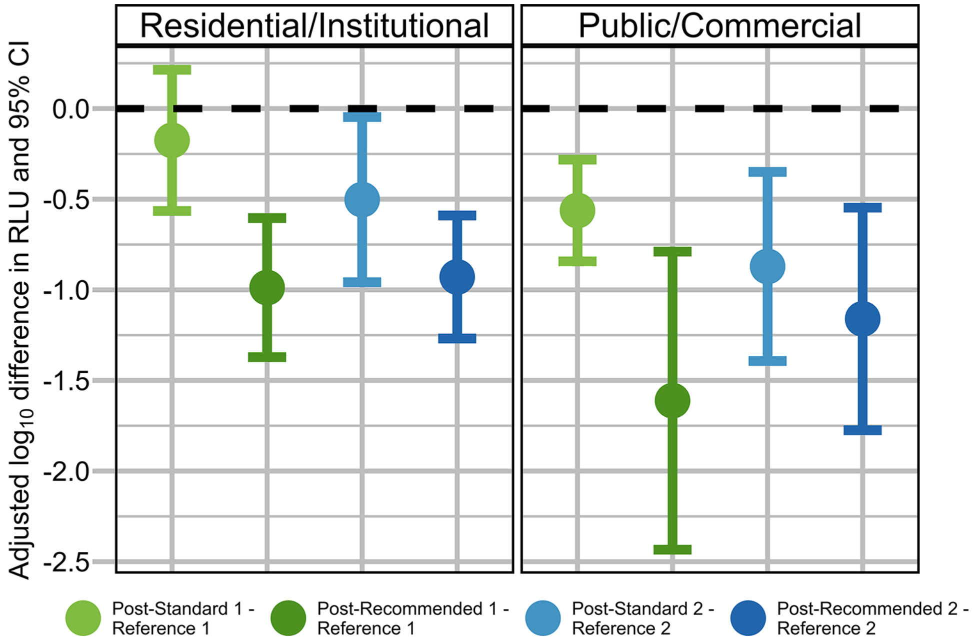

Figure 2. Reductions in organic surface contamination following cleaning and disinfection on high-touch surfaces in shared toilets, Kisumu, Kenya

Mean differences in $\log_{10}$ Relative Light Unit per $100cm^2$ and 95% confidence interval, adjusted for time of day and surface composition, by type of toilet site. References are ATP samples from surfaces when no cleaning or disinfection occurred. Comparison of ATP samples following standard and recommended cleaning procedures to references levels are shown by dots and error bars. Details of cleaning procedures are provided in Table 2.

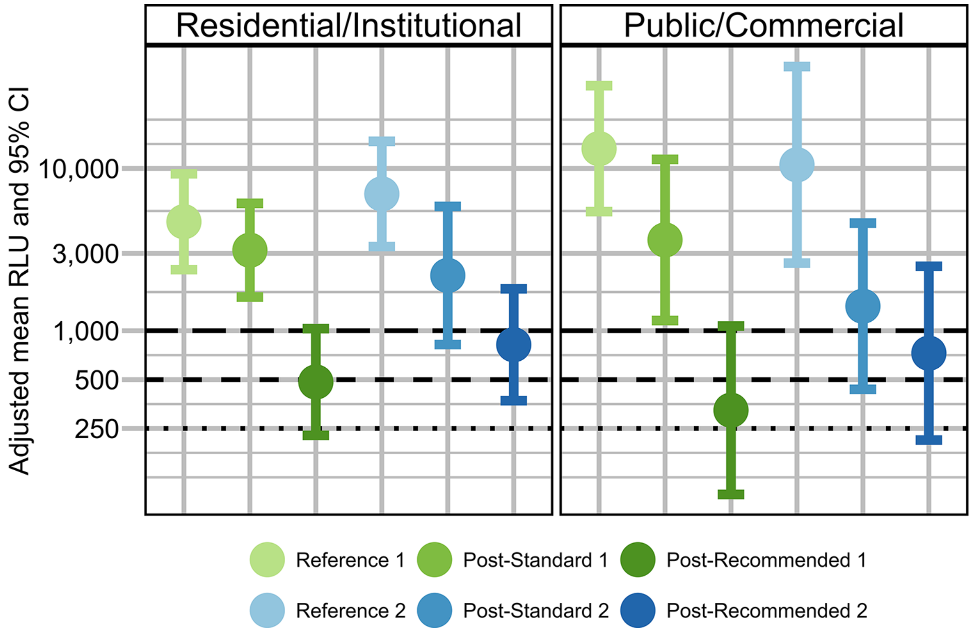

Figure 3. Estimates of organic surface contamination on high-touch surfaces in shared toilets within cleaning and disinfection assessment, Kisumu, Kenya

Mean Relative Light Unit (RLU) per $100cm^2$ and 95% confidence interval, adjusted for time of day and surface composition, by type of toilet site. Details of cleaning procedures are provided in Table 2. Horizontal reference lines at 250, 500, and 1,000 RLU show ATP cleanliness thresholds from manufacturer and previous studies that sampled from toilets.

Table 1:

Study procedures for cleaning and disinfection assessment by round

Reference Round 1	(November 2021) No cleaning or disinfection
Post-Cleaning Round 1	(Observed) Standard: (November 2021) Cleaning with water and soap following standard practice of representative at site (Supplemental Table 4) Recommended: (January 2022) Cleaning with detergent solution, spray application of 0.5% sodium hypochlorite disinfectant solution, allowing ≥1 minute contact, wiping dry with clean paper towel; adapted from recommendations for laboratory settings [36]
Reference Round 2	(June 2022) No cleaning or disinfection
Post-Cleaning Round 2	Standard: (June 2022) Cleaning with detergent solution, disinfection according to common practice (using solution mixed by adding 1 capful of 3.5% sodium hypochlorite (approximately 6 mL) to 1 L of water, yielding an approximate 0.02% chlorine solution) Recommended: (June 2022) Cleaning with detergent solution, spray application of 0.1% sodium hypochlorite disinfectant solution, allowing ≥1 minute contact time, wiping dry with clean paper towel; adapted from recommendations for healthcare facilities [37,38]

Table 2:

Associations between toilet characteristics and organic surface contamination on high-touch surfaces in shared toilets from initial assessment, Kisumu, Kenya

	Adjusted difference in mean $\log_{10}$ RLU/100 cm^2 (95% CI)	Adjusted mean RLU/100 cm^2 (95% CI)
Site Category		
Residential	Ref.	1,536 (847–2,787)
Public/Commercial	**0.33 (0.02, 0.64)**	3,302 (1,917–5,691)
Institutional	0.03 (−0.28, 0.35)	1,657 (1,117–2,458)
Toilet Type		
Flush/pour-flush	Ref.	1,569 (1,075–2,291)
Pit latrine	0.17 (−0.19, 0.53)	2,311 (1,192–4,481)
Container-based toilet	0.17 (−0.12, 0.45)	2,317 (1,405–3,821)
Designation		
None	Ref.	2,046 (1,450–2,886)
Female	−0.06 (−0.48, 0.37)	1,796 (859–3,756)
Male	0.05 (−0.34, 0.44)	2,287 (1,177–4,442)
Time of Day		
Early Morning < 8:29 hrs	Ref.	2,154 (1,434–3,236)
Mid-Morning 8:30 – 11:59 hrs	−0.17 (−0.40, 0.06)	1,451 (925–2,277)
Afternoon 12:00 – 15:59 hrs	−0.07 (−0.29, 0.15)	1,843 (1,172–2,898)
Early Evening 16:00 – 17:59 hrs	0.05 (−0.17, 0.27)	2,417 (1,593–3,667)
Late Evening >18:00 hrs	0.06 (−0.24, 0.36)	2,496 (1,420–4,388)
Toilet conditions present (Ref.=Not present)		
Intact feces	0.07 (−0.18, 0.33)	--
Feces traces	−0.02 (−0.26, 0.22)	--
Urine/water puddling	−0.06 (−0.25, 0.12)	--
Rubbish	**−0.15 (−0.26, -0.03)**	--
Toilet blocked/unusable	0.02 (−0.19, 0.23)	--

RLU: Relative light unit

Significant associations shown in bold text.

Table 3:

Agreement between visual and ATP-based assessments of surface cleanliness by cleaning and disinfection procedure[1] for high-touch surfaces in shared toilets, Kisumu, Kenya

	Visual Assessment				
Pass/Fail at 1,000 RLU/100 cm^2	**Not visibly dirty**	**Visibly dirty**	**Total**	**Kappa**	**P value**
Reference 1 & 2					
Pass	1	1	2	0.04	0.50
Fail	18	46	64		
Total	19	47	66		
Post-Standard 1 & 2					
Pass	20	0	20	0.03	0.34
Fail	44	2	46		
Total	64	2	66		
Post-Recommended 1 & 2					
Pass	27	16	43	-0.16	0.20
Fail	18	5	23		
Total	45	21	66		

[1]Cleaning and disinfection procedures described in Table 2

RLU: Relative light unit