

Decontamination of high-touch environmental surfaces (HITES) by wiping: quantitative assessment of a carrier platform simulating pathogen removal, inactivation and transfer in the field

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Abstract

We report here a carrier platform (Teflon; 30.0 × 60.0 × 0.9 cm) and a carrier retrieval device to assess pathogen decontamination of high-touch environmental surfaces (HITES) by wiping. Each one of the nine metallic disks (1 cm diameter and 0.7 mm thick) received 10 µL of the microbial suspension in a soil load, the inocula dried and the platform then wiped with a piece of fabric presoaked in a control or disinfectant fluid; the used wipe was immediately applied on a second platform with sterile disks to assess microbial transfer. Each test and control disk from a given platform was separately and simultaneously retrieved into 10 mL of an eluent/neutralizer for assays at the end of the contact time (total of 5 min, starting from the beginning of the wiping). *Staphylococcus aureus* and *Acinetobacter baumannii* were used as representative HITES-borne pathogens. The wipes tested separately contained 0.26% of a quaternary ammonium compound (Product A), and 250 ppm sodium hypochlorite at neutral pH (Product B). The control fabric (Product C) was dampened with a buffer containing a detergent. Product A achieved a >4 log₁₀ (>99.99%) reduction in the viability of the bacteria on wiping with a barely detectable level of transfer of CFUs to clean disks. Product B achieved a >2 log₁₀ (>99.00%) reduction in the viability of the test microbes while transferring a higher level of CFUs as compared to Product A. With Product C, there was a <1 log₁₀ (<86.2%) reduction in the viability of the test microbes while transferring >1% of the contamination.

Significance and impact of the study:

This versatile platform is usable with all major classes of high-touch environmental surfaces-borne pathogens and suitable for work with all types of hard, nonporous materials to assess pathogen inactivation, removal, and transfer.

Keywords: biocides, disinfection, environmental health, microbial contamination, microbicide, presoaked

Introduction

There is mounting appreciation of high-touch environmental surfaces (HITES) as vehicles for pathogens in healthcare and other settings (Weber et al. 2010; 2013, Carling 2016, Donskey 2019). There is also a corresponding increase in products and procedures for HITES decontamination. Nonetheless, such routine practices remain suboptimal (Sattar and Maillard 2013, Carling 2016, Jacobshagen et al. 2020) and, indeed, counter-productive as cursory wiping of surfaces can spread pathogens over a wider area (Ramm et al. 2015) while loading the environment with potentially unsafe chemicals.

Barring no-touch technologies, chemical decontamination of HITES invariably incorporates wiping (Sattar and Maillard 2013, European Norm EN 16615-2015). However, current and widely accepted methods to assess environmental surface disinfectants do not incorporate that physical action (Sattar 2010) so critical for dislodging and removing dried contamination to allow better access to and action by the disinfectant. Thus, testing with ‘static’ (without any wiping action) protocols, and label claims based on them, only show the

microbicidal potential of a given formulation without indicating its ability to perform under actual field use. There is, therefore, a need to generate test data on such formulations via a “dynamic” (combining physical action of wiping with chemical disinfection process) test protocol to better reflect field use of the process. Such information would better inform disinfectant manufacturers, government regulators as well as infection preventionists.

We report here a carrier platform and a carrier retrieval device to better assess the decontamination of HITES by wiping. The basic objectives were to conduct laboratory-based testing of commercial wipes separately prewetted with a quaternary ammonium compound (QAC; Product A) and a sodium hypochlorite (SH; Product B) disinfectant for microbial decontamination of hard, nonporous environmental surfaces representing those found in healthcare and other settings. J-Cloth (Product C) was dampened with a detergent-containing buffer and used as a control. The aim here was to demonstrate that the platform and the test protocol using it could yield reproducible data employing *Staphylococcus aureus* and *Acinetobacter baumannii* as test microbes and two types of

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Table 1. Details on the materials and pieces of equipment used in this study.

Item	Source and catalog #, if available
A sealed bag of metallic glass gravel with Velcro tape: to place over the hand of the operator to standardize the pressure during the wiping of the disks.	Walmart
Air displacement pipettes: Eppendorf or equivalent, 100 to 1000 μ L with disposable tips.	Ranian and Fisher Scientific
An aerobic incubator: for incubation of culture plates at $36 \pm 1^\circ\text{C}$.	Forma 1025
Analytical balance: to weigh chemicals and to standardize inoculum delivery volumes by pipettes.	Denver Instrument: APX-150
Biological safety cabinet, Class II (Type A): for handling biohazardous materials and to protect items being handled from extraneous contamination.	Baker SG-600 S/N: SL 36021V
Block magnet: strong enough to hold the magnetized disk carriers in place in the plastic vials while the liquid is being poured out of them for membrane filtration.	VWR Cat No: 470149–774
Centrifuge: to allow for the sedimentation of the cells of the test organism(s) for concentration, or washing, or both.	Beckman J2 S/N: 3097
Disposable gloves: nitrile for handling any biohazardous materials.	VWR Cat: 76 411
Filter sterilization system for media and reagents: a membrane or cartridge system (0.22 μm pore diameter) is required for sterilizing heat-sensitive liquids.	Filter: Pall Life Sciences Cat #4658 Syringe: EXELINY Cat # 26 290
Forceps, straight, or curved: (1) with smooth tips to handle membrane filters, and (2) to pick up the metal disk carriers for placement in plastic vials.	VWR Cat: 89259–954
Freezers: a freezer at $-20 \pm 2^\circ\text{C}$ for the storage of media and reagents. A second freezer at -80°C or lower is required to store the stocks of test organisms.	KENMORE: Model: 253.9281110 S/N: WB91016479
Glassware: 1-L flasks with a side-arm and appropriate tubing to capture the filtrates from 47-mm diameter membrane filters; 250-mL Erlenmeyer flasks for making culture media and reagents.	Filtering Flask PYREX Cat#: 5340 VWR Erlenmeyer Flask Cat#: 4980
Magnetic stir plate and stir bars: large enough for a 5-L beaker or Erlenmeyer flask while preparing culture media or other solutions.	VWR Cat#: 76447–044 VWR Cat#: 58948–080
Membrane filtration system for capture of the test organisms: sterile 47-mm diameter membranes (0.22 μm pore diameter) with glass, plastic or metal holders.	Pall Corporation Cat# 66 234
Miscellaneous labware: pipette tips, plastic vials, dilution tubes.	VWR, Fisher brand Nalgene
Petri plates (Pyrex glass; 150 mm in diameter): for holding and autoclave-sterilization of the metallic disk carriers.	Pyrex Cat# 3160–150
Positive displacement pipette: a pipette and pipette tips fitted with “plungers” that can dispense accurately 10- μ L volumes for inoculation of carriers without the aerosol generation that occurs when air displacement pipettes are used.	Pipette: Eppendorf Tips: Eppendorf Cat#022 354 159
Refrigerator: at $5 \pm 3^\circ\text{C}$ for cold storage of media, culture plates, and reagents.	SANYO Model: MPR-1410R S/N: 40 100 997
Serological pipettes: sterile reusable or single-use pipettes of 1.0, 5.0, and 10.0 mL capacity for dispensing liquids or for making dilutions.	1ml: VWR: Cat# 76093–882 5 mL: Costar: Cat#: 4487 10 mL: VWR: Cat#:8130–898
Sterile dispenser: 10 mL, for dispensing diluent/eluent.	VWR: Cat#: 75856–470
Sterile disposable plastic Petri dishes (100 \times 15 mm): for holding culture media.	VWR Cat#: 89022–320
Sterile polypropylene centrifuge tubes with caps; 25 and 50 mL capacity): for holding and centrifugation of bacterial suspensions.	VWR Cat#: 10025–698
Timer: any stopwatch for reading time in minutes and seconds.	VWT Cat#: 100493–814
Vacuum source: a vacuum pump or access to an in-house vacuum line for membrane filtration of reagents or culture media.	Thermo Scientific Model: 13–310-900
Vials (Nalgene): wide mouth, 30 mL capacity to collect the retrieved disks.	Thermo Scientific™ Cat # 11–815-10A
Vortex mixer: to mix the eluate and rinsing fluid in the carrier vials to ensure efficient recovery of the test organism(s).	Fisher Scientific Cat# 12–812

commercial wipes. Each test was repeated at least three times with each organism and test sample (including the control) to generate enough data for proper statistical analyses.

Materials and equipment

Table 1 gives the details on the materials and pieces of equipment used in this investigation.

All items requiring sterilization prior to use were autoclaved at 121°C for 25 min. All used disposable labware was autoclaved and discarded as biomedical waste.

The soil load (SL)

A SL was added to microbial suspensions (Springthorpe and Sattar 2007, ASTM 2017) to simulate the presence of body fluids. The SL was a mixture of bovine serum albumin

(BSA; Sigma-Aldridge), mucin from the bovine submaxillary gland (Sigma-Aldridge), and yeast extract powder (VWR). Their stock solutions were prepared separately by dissolving 0.5 g, 0.04 g, and 0.5 g, respectively, in 10 mL of Dulbecco's phosphate buffered saline (DPBS; pH 7.2 ± 0.2). The solutions were individually passed through a syringe-mounted polyethersulfone (PES; Sterlitech) membrane ($0.2 \mu\text{m}$ in pore diameter), aliquoted in 1.5-mL volumes, and stored at $-20 \pm 2^\circ\text{C}$ with a shelf life of 1 year. The SL was tested and proved harmless to the test organisms as it did not alter their viability titer after a contact of 1 h at room temperature.

Sterility controls

All culture media and reagents were tested for sterility prior to use.

Test substances

Commercial pre-wetted wipes separately containing a QAC-based disinfectant (Alkyl 5%, C14 4%, C12 10% C6, Dimethyl benzyl ammonium chloride–0.26% w/w), (A) and a sodium hypochlorite-based disinfectant containing 250 ppm at neutral pH (B), and phosphate-buffered saline +0.1% polysorbate-80 (PBST) as a control (C). Product A was purchased in the open market a week before the commencement of the study. Product B was a gift from its manufacturer. The labels on both the products were masked for “blinding” the operator. The products were stored at room temperature ($22 \pm 2^\circ\text{C}$) at CREM Co Labs in a secure place with access only to authorized individuals.

A box of J-Cloth (E.D. Smith Foods, Ltd.; <http://www.jcloth.com>) was also purchased in the open market. This absorbent fabric is made with wood fiber and is biodegradable. It was selected because its freedom from any antimicrobial activity and also because it is frequently used in the wiping of HITES in a variety of settings. Just prior to testing, a piece of J-Cloth was retrieved from its container, and wetted with 15 mL of sterile phosphate-buffered saline with 0.1% polysorbate-80 (PBST). The volume used was just enough to evenly wet the fabric without any dripping.

All wipes were discarded as biomedical waste at the end of each experiment.

Test carriers

Disks (1 cm in diameter and 0.7 mm thick) of brushed and magnetized stainless steel (AISI 430) were purchased from Pegen Industries, Stittsville, ON, Canada; www.pegenindustries.com/). The disks were washed in a detergent solution to clean and degrease them. They were then autoclave sterilized. Each disk was used only once.

Test organisms

S. aureus (ATCC 6536) and *A. baumannii* (ATCC 19606) were grown and recovered in Trypticase soy broth (TSB) and Trypticase soy agar (TSA), respectively (OXOID, Thermo Fisher, Mississauga, ON, Canada; <https://www.hygienea.com/other-sales/canada.html>). To start a broth culture 100 μL of the stock was added to 10.0 mL of sterile TSB in a tube and incubated aerobically for 18 ± 2 h at $36 \pm 1^\circ\text{C}$. For carrier inoculation, the culture was mixed directly with the SL (see below).

Air temperature

All testing was conducted under ambient conditions ($22 \pm 2^\circ\text{C}$).

The test platform

The platforms and the carrier retrieval device are shown in Figs. 1A and 1E, respectively (Navid Manufacturing, Guelph, ON, Canada; <https://navidmfg.ca/>). The retrieval device permitted the collection of all the carriers simultaneously in separate vials containing an eluent/neutralizer immediately at the end of the wiping time. The eluates were assayed for viable organisms.

The test procedure simulated the style of HITES wiping in the field based on our own observations at a neighboring healthcare facility. Starting with the contaminated platform, the surfaces of both platforms were wiped in two steps in a predetermined manner with a single wipe. Prior to testing, 2 wipes were pulled out from the wipe dispenser and discarded. Aseptically and using a gloved hand, one wipe was then taken and directly used on both the platforms. In the first step, the contaminated platform was wiped twice transverse, back and forth, with 10%–20% overlap with the previous pass and in the second step; wiping was continued with the same wipe from the beginning of the contaminated platform to the end of transfer platform. The used wipe was discarded as biomedical waste.

To standardize the pressure during wiping, a plastic bag with 1 kg of sand was taped over the top surface of the operator's gloved hand (not shown in the figures). This level of weight was chosen based on how members of the environmental services staff (EVS) wiped HITES at a neighboring healthcare facility. Depending on the size of the operator's hand, this generated a pressure of 6–10 gram/cm^2 . The operator moved the hand over the platform without any additional pressure.

The test protocol

One clean and sterile carrier was placed in each one of the holes on both the platforms (Fig. 1A). For wipe testing, each carrier on one platform received 10 μL of the test inoculum (Fig. 1B) with an added SL, and the inocula dried under an operating biosafety cabinet (BSC) for 120 ± 10 min. The second carrier-loaded platform was used as a clean surface to determine if, and how much, microbial contamination could be transferred by wiping to uncontaminated surfaces in the immediate vicinity.

The contaminated platform was wiped using either the prewetted test or control fabric (Fig. C). On completion of the wiping, the used fabric was applied on the second platform to assess any microbial transfer. At the end of the wiping, the platforms were left undisturbed for the remaining of contact time (total of 5 min, starting from the beginning of the wiping).

The two platforms were separately placed on the carrier collection vials (Fig. 1D). Using the retrieval mechanism (Fig. 1E), the disks were collected simultaneously into individual vials (Fig. 1G) containing 10 mL of a neutralizer/eluent/diluent and vortex mixed for 30 ± 5 sec to recover the inocula from the carriers (10^0 dilution). Ten-fold dilutions were prepared for each carrier. Depending on the initial inoculum level and the level of microbicidal activity expected,

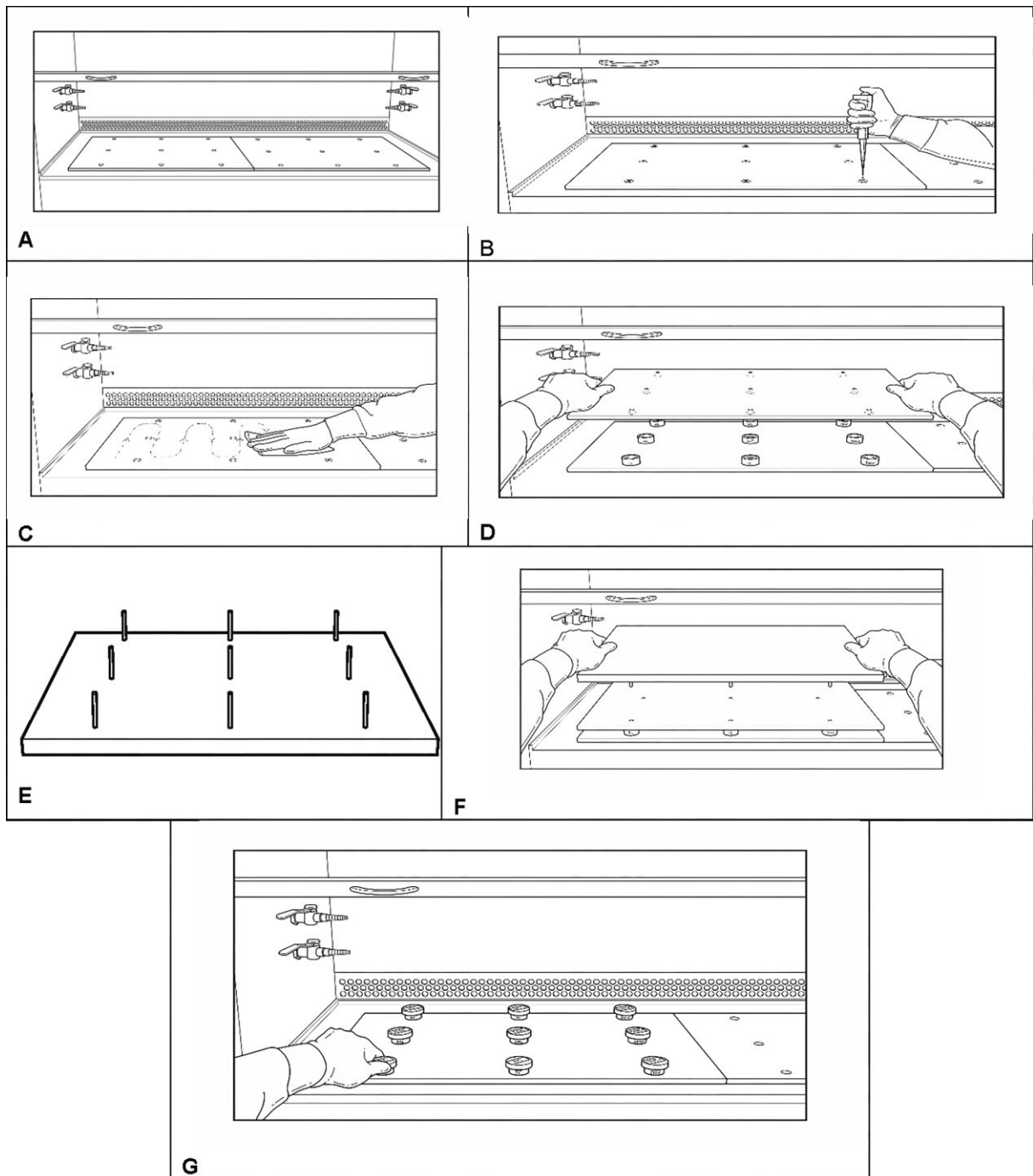


Figure 1. Wipe testing using the Teflon platform and disk carrier retrieval device. (A) Two carrier-loaded platform sitting inside an operating biosafety cabinet. (B) The carriers on the platform (contaminated platform) to the left receiving test microbial inoculum for subsequent drying. (C) The platform with the contaminated carriers is being wiped with a prewetted fabric in a standardized fashion. The used wipe is then applied over the platform with the clean carriers to study microbial transfer. (D) Each platform is placed over a tray containing nine plastic vials to separately capture the carriers directly into an eluent/neutralizer. (E) Carrier retrieval device with nine prongs to push the carriers into the collection vials. (F) The carrier retrieval device being placed on the platform for carrier collection. (G) The tray with the vials containing the retrieved carriers for viability assays.

the number of dilutions was different for test and control eluates. The selected dilutions of treated carriers were membrane-filtered, then the vial was rinsed with 10 mL of PBS. The membranes were washed with 10 mL PBS first and washed with 40 mL of PBS after pouring the contents of each vial. Finally, each membrane was placed aseptically on the surface of a TSA plate. The plates were incubated aerobically at $36 \pm 1^\circ\text{C}$ for 48 ± 4 h and the CFU counted. The plates were reincubated

for three more days to allow any injured or stressed organisms to grow. A final CFU count was recorded at the end of the fifth day.

Each test included three controls to estimate the microbial input level. One control was eluted before processing the test carriers, the second after all test carriers were processed and the third once the processing the transfer carriers was done. This spacing of the controls was to account for any possible

Table 2. Results for neutralization validation for Samples A and B against *S. aureus*.

Sample ID	Input control (IC)			Neutralizer toxicity (NT)			Neutralization effect (NE-A)			Neutralization effect (NE-B)			
	1	2	3	1	2	3	1	2	3	1	2	3	
Replicate No.	1	2	3	1	2	3	1	2	3	1	2	3	
No. of CFU per carrier	117	122	135	135	127	124	131	113	125	123	131	115	
Mean of CFUs recovered		124.7			128.7			123.0			123.0		
Index				103.2077				95.5711			95.5711		
Neutralization effectiveness				Yes				Nontoxic			Neutralized		

Table 3. Percentage reductions and percentage transferred in CFUs by the two test samples (A and B) and the control wipe (C) against *A. baumannii*.

	CFU/cm ²			Percent		Mean Percent ± Standard Deviations	
	Control	Contaminated	Transfer	Reduction	Transfer	Reduction	Transfer
Test sample A							
Test #1	4.33E + 04	2.55E-01	2.55E-01	99.9994	0.00058	99.994 ± 0.0085	0.0095 ± 0.0083
Test #2	1.63E + 04	2.55E + 00	1.78E + 00	99.9844	0.011		
Test #3	1.95E + 04	2.55E-01	3.30E + 00	99.9987	0.017		
Test sample B							
Test #1	1.69E + 04	2.060E + 02	1.44E + 01	98.78	0.086	99.318 ± 0.5038	0.0490 ± 0.0317
Test #2	3.61E + 04	2.1911E + 02	1.15E + 01	99.39	0.032		
Test #3	3.25E + 04	7.180E + 01	9.68E + 00	99.78	0.030		
Test sample C							
Test #1	3.51E + 04	2.58E + 03	4.75E + 02	92.65	1.350	86.242 ± 12.1884	1.075 ± 0.5313
Test #2	6.24E + 04	3.82E + 03	8.79E + 02	93.88	1.410		
Test #3	3.82E + 04	1.06E + 04	1.77E + 02	72.19	0.460		

loss in the input level of the test organism during the experiment.

Experimental design

a) Input

The initial CFU titers of *S. aureus* and *A. baumannii* stock cultures were determined before start of the testing.

b) Neutralization validation

The neutralizer used contained lecithin and sodium thiosulfate to quench the activity of the quat and SH, respectively.

Data analyses

Calculation of log₁₀ and % reductions and transfers

$$\text{Percent Reduction} = \left(1 - \frac{\frac{CFU_{contaminated}}{A_{disk}}}{\frac{CFU_{initial}}{A_{platform}}} \right) \times 100,$$

$$\text{Percent Transfer} = \left(\frac{\frac{CFU_{transfer}}{A_{disk}}}{\frac{CFU_{initial}}{A_{platform}}} \right) \times 100,$$

where,

CFU_{initial} = average of CFU on the three control disks

CFU_{contaminated} = average of CFU on the five disks retrieved from contaminated platform

CFU_{transfer} = average of CFU on the five disks retrieved from transfer platform

A_{platform} = area of the platform (cm²)

A_{disk} = area of the disk (cm²)

Results and discussion

Input level

The initial titer of the stock cultures was about 10⁹ CFU/mL. In the neutralization tests (Table 2), the inoculum was diluted to give countable CFU on each disk after drying the inoculum. This resulted in an average of 125 CFU/disk.

In each test suspension prepared from the stock culture was used directly in the efficacy tests with the SL added. The test suspension was diluted in the neutralization test (Table 2) to get countable colonies in the recovery medium. As shown in Table 3, the average level for *A. baumannii* contamination on each control disk after the drying of the inoculum was 3.23 × 10⁴ CFU (range 1.63 to 6.24 × 10⁴); as can be seen from Table 4, the corresponding value for *S. aureus* was 2.57 × 10⁴ CFU (0.59 × 10 × 4.03 × 10⁴ CFU).

Neutralization validation test

All tests included three carriers each as input control (IC), neutralizer toxicity (NT) determination, and neutralization efficacy (NE) assessment. PBS as IC and the neutralizer were included individually to rule out any microbicidal or microbistatic action of the neutralizer itself. The neutralization test was performed using a 1:200 dilution of each test substance with a contact time of 10 min under ambient conditions. This was assuming that each disk on the wiped platform had received no more than 50 μL of test substance on its surface during the wiping. Therefore, placement of each disk in 10 mL of the eluent would represent a 1:200 dilution.

As shown in Table 2, the numbers of CFUs recovered from each control and test carrier were similar, thus indicating that the neutralizer was harmless to the test organism while being effective in quenching the microbicidal activity in the wipes tested. Therefore, the selected neutralizer was used throughout this study.

Table 4. Percentage reductions and percentage transferred in CFUs by the two test samples (A and B) and the control wipe (C) against *S. aureus*.

	CFU/cm ²			Percent		Mean Percent \pm Standard Deviations	
	Control	Contaminated	Transfer	Reduction	Transfer	Reduction	Transfer
Test sample A							
Test #1	2.22E + 04	0.00	2.55E-01	100.000	0.001	99.994 \pm 0.0085	0.0095 \pm 0.0083
Test #2	2.43E + 04	4.84E + 00	7.64E-01	99.980	0.003		
Test #3	1.77E + 04	5.80E + 01	1.27E + 00	99.67	0.007		
Test sample B							
Test #1	3.71E + 04	3.17E + 01	1.54E + 01	99.915	0.042	99.318 \pm 0.504	0.049 \pm 0.032
Test #2	1.04E + 04	2.68E + 01	1.47E + 01	99.742	0.141		
Test #3	9.59E + 03	4.84E + 00	4.08E + 00	99.950	0.042		
Test sample C							
Test #1	3.86E + 04	4.78E + 03	7.68E + 02	87.6	1.99	88.88 \pm 7.469	1.605 \pm 0.575
Test #2	4.03E + 04	1.24E + 03	7.58E + 02	96.9	1.88		
Test #3	3.49E + 04	6.23E + 03	3.30E + 02	82.1	0.944		

Efficacy tests

Table 3 and Table 4 show the results of percentage of reduction and percentage of transfer of the two test samples (A and B) and the J-Cloth control wipe (C) against *A. baumannii* and *S. aureus*, respectively. Product A was able to achieve a $>4 \log_{10}$ ($>99.99\%$) reduction in the viability of the test organisms on wiping, possibly as a combination of killing and removal. At the same time, there was a barely detectable level of transfer of CFUs to clean disks. This suggests that most of the contamination removed was inactivated or sequestered in the applicator itself.

Product B achieved a $>2 \log_{10}$ ($>99.00\%$) reduction in the viability of the test organisms while transferring a higher level of CFUs as compared to Product A.

As would be expected, Product C (J-Cloth) achieved $<1 \log_{10}$ ($<86\%$) reduction in the test organisms' viability while transferring $>1\%$ of the contamination. In this instance, the reduction in the level of viability was most likely due to mechanical removal of the contamination. The level of transfer from the control fabric was also higher due to the absence of any microbicidal activity in it.

The organisms used in this study are among well-recognized HITES-borne pathogens (Howard et al., 2012). They were selected also for their relative stability during the inoculum drying process.

The commercial wipes tested represented two types of common microbicidal agents, namely, QAC and SH. The latter was formulated to contain 250 parts per million (ppm) of available chlorine at a neutral pH. The control wipe (J-Cloth) was a commercial fabric frequently employed in the wiping of HITES. It was wetted with a buffer containing 0.1% of polysorbate-80, a solution harmless to the test organisms while possessing detergent activity only.

The commercial SH-based formulation used was deliberately selected for the low level of its active to enhance workplace safety and reduce any potential corrosivity while also reducing the chemical loading of the environment. Higher levels of SH would most likely perform as well as or better than the QAC-based wipes. Yet another reason for testing SH at neutral pH was its potential to rapidly breakdown into innocuous by-products.

The neutralizer used in this study contained a mixture of chemicals that could quench the microbicidal activities of both test formulations effectively while being harmless to the test organisms.

The custom-built carrier retrieval system allowed for the collection of all the disks from the platforms simultaneously in an eluent/neutralizer, thus avoiding any time lag during disk retrieval and its potential impact on the consistency of the data.

The wiping procedure used was based on observations on how it is applied in healthcare and other settings. Every effort was made to keep the pressure during wiping and transfer as consistent as possible. This was done by placing a 1-kg sand bag over the operator's gloved hand (not shown in the diagrams). The amount of pressure to be applied during wiping and transfer was based on our own observations of EVS at a neighboring healthcare facility as well as based on our previously published studies (Sattar et al. 2001).

SH and QACs continue to be among the most common microbicides in HITES wipes. However, their respective concentrations in prewetted wipes can vary widely along with the nature of the applicator itself. In this study, the wipe substrates were as supplied by the respective manufacturers, the SH-based wipe being a microfiber cloth. While the use of three separate types of wipe materials used in this study may limit any direct comparisons between the formulations tested, our primary focus in this investigation was to create a standardized test protocol to simulate as closely as possible the way HITES are wiped in the field. In our view, this test protocol is not only quantitative but also versatile enough to allow testing with all major classes of HITES-borne pathogens and disinfectants used in decontaminating wipes. Further, the platform can not only test prewetted wipes but also spray-and-wipe systems. This is an added feature of the described platform with multiple disks as compared to other wipe test methods available (Sattar et al. 2015, Jacobshagen et al. 2020). Teflon was chosen to make the platform for its high heat- and chemical-resistance as well as easy cleanability between uses.

In this study, the input level of the test organisms used could demonstrate up to a 4-log_{10} reduction in their viability. However, higher levels of reduction (e.g. $5 \log_{10}$ or greater) in bacterial viability can be readily tested simply by increasing the number of viable organisms in the input inocula.

The findings presented here show that the test platform and the protocol based on it have considerable promise as a versatile and quantitative approach to testing wiping for the decontamination of HITES. It is also a potential candidate as an

international standard after generating data in a multilaboratory collaborative.

Overall, the platform described here offers a combination of the following advantages over the existing protocols for testing of surface disinfecting wipes: (1) It can accommodate disks of all types of hard nonporous HITES; even the non-magnetized ones can be magnetized by covering one of their sides with a nontoxic magnetic paint (e.g. Rust-Oleum brand); (2) It can handle all major classes of HITES-borne pathogens and the same basic test protocol, thus allowing for a level playing field to assess the breadth of decontaminating activity of the wipes being tested; (3) The ability of the unique disk-retrieval system permits the separate yet simultaneous collection of all the disks from a given platform in vials with an eluent/diluent/neutralizer; the volume of such a collecting fluid can be reduced from 10 mL to 1 mL/tube to better suit work with human and animal pathogenic viruses; (4) In addition to allowing for testing of disinfectant presoaked wipes, the platform can be utilized for testing spray-and-wipe technologies; (5) The pressure and contact time for wiping can be adjusted within certain limited to test their influence on the HITES decontamination process; (6) The use of a platform with clean disks permits a quantitative assessment of microbial transfer during wiping of HITES; (7) While the system described here was designed to work with disks of 1.0 cm diameter, platforms to accommodate disks with larger diameters can be readily made and used without changing the rest of the protocol; (8) The input level of microbial contamination on each disk can be adjusted to reflect the extent of log₁₀ reduction required by wiping; however, how high the input can go is limited by the type of microbe being tested as well as its stability during the drying of the inocula.

The data generated demonstrated the ability of the platform to assess HITES decontamination as well as the ability of the used wipes to transfer pathogens to clean surfaces. Such microbial transfer was the highest with the control wipe wetted with a buffer.

Another possible use of the platform is to train EVS in the wiping of HITES. For such training, the carrier disks could be contaminated with a fluorescent dye (Carling 2016) to monitor the efficiency of the wiping process.

The limitations of the study include the fact that it was based on relatively small (1 cm in diameter) carriers with a flat surface. While such flat surfaces are predominant in most healthcare and other settings, consideration may be given to adapting the method for work with irregularly shaped carriers and those with uneven surfaces.

Also, neutralization tests were not conducted with *A. baumannii* due to resource limitations.

The device and the protocol described for its use form the basis for a quantitative and field-relevant means of assessing the HITES decontaminating activity of wipes. With additional experimentation using a wider variety of pathogens and wipe technologies, the system has the potential to become an international standard, such as that for ASTM International (www.astm.org).

Acceptance criteria

All sterility tests were to be negative (no microbial growth) before proceeding with the wipe testing. Since this study was designed primarily to assess the reproducibility of

the test protocol, no product performance criterion was specified.

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Conflict of interest

The authors declare no conflict of interest.

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Author contributions

Bahram Zargar (Conceptualization, Data curation, Methodology, Project administration, Supervision, Writing – review and editing), and Syed A Sattar (Conceptualization, Methodology, Project administration, Writing – original draft, Writing – review & editing).

References

- ASTM International. *Standard quantitative disk carrier test method for determining the bactericidal, virucidal, fungicidal, mycobactericidal and sporicidal activities of liquid chemicals (E-2197-17)*. West Conshohocken (PA): ASTM, 2017.
- Carling PC. Optimizing health care environmental hygiene. *Infect Dis Clin North Am* 2016;30:639–60.
- Donskey CJ. Beyond high-touch surfaces: portable equipment and floors as potential sources of transmission of health care-associated pathogens. *Am J Infect Control* 2019;47:A90–5.
- European Norm 16615. Chemical disinfectants and antiseptics. Quantitative test method for the evaluation of bactericidal and yeasticidal activity on non-porous surfaces with mechanical action employing wipes in the medical area (4- field test). 2015; https://www.techstrete.com/searches/33527680?gclid=Cj0KCQiAnuGNBhCPARIsACbnLzrv_o7lR7sLgkZhm2SK8izVdRcRL7PZj1z-T4SMl8IAe_TKcA9WCQaArC4EALw_wcb&searchText=EN+16615&sid=goog, (8 January 2023, date last accessed).
- Howard A, O'Donoghue M, Feeney A *et al.* *Acinetobacter baumannii*, an emerging opportunistic pathogen. *Virulence* 2012;3:243–50.
- Jacobshagen A, Gemein S, Exner M *et al.* Test methods for surface disinfection: comparison of the Wiperator ASTM standard E2967–15 and the 4-field test EN 16615. *GMS Hyg Infect Control* 2020;15:1–9. DOI: 10.3205/dgkh000339
- Ramm L, Siani H, Wesgate R *et al.* Pathogen transfer and high variability in pathogen removal by detergent wipes. *Am J Infect Control* 2015;43:724–8.
- Sattar SA, Springthorpe VS, Mani S *et al.* Transfer of bacteria from fabrics to hands and other fabrics: development and application of a quantitative method using *Staphylococcus aureus* as a model. *J Appl Microbiol* 2001;90:962–70.
- Sattar SA, Maillard J-Y. The crucial role of wiping in decontamination of high-touch environmental surfaces: review of current status and directions for the future. *Am J Infect Control* 2013; 41: S97–104.
- Sattar SA. Promises and pitfalls of recent advances in chemical means of preventing the spread of nosocomial infections by environmental surfaces. *Am J Infect Control* 2010;38:S34–40.

- Sattar SA, Bradley C, Kibbee R *et al.* Disinfectant wipes are appropriate to control microbial bioburden from surfaces - the use of a new ASTM standard test protocol to demonstrate efficacy. *J Hosp Infect* 2015;**91**:319–25.
- Springthorpe VS, Sattar SA. Application of a quantitative carrier test to evaluate microbicides against mycobacteria. *J AOAC Int* 2007;**90**:817–24.
- Weber DJ, Anderson D, Rutala WA. The role of the surface environment in healthcare-associated infections. *Curr Opin Infect Dis* 2013;**26**:338–44.
- Weber DA, Rutala WA, Miller MD, *et al.* Role of hospital surfaces in the transmission of emerging health care-associated pathogens: norovirus, *Clostridium difficile*, and *Acinetobacter* species. *Am J Infection Control* 2010;**38**(5 Suppl 1):S25–33. DOI: 10.1016/j.ajic.2010.04.196.