



## Occurrence of bacteria and biochemical markers on public surfaces

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### Abstract

From 1999–2003, the hygiene of 1061 environmental surfaces from shopping, daycare, and office environments, personal items, and miscellaneous activities (i.e., gymnasiums, airports, movie theaters, restaurants, etc.), in four US cities, was monitored. Samples were analyzed for fecal and total coliform bacteria, protein, and biochemical markers. Biochemical markers, i.e., hemoglobin (blood marker), amylase (mucus, saliva, sweat, and urine marker), and urea (urine and sweat marker) were detected on 3% (26/801); 15% (120/801), and 6% (48/801) of the surfaces, respectively. Protein (general hygiene marker) levels  $\geq 200 \mu\text{g}/10 \text{ cm}^2$  were present on 26% (200/801) of the surfaces tested. Surfaces from children's playground equipment and daycare centers were the most frequently contaminated (biochemical markers on 36%; 15/42 and 46%; 25/54, respectively). Surfaces from the shopping, miscellaneous activities, and office environments were positive for biochemical markers with a frequency of 21% (69/333), 21% (66/308), and 11% (12/105), respectively. Sixty samples were analyzed for biochemical markers and bacteria. Total and fecal coliforms were detected on 20% (12/60) and 7% (4/60) of the surfaces, respectively. Half and one-third of the sites positive for biochemical markers were also positive for total and fecal coliforms, respectively. Artificial contamination of public surfaces with an invisible fluorescent tracer showed that contamination from outside surfaces was transferred to 86% (30/35) of exposed individual's hands and 82% (29/35) tracked the tracer to their home or personal belongings hours later. Results provide information on the relative hygiene of commonly encountered public surfaces and aid in the identification of priority environments where contaminant occurrence and risk of exposure may be greatest. Children's playground equipment is identified as a priority surface for additional research on the occurrence of and potential exposure to infectious disease causing agents.

**Keywords:** *Fomites, hygiene, biochemical markers, playgrounds, offices, coliforms, bacteria*

### Introduction

Inanimate objects (fomites) have been shown to play a role in the transmission of human pathogens either directly, by surface-to-mouth contact, or indirectly, by contamination of fingers and subsequent hand-to-mouth contact (Butz et al. 1993; Haas et al. 1999; Sattar et al. 2000). Other routes of exposure include the eyes, nose, and cut or abraded skin (Hall &

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Douglas 1981; Beltrami et al. 2003). Post-infection, pathogenic organisms, i.e., viruses, bacteria and protozoa, may be excreted in large numbers in biological substances including blood, mucus, saliva, feces and urine (Hall & Douglas 1981; Hall et al. 1981a; Feachem et al. 1983; Weber et al. 1994; Uhnoo et al. 1990; Islam et al. 2001). Some microbes are infectious at very low doses and can survive for hours to weeks on nonporous surfaces, such as countertops and telephone handpieces (Mahl & Sadler 1975; Bean et al. 1982; Noskin et al. 1995; Sattar & Springthorpe 1999; Bures et al. 2000; Barker et al. 2001; Abad et al. 2001). A number of viruses, including influenza A virus, hepatitis A virus, and herpes simplex virus can be found in oral secretions of those infected and survive 2–24 hours on hard surfaces (reviewed in Beumer et al. 2002).

Fomites are thought to play a role in the spread of the SARS virus, where they are known to survive for up to 96 hours on environmental surfaces and longer in the presence of biological substances (Duan et al. 2003). Likewise, contaminated fomites have been implicated in the persistence of Norovirus outbreaks between guests in a hotel and on cruise ships (McEvoy et al. 1996; Cheesbrough et al. 2000). A Norovirus surrogate survives 21–28 days, at room temperature, when dried (Doutree et al. 1999). Pathogens are readily transferred to hands from contaminated fomites and to the mouth from contaminated hands (Gwaltney & Hendley 1982; Abad et al. 2001; Rusin et al. 2002). Rotavirus contaminated 16–30% of surfaces in day care centers (Keswick et al. 1983; Wilde et al. 1992; Butz et al. 1993) and Rhinovirus was isolated from 39% of the hands of adults with colds (Hendley et al. 1973) and 43% of surfaces touched with experimentally contaminated hands (Gwaltney & Hendley 1982).

The presence of microbial contamination on home surfaces (Rusin et al. 1998) and clinical settings (Gurevich et al. 1988) has been previously studied, however, little is known about the relative hygiene of commonly encountered surfaces, outside the home. Bellamy et al. (1998) used biochemical markers to indicate the general hygiene of home environments, finding that nearly 98% were positive for protein, 2% for hemoglobin and 29% for amylase. The purpose of this study was to identify public environments where the occurrence of and exposure to contaminated fomites may be greatest. Determining occurrence and exposure levels to contaminants from specific routes, is the area of greatest uncertainty in microbial risk assessment.

This study provides novel information on the occurrence of hygienic markers on public surfaces and identifies the relative importance of surfaces where exposure rates may be highest and preventative health measures (i.e., handwashing following exposure, cleaning, and biocide treatment) may be targeted. Frequently contacted public surfaces from daycare facilities, offices, shopping centers, miscellaneous activities, and other sites were monitored for the occurrence of fecal and total coliform bacteria, heterotrophic plate count (HPC) bacteria, protein, and biochemical markers for biological substances. In addition, a fluorescent copolymer resin tracer was used to evaluate the potential transfer, and priority exposure routes, of surface contaminants from public places to hands and other environments. The use of biochemical markers or bacteria provides a cost effective and time efficient approach to comparatively evaluate the hygiene of a large number of samples.

## **Methods**

### *Site selection and sample collection*

From 1999–2003, 1061 samples were collected in four US cities (Chicago, IL, Tucson, AZ, San Francisco, CA and Tampa, FL) from surfaces in five general categories including shopping, daycare, office, and personal environments, and miscellaneous activities (Table I).

Table I. Sites sampled for the presence of protein and biochemical markers. Sample number (n) (Percent + ve for: > 200 µg/10 cm<sup>2</sup> protein; biochemical marker).

<i>Shopping 333 (44;21)</i>	Malls 163 (49;24)	Telephones, tables, handles (strollers, doorknobs, trash cans, stairs, escalators), elevator buttons, restrooms*, display cases, chairs, highchairs, countertops
	Grocery stores 90 (36;17)	Telephones, shopping cart handles, food items (fruit, meat packages), refrigerator handles, common use pens
	Vending machines 43 (47;14)	Newspaper, soda, food, ATM buttons, arcade games, candy machines, water machines,
	Banks 28 (46;29)	ATM buttons, common use pens, counter tops
<i>Activities 308 (51;21)</i>	Copy store 9 (11;11)	Copy buttons, countertops
	Gyms 18 (28;28)	Pool surfaces (benches, counter, locker handles), exercise equipment, handrails, water fountain, doorknobs, restrooms*
	Air travel 25 (60;4)	Arm rests, food trays, luggage carts, water fountains, escalators, phones, restrooms*
	Playgrounds 42 (74;36)	Children's playground equipment (indoor and outdoor play equipment), children's rides, park surfaces (concession counters, restrooms*)
	Bus travel 31 (61;35)	Benches, handrails, call button, arm rests, vending machines
	Restaurants 51 (61;14)	Menus, tables, table condiments, restrooms*
	Doctor's offices 39 (44;10)	Arm rests, children's play areas, elevator buttons, waiting room phone
	Movie theatres 57 (39;26)	Arm rests, water fountains, restrooms*, video games, phones, doorknobs
	Miscellaneous 45 (18;38)	Parks (benches, water fountains), swimming pools (locker room benches, tables)
	<i>Daycare 54 (65;46)</i>	Kitchen surfaces, highchairs, toys, cups, changing tables, play tables, restrooms
<i>Office 105 (46;11)</i>	Copier buttons, computer keyboards, file cabinet handles, phones, fax machines, doorknobs, restrooms*, cash registers, elevator buttons, kitchen appliances	
<i>Personal items 20 (40;5)</i>	Purses, backpacks, briefcases, home surfaces (sink, refrigerator, toys, washing machines)	

\*Faucets, doorknobs, baby changing tables, toilet flush handles and seat, soap and towel dispenser handles.

Sites were swabbed with sterile, cotton-tipped applicators moistened either with saline, for the collection of biological substances, or tryptic soy broth (TSB) for the collection of bacteria, each containing 100 mg/ml sodium thiosulphate neutralizer. Protein was collected using swabs and reagents from the Assure Protein colorimetric field test kit (Biocontrol Systems, Bellevue, WA). A maximum 10 cm<sup>2</sup> area was sampled and the swabs were immersed in plastic test tubes with 1 ml of sterile saline, or TSB eluting solution. Samples were transported, on ice, to the test laboratory where they were mixed vigorously, using a vortex mixer, for 10 s and allowed to stand for 1 min prior to swab removal. Within 24 h, samples were analyzed for the presence of hemoglobin (indicating the presence of blood), alpha-amylase (indicating the presence of mucus, saliva, sweat and urine) and total protein (indicating general hygiene) using methods previously validated by Bellamy et al. (1998). In addition, samples were analyzed for urea, using the blood urea nitrogen test (BUN, indicating the presence of urine and sweat). HPC bacteria were cultured with tryptic soy agar (TSA) and total and fecal coliforms were cultured with mEndo and mFC media, respectively (Difco, Becton Dickinson, Franklin Lakes, NJ).

*Hemoglobin detection.* Hemastix test strips (Bayer Corporation, Tarrytown, NY), were used to quantitatively detect hemoglobin in environmental samples. The strip was completely immersed in swab eluent, immediately removed, and examined after one-minute incubation at room temperature. Based on the detection limits of the test, hemoglobin concentrations of  $> 0.015$  mg/ml, or approximately 5–20 intact red blood cells, were apparent by comparison of the sample strip with color chart controls supplied with the test kit. Sheep's blood (supplied by Dr Patricia Rusin, The University of Arizona) was used as a positive control.

*Alpha-amylase activity.* The presence of alpha-amylase in swabbed surface eluants was quantitatively detected using a commercial enzymatic test kit and spectrophotometric analysis (Sigma-Aldrich Corporation, St. Louis, MO). Sample volumes of  $20 \mu\text{l}$  were added to 1 ml of amylase reagent at  $30^\circ\text{C}$  and incubated for 2 min. Absorbance was read at 405 nm after 1 and 2 min and the amylase activity and the reaction rate were calculated according to manufacturer's instructions. Human urine and diluted serum, with known concentration of amylase, were used as positive control reagents (Sigma-Aldrich Corporation, St. Louis, MO). Based on the detection limits of the test, only values  $> 10$  U/ml were considered positive.

*Urea detection.* The blood urea nitrogen (BUN) test was performed according to manufacturer's instructions to enzymatically detect the presence of urea nitrogen in eluted swabs as an indicator of urine and sweat (Sigma Diagnostics, St. Louis, MO). Ten microliters of sample was added to 1 ml of BUN reagent, mixed and incubated at  $30^\circ\text{C}$  for 1 min. Absorbance at 340 nm was read, using a spectrophotometer at 30 sec intervals, and the BUN rate calculated relative to commercially available urea positive control standards (Sigma Diagnostics, St. Louis, MO). Values of 5–100 mg/ml were within the linear range of the test and considered a positive result.

*Protein detection.* The Assure Protein colorimetric field test kit was used to detect total protein on swabbed surfaces (Biocontrol Systems, Bellevue, WA). Swabs were moistened with supplied wetting solution, used to sample the test site ( $10 \text{ cm}^2$ ), and then placed in 1 ml volumes of eluting solution followed by the addition of three drops of reactive color indicator reagent. After a 10 min incubation, samples were compared to a color chart. A green color indicated negative protein levels and increasing intensities of gray to purple indicated an increasing concentration of protein. Samples yielded a visual result corresponding to a scale of 1 to 4 in color intensity (1 = 0–25, 2 = 55–100, 3 = 200–420, 4 =  $> 600 \mu\text{g/ml}$ ). Results measuring a 3 or 4, indicating  $> 200 \mu\text{g/ml}$  protein, were considered heavily contaminated based on the fact that surfaces were often visibly soiled.

*Bacterial assays.* Total and fecal coliforms were cultured using m-Endo agar incubated 24 h at  $35^\circ\text{C}$  and mFC agar incubated 24 h at  $44.5^\circ\text{C}$ , respectively. HPC bacteria were grown on TSA media following incubation at  $35^\circ\text{C}$  for 24 h.

*Tracer studies.* Three separate office environments and 35 employees were monitored. A commonly used surface (i.e., telephone, faucet, copier button, or doorknob) was inoculated every hour with either powdered or spray fluorescent resin, visible only under black light (Glo Germ<sup>TM</sup>, Brevis Corporation, Salt Lake City, UT). Surfaces at the employee's work and five volunteer's home environments were examined at the end of the day for the presence of the invisible dye by exposure to black light.

## Results

Surfaces sampled from public environments ( $n = 1061$ ), were categorized as: (1) Shopping-malls, grocery stores, banks, vending machines, and other retail stores; (2) Miscellaneous activities – gyms, air travel environments, playgrounds, bus travel environments, restaurants, doctor's offices, and movie theaters; (3) Daycare facilities; (4) Work environments; and (5) Personal items (Table I). All samples were collected during the summer months (May to August).

Initially, 801 samples were collected and analyzed for biochemical markers and protein. One in four (200/801) surfaces sampled were considered heavily soiled, testing positive for  $> 200 \mu\text{g}/10 \text{ cm}^2$  of protein, while one in five (168/801) tested positive for at least one biochemical marker (hemoglobin, amylase, or urea; Figure 1).

Hemoglobin was detected in 3% (26/801) of the surfaces sampled. Children's playground equipment was the most frequently positive site ( $n = 5/42$ ). Other positive surfaces were from the gym (water fountain), mall (restroom faucet, 2-trash can handles, baby changing table, toilet seat and tank, 3-escalators, elevator button, handrail), office (calculator, cash register), grocery store (meat counter), bus (bench, hand railing), and restaurant (toilet rim, table bottom) environments. In addition, 1-purse bottom tested positive.

Alpha-amylase was found on 15% (120/801) of the samples analyzed (Figure 1). The five most frequently positive sites were: children's playground equipment, daycare surfaces, public phones, computer keyboards, and vending machines.

Urea was detected in 6% (48/801) of the samples (Figure 1). The five most frequently positive sites included: public restrooms, daycare facilities, restaurants, children's playground equipment, and shopping cart handles.

Although 24% (41/168) of the samples positive for biochemical markers were from bathroom surfaces, the remaining 76% (127/168) were from other surfaces. The daycare environment was positive for biochemical markers, and protein levels  $> 200 \mu\text{g}/\text{ml}$ , most frequently (46%; 25/54 and 65%; 35/54, respectively) compared to other categories such as

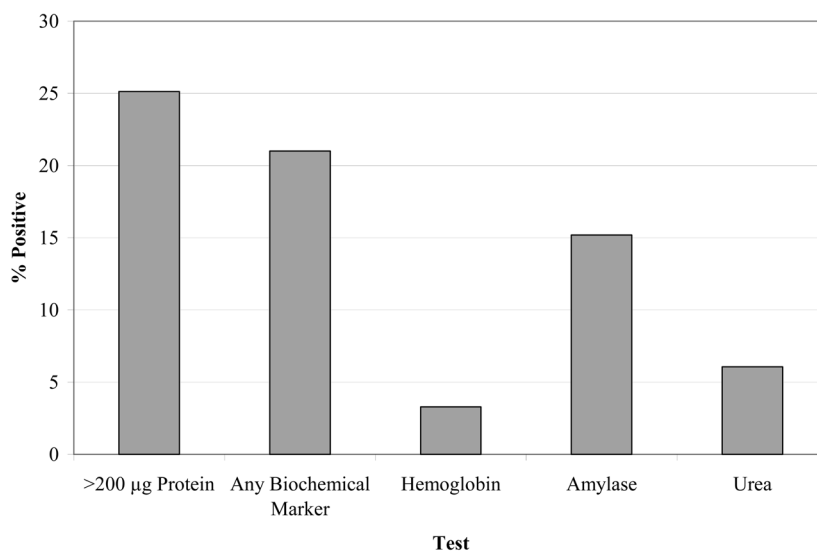


Figure 1. Percentage of surface samples positive for protein and biochemical markers ( $n = 801$ ).

shopping environments (21%; 69/333 and 44%; 147/333), miscellaneous activities (21%; 66/308 and 51%; 157/308), and office environments (11%; 12/105 and 46%; 48/105) (Table I). The top ten specific surfaces most frequently positive for biochemical markers, were also the top 10 sites positive for protein detection at levels  $> 200 \mu\text{g/ml}$  (Table II). Children's playground equipment was the site most likely to test positive for biochemical markers, at a rate of 36% (15/42) positive, followed by bus rails/armrests (35%; 11/31) and public bathroom surfaces (25%; 41/165).

Following the initial survey, an additional sixty sites were analyzed for both biological substances and total and fecal bacteria. Approximately ten samples each were collected from the following sites in Tucson: the airport, bus station, public bathroom, home, children's playground equipment and shopping mall. Twenty percent (12/60) of the sites were positive for a biochemical marker, 20% for coliform bacteria, and 7% (4/60) for fecal coliforms. Samples positive for total coliforms were also positive for biochemical markers 50% (6/12) of the time. Although all fecal coliform positive samples (4/4) were also positive for biochemical markers, eight samples were positive for biochemical markers and negative for fecal coliforms. Fecal coliform bacteria were detected on three mall sites (escalator, table, and highchair) and on one piece of outdoor playground equipment.

To further evaluate general hygiene, the presence of total and fecal coliforms and heterotrophic plate count (HPC) bacteria were monitored from an additional 200 environmental surfaces at malls, shopping centers, and restaurants, in Tucson, AZ and Tampa, FL. Twenty samples each were collected from diaper changing tables, shopping cart handles, chair armrests, children's playground equipment, tabletops, restaurant condiments, doorknobs, ATM buttons, elevators, and escalators. Six sites were positive for total coliforms (shopping cart, 2-children's playground equipment, table top, condiment and ATM buttons) however no fecal coliforms were isolated from any sample. Most (93%; 186/200) sites tested positive for some level of HPC bacteria, ranging from none detected to  $2.1 \times 10^6$  CFU/10  $\text{cm}^2$  area, with a Geomean range of 50–415 CFU.

*Transfer potential.* Three office environments were monitored after artificial inoculation of common use surfaces with a copolymer resin tracer. After touching contaminated surfaces, 86% (30/35) of office workers, from three separate offices, transferred the resin tracer to their hands while 82% (29/35) transferred the resin to additional surfaces. Following inoculation of

Table II. Percentage of specific surfaces positive for protein and biochemical markers.

Surface (n)	% $> 200 \mu\text{g}/10 \text{ cm}^2$ Protein Test (n)*	% Positive for Biochemical Markers (n) <sup>†</sup>
Playground equipment	(42) 74 (31)	36 (15)
Bus rails/armrests	(31) 61 (19)	35 (11)
Shopping cart handles	(24) 54 (13)	21 (5)
Chair/seat armrests	(68) 51 (35)	21 (14)
Vending machine buttons	(43) 47 (20)	14 (6)
Escalator handrails	(37) 46 (17)	19 (7)
Public bathroom surfaces	(165) 46 (76)	25 (41)
Customer-shared pens	(19) 42 (8)	16 (3)
Public telephones	(47) 34 (16)	13 (6)
Elevator buttons	(21) 29 (6)	10 (2)

\*Positive protein results reading of  $\geq 3$  ( $> 200 \mu\text{g/ml}$ ) with the visual assure kit. <sup>†</sup>Positive for at least one of the following: amylase, urea, or hemoglobin.

a bathroom faucet and exit doorknob, the resin spread most commonly to employees' hands, faces, phones, and hair. From an inoculated community phone, the resin spread most frequently to hands, face, hair, desktop surfaces, drinking cups, keyboards, pens, and doorknobs. Resin was also found on a nearby drinking water fountain. Finally, from an inoculated copy machine button, resin transferred to copied and original documents, computer equipment, and employees' hands and faces. Five of the volunteers were accompanied to their homes for additional sampling. Twenty minutes after arriving home from the office, resin was commonly (5/5) found on volunteers' hands, personal items (i.e., backpacks, keys, purses) and home surfaces (doorknobs, light switches, counter tops, and kitchen appliances).

## Discussion

This study is the first to identify the relative ranking of select public surfaces with regard to the frequency of hygienic markers present. Monitoring the occurrence of bacteria, protein and biochemical markers on environmental surfaces provides baseline information regarding the areas of greatest potential exposure and public health risk. The biochemical tests used to evaluate surface hygiene were chosen based on their previous success in the monitoring of biological substances in the home environment (Bellamy et al. 1998). The addition of the blood urea nitrogen test (primarily a marker of urine but also a marker of sweat) offered a means to further define the source of amylase (a marker of urine, sweat, mucus and saliva) positive samples. The various biochemical tests used in this study are not specific markers of human biological substances and thus provide a broader analysis of substances that may originate from either animal or human origin.

Hemoglobin is a protein component of vertebrate (i.e., human or animal) blood, comprising 33% of red blood cells. Bloodborne pathogens may be carried by humans (i.e., human immunodeficiency virus, hepatitis B virus), or animals and animal products such as packaged raw meats, (i.e., *E. coli* in raw ground beef).

Urea is the chief solid component of mammalian urine and may be found in human and animal excreta. Urea may also be detected in sweat and in the epidermis of healthy human skin (i.e., sweat). In addition, urea is commercially produced as an additive to skin moisturizers, animal feeds, plastics, fuels and oils. Amylases are normally present in human and animal (i.e., mice, pig, etc.) mucosa, saliva, and urine at levels ranging from (53–123; 0–375; and 0–300 U/l, respectively). They have also been used as additives to foods and vitamins and are natural byproducts of fungus (i.e., *Aspergillus*).

Proteins are complex combinations of amino acids and are essential building blocks of living cells. Found in skin, hair, and human cells, protein is the most abundant dry substance in the human body. It is also a major component of food products and other organic matter. In studies by Bellamy et al. (1998), 97.8% of surfaces in the home environment were positive for detectable levels of protein, with only 6% positive for > 20 µg/ml. In our study, 25% of the sites sampled from public surfaces were positive for protein at a 10-fold increase in concentration, compared to home surfaces. The top 10 sites positive for protein > 200 µg/ml were also the top 10 sites positive for biochemical markers, in approximately the same descending order. The bathroom environment, however, ranked number seven in frequency for protein levels > 200 µg/ml and number three for presence of biochemical markers. This may be due to the fact that public restrooms are cleaned regularly, as they are prone to frequent recontamination with biological substances. Other public surfaces may be rarely cleaned and could remain soiled for long periods of time.

HPC bacteria were isolated from 93% of the tested public surfaces with a geometric range of 50–415 CFU/10 cm<sup>2</sup>. Since such a large percentage of samples were positive for HPC bacteria with no consistent differences between sites, their prevalence provided little relative information on the hygiene of various environments. Fecal and total coliforms, on the other hand, were rarely isolated (1.5%, 4/260 and 7%, 16/260 samples, respectively). More research, however, is needed to determine if coliform bacteria, or the biochemical markers for that matter, would be reliable indicators of pathogen occurrence.

The artificial tracers used in this study were 5 µm melamine copolymer resin beads. This product has been used to teach aseptic technique in hospitals, and hygiene in industry and schools. Tracers, as they were used here, were not designed to mimic the transfer rates of human pathogens but rather to aid in the identification of high use areas in home and office environments, and thus were inoculated on test surfaces every hour. This protocol may exaggerate exposure levels but aids in the identification of priority exposure sites.

Although it is not known how well the transfer rates of polymer tracers relate to various human pathogenic microbes, it is well documented that contaminated surfaces spread infectious doses of pathogens to the mouths of exposed individuals following handling (Bloomfield & Scott 1997; Rusin et al. 2002). Transfer of *Escherichia coli* was 40% from a laminate surface to fingers up to 2 h following contamination (Scott & Bloomfield 1990). Rheinbaben et al. (2000) documented that at least 14 successive persons transferred ΦX174 bacteriophage to their hands and to an additional six successive contact persons, from an inoculated door handle. Following exposure to contaminated coffee cups, 54% (14/26) of healthy young adults became infected with rhinovirus (Gwaltney & Hendley 1982).

Pathogen survival on fomites is an important factor in evaluating exposure potential. Influenza viruses survive up to 48 h on dry surfaces (Bean et al. 1982) and are detected on more than 50% of fomites in homes of children with flu-like symptoms and day care centers during influenza season (Boone & Gerba 2004). A Norovirus surrogate may survive for weeks at room temperature after being dried (Doulton et al. 1999).

The results of this study suggest further evaluation of children's playground equipment as a priority target site, based on the frequency of samples positive for both protein levels > 200 µg/10 cm<sup>2</sup> and biochemical markers and the potential for exposure to children, a population recognized as being more susceptible to adverse outcomes following exposure to pathogenic microbes (Nwachuku & Gerba 2004; Gerba et al. 1996). Exposure to contaminated fomites is particularly important with children who have not yet developed proper sanitary habits (i.e., use of toilet facilities, handwashing, and frequent hand-to-mouth or fomite-to-mouth contact) (Springthorpe & Sattar 1990). Hutto et al. (1986) found the median number of fomite or hand-to-mouth contacts per hour, among children, were as follows: 1–12 months, 64; 13–24 months, 34; 25–30 months, 27; 31–36 months, 5 and 37–48 months, 10. Approximately 50% of the children's playground equipment sampled were from indoor environments. We could find no published information on the evaluation of pathogen survival on indoor versus outdoor fomites.

While the presence of biochemical markers and protein provides information on the relative hygiene of various environments, little is known regarding their correlation with infectious microbes. This study suggests that they do not correlate with fecal coliform bacteria. The risk of disease transmission via surfaces involves a number of factors including the: (1) frequency of site contamination and exposure; (2) level of pathogen excreted by the host; (3) likelihood of transfer of the infectious agent to a susceptible individual; (4) virulence of the organism, (5) immunocompetence of the persons in contact; (6) the practice of control measures (i.e., disinfectant use and personal hygiene) and other factors. Even detection of a pathogen does not determine the risk of infection and disease manifestation. Identifying key surface sites



most likely to serve as intermediate routes of disease transmission, either due to their frequent contamination, frequent contact with susceptible humans, or infrequent implementation of effective control measures (i.e., cleaning, disinfecting, and handwashing) is important in order to increase awareness of the need for an integrated hygiene control regimen.

## Conclusion

This study identified the relative hygiene of various public environments and areas of high level of contact and exposure based on biochemical markers of biological substances. Twenty-five percent of samples collected were positive for protein at levels  $> 200 \mu\text{g}/10 \text{ cm}^2$ , 20% tested positive for biochemical markers, 93% for HPC bacteria, 7% for total coliforms, and 1.5% for fecal coliforms. Although collectively, the use of biochemical markers and bacterial indicators provide an overall picture of the relative hygiene of specific environmental surfaces and target priority exposure sites, more research is needed to evaluate the relationship between the presence of HPC bacteria, fecal and total coliform bacteria, and biochemical markers on public surfaces and the risk of infection from a microbial pathogen. Based on the results of this study, children's playground equipment is identified as a priority surface for additional research on the occurrence of and potential exposure to infectious disease causing agents.

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