MICROBIOLOGICAL METHODS

Theoretical and Experimental Aspects of Microbicidal Activities of Hard Surface Disinfectants: Are Their Label Claims Based on Testing Under Field Conditions?

NAVID OMIDBAKHSH
Virox Technologies, 2770 Coventry Rd, Oakville, ON, Canada

High-touch environmental surfaces are important in the spread of many nosocomial pathogens. Although such surfaces are routinely disinfected, the testing and label claims of many common disinfectants do not reflect the realities of field use. A study was conducted to determine the influence of several crucial factors on the action of disinfectants in general, and to assess the killing efficiency of selected chemistries against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, related to their drying times (i.e., after one application) and label-specified contact times using a quantitative carrier test. The products were also tested for their ability to wet a hydrophobic (epoxy resin) surface. The hard-surface disinfectants (in-use concentration in ppm) tested were: (a) chlorine bleach (500); (b) quaternary ammonium compounds (quat; 600) alone; (c) quat (3000) with 17% isopropanol (v/v); (d) quat (3000) with 60% ethanol (v/v); (e) phenolic (800) alone; (f) quat (2000); phenolic (3000) with 70% ethanol (v/v); and (g) accelerated hydrogen peroxide (AHP; 5000 of H_2O_2). The arbitrarily set criterion of bactericidal activity was 6 $\log_{10}$ reduction in the viability of both species tested. All surfaces tested with all products dried in <5 min, with alcohol-based surfaces drying significantly faster. Even though the alcohol-free quat and phenolic claim a contact time of 10 min, they dried in <4 min after a single application and failed to meet the performance criterion. Bleach (500 ppm) dried in about 3 min and was effective. AHP also dried in about 3 min and met its label claim even at 1 min of contact. Quat (3000) with 17% isopropanol dried at 1 min and was effective. Quat (3000) with 60% ethanol and quat (2000), phenolic (3000) with 70% ethanol dried in <1 min, and were ineffective. AHP, alcohol-containing quats, and quat-phenolic-alcohol gave acceptable wettability, while quat and phenolic alone, as well as bleach, covered the treated surface unevenly. The findings show that label claims, especially those for contact times, fail to reflect the way many hard-surface disinfectants are used in the field.

Received February 1, 2010. Accepted by AH May 25, 2010.
Corresponding author's e-mail: navid@virox.com

Healthcare-associated infections (HAI) continue to exact a heavy toll on our health and economy (1); among the important means to reduce the risk of spread of HAI is the proper and regular disinfection of high-touch environmental surfaces (2). Regulatory bodies, such as the U.S. Environmental Protection Agency (EPA) and Health Canada, require a formal review of the test methodology and label claims of products used for that purpose prior to their registration for marketing. However, it is increasingly obvious that certain widely accepted methods to assess the microbicidal activities of environmental surface disinfectants and the label claims based on them often fail to reflect the realities of field use (3). Thus, they may generate a false sense of security in the minds of users, while exposing humans and the environment to potentially harmful and possibly ineffective chemicals (4). The use of products with weak microbicidal activity may indeed be counter-productive by spreading viable pathogens over a wider area during the decontamination of targeted environmental surfaces (5).

Why does this disparity between label claims and the demands of actual field use continue to persist? Which aspects of the test methods widely accepted for registration of environmental surface disinfectants need re-examination to make them more relevant to the field use of disinfectants? Are there factors in assessing the microbicidal activities of disinfectants that are crucial but not included in the testing for generating label claims? What, if any, improvements can make the testing and label claims of environmental surface disinfectants more relevant to their field applications? Our objective was to assess the bactericidal activity of selected environmental surface disinfectants using conditions reflective of their label claims and field use.

This report discusses the theoretical aspects of field conditions such as temperature and dilution rates and their effect on disinfection activity. Laboratory-based data for wetting capability of selected chemistries, as well as their bactericidal activity, are also presented. However, this study included only regular surfaces. Those with built-in

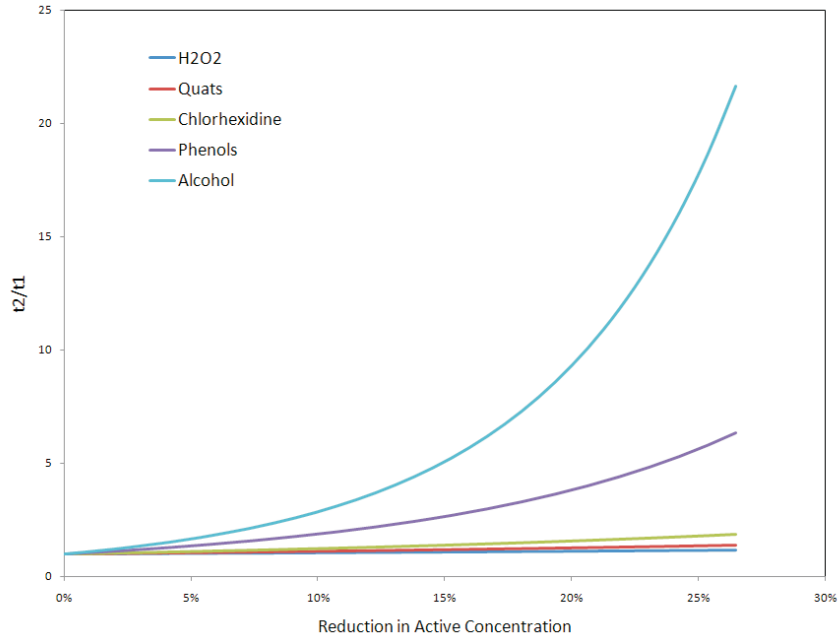

Figure 1. $\frac{t_2}{t_1}$ **versus active concentration reduction for five different microbicidal actives.** t_1 **and** t_2 **are the respective times to inactive microorganisms to the same level.**

antimicrobial activity were beyond the scope of this investigation.

Some Theoretical Considerations

Air Temperature

Air temperature is a crucial factor affecting microbicidal activity. Although environmental surface disinfectants are generally used under ambient conditions, there can be wide variations in air temperature from place to place and at the same site, depending on season, climate, and air-handling systems. Even though one would expect the influence of air temperature to be relatively insignificant for the short (seconds to a few minutes) contact times normally used in testing environmental surface disinfectants, we examined the theoretical basis to evaluate the impact of this factor on microbicidal action.

Table 1. Dilution coefficients for selected microbicidal actives (modified from ref. 12)

Microbicidal agent	Dilution coefficient ( )
Aliphatic alcohols	6.0–12.7
Hydrogen peroxide	0.5
Iodine	0.9
Phenolics	4.0–9.9
Quaternary ammonium compounds	0.8–2.5
Silver nitrate	0.9–1.0

The microbicidal activity of most disinfectants is directly proportional to air and/or surface temperature as long as the temperature remains above the freezing point or not high enough to cause an immediate break-down or evaporation of the active ingredient(s) in it. There has been very limited testing of microbicidal activity of liquid disinfectants at sub-zero temperatures (6, 7). In many tropical and subtropical settings, it is not uncommon to encounter indoor air temperatures of 40 C or higher.

Although the air temperature in the test environment is required to be 20 1 C, the comfort level in most climate-controlled settings in the United States and Canada is generally lower than 23–25 C. Depending on the conductive nature of the surface to be disinfected, the actual temperature on its surface may be lower than the temperature of the surroundings. The relatively short contact times that are relevant for testing environmental surface disinfectants may not be influenced by either the surface or air temperature, even though good test protocols propose that the temperature of the test substance be brought to that required in the testing. However, the following equation can be used to assess the impact of temperature on the speed of microbicidal action of disinfectant chemicals:

$$\frac{t_1}{t_2} \qquad (1)$$

where t_2 and t_1 are the respective times to completely inactivate organisms at T_2 and T_1 (8) and is the temperature coefficient, refers to the effect of temperature for each 1 C

increase, and is usually between 1 and 1.5 (9). It is more useful to compare the disinfection activity for every 10 C ($_{10}$), which can be shown as (10):

$$_{10} \quad \frac{\textit{Time to kill at } T}{\textit{Time to kill at } \ T \quad 10} \quad _{10} \qquad (2)$$

While $_{10}$ values of chemical and enzyme-catalyzed reactions vary between 2 and 3, values for disinfection vary more widely; for example, 45 for ethanol, 4 for phenol, and 1.5 for formaldehyde (8, 10). Based on equation 2, for ethanol, phenol, and formaldehyde will be 1.46, 1.15, and 1.04, respectively. This means that a decrease in temperature by 1 C will result in a slowing of the microbicidal activities of ethanol, phenol and formaldehyde by 46, 15, and 4%, respectively. Therefore, while the disinfectants are applied, the operating temperature must be taken into account. Furthermore, chemistries with higher $_{10}$ must be tested in the lower bound of the temperature range due to their significant activity reduction by temperature drop.

Product Dilution

With the exception of alcohols, the higher the concentration of an active ingredient, the faster is its microbicidal action. The increase in the activity versus concentration is shown to be exponential (10) and can be expressed as:

$$\frac{\log t_2 \quad \log t_1}{\log C_1 \quad \log C_2} \qquad (3)$$

where is the dilution coefficient, C_1 and C_2 represent two different concentrations, and t_1 and t_2 are the respective times to inactive microorganisms to the same level. Equation 3 can be rewritten as:

$$\frac{t_2}{t_1} \quad 10 \quad ^{\log \frac{C_1}{C_2}} \qquad (4)$$

By substituting values of for each active ingredient, $\frac{t_2}{t_1}$ can be drawn versus the reduction in the active concentration as shown in Figure 1.

Table 1 shows that the microbicidal activity of alcohols is very sensitive to their concentrations. Ethanol's activity drops sharply below 50%; at 30% concentration, the activity is almost negligible (11). Figure 1 shows the $\frac{t_2}{t_1}$ versus active concentration reduction where t_1 and t_2 are the respective times to inactive microorganisms to the same level. As shown in Figure 1, alcohol and phenol disinfection times are highly dependent on their concentration, while peroxide, quaternary ammonium compounds (quats), and chlorhexidine have much less dependence. It is also shown that hydrogen peroxide disinfection has the lowest change versus its concentration.

These dilution coefficient values are very important, especially when working from concentrates. Table 1 and Figure 1 show that if a phenolic concentrate is diluted only 10% more than its recommended level, its disinfection rate will slow to about 77%, while for quats and hydrogen peroxide, this slowdown is 10 and 5%, respectively. Because alcohol-based formulations are always sold as ready-to-use, they are devoid of any risks from incorrect dilution. However, if alcohol is applied on a wet surface, the resulting dilution with water could seriously compromise its microbicidal action.

Materials and Methods

Disinfectants Tested

The seven hard-surface disinfectants included in this study are all registered for sale in Canada and the United States, and represent a variety of commonly used actives. They are designed to decontaminate environmental surfaces in healthcare facilities. Table 2 summarizes the relevant details on the tested products, which were obtained as sold to healthcare institutions. The dilutions and contact times in this table represent the label claims of the respected products for hard-surface disinfection.

Test Organisms and Their Culture

Standard strains of *Pseudomonas aeruginosa* (ATCC 15442) and *Staphylococcus aureus* (ATCC 6538) were used in this study as representative Gram-negative and -positive organisms, respectively. The organisms used are those required to assess the bactericidal activities of environmental surface disinfectants for regulatory purposes. *S. aureus* was grown in tryptic soy broth (TSB) for 24 h at 36 1 C. *P. aeruginosa* was grown in a 1:1000 dilution of TSB for 72 h at 36 1 C. Further details on the preparation of the test bacterial suspensions were as detailed earlier (12).

Standard Hard Water

When required, water with 400 ppm hardness as $CaCO_3$ was used to prepare any use-dilutions of the tested disinfectants (13).

Drying and Wettability Tests

The disinfectants were tested for their drying time on a surface under ambient conditions. After their average drying time was measured, they were tested for their bactericidal activity in that time. They were also assessed for their wettability on a hydrophobic surface (epoxy resin).

In the absence of a standardized and generally recognized test to assess drying, the following protocol was used: 5 mL of each test solution was applied to a 30 cm^2 surface using a 20 cm^2 paper towel. The towel was folded twice; the solution was pipetted onto the surface, and the towel was used to uniformly distribute the solution. The time for drying of 50% of the surface was recorded as the drying time. The test was repeated 10 times for each solution on each surface. The surface was rinsed with deionized water and dried between

Table 2. Formulations tested in this study[a]

Formulations tested	Active ingredient(s), ppm	Recommended uses and use dilution	Label claims of microbicidal activities	Contact time(s) on label, min
AHP RTU	Formulated hydrogen peroxide (5000)	RTU	Bactericide, virucide, fungicide, mycobactericide	1
Chlorine bleach	Sodium hypochlorite (52500)	1:100	Bactericide, virucide	10
Concentrated quat	Quat (75000)	1:128	Bactericide, virucide	10
Quat/IPA	Quat (3000) in 17% IPA	RTU	Bactericide, virucide, mycobactericide	3
Concentrated phenolic	Phenol (110000)	1:128	Bactericide, virucide, fungicide	10
Alcohol/quat/phenol blend	Phenol (3000), and quat (2000) in 70% ethanol	RTU	Bactericide, virucide, fungicide, mycobactericide	1
Alcohol/quat	60% ethanol, quat (3000)	RTU	Bactericide, virucide, mycobactericide	10

[a] AHP = Accelerated hydrogen peroxide; RTU = ready-to-use; IPA = isopropyl alcohol.

tests. Three different surfaces were used, namely, ceramic, stainless steel, and porcelain; the results were averaged. The test solutions were labeled by numbers and were tested randomly to avoid any test bias.

To test the wettability of disinfectants in this study, saturated paper towels (20 cm^2) were applied to an epoxy resin countertop, and the distribution of the solution on the surface was visually observed. Photographs were taken before the solutions started drying to show the spreading of each disinfectant.

Testing for Bactericidal Activity

The second tier of the quantitative carrier test, a standard of American Society for Testing and Materials International, was used to evaluate the bactericidal activities of the tested formulations (12). Stainless steel disks (1 cm diameter, 0.7 cm thickness) were used to simulate hard, nonporous surfaces. A soil load was added to the bacterial culture to simulate the presence of body fluids under field conditions. To prepare the soil load, 100 L 0.4% bovine mucin (Sigma Chemical Co., St. Louis, MO) and 35 L 5% tryptone (Difco, Detroit, MI) were mixed with 340 L of the bacterial suspension all in 0.3 mmol/L potassium phosphate buffer, with 0.05% magnesium sulfate. Each carrier then received 10 L of the inoculum. The disks were placed in a laminar-flow cabinet and dried for 20 min. They were then placed in a desiccator and dried further for 2 h under vacuum. Each disk was placed in a sterile 30 mL plastic vial, and 50 L of the test solution was added on top of the dried inoculum and held at room temperature (22 2 C) for the specified contact time. Then, 9.95 mL of an eluent/neutralizer was added to each vial immediately at the end of the contact time to arrest the disinfection process. Control disks were exposed to 50 L Tween-80 (Saline-T), but were otherwise treated in the same manner as the test carriers. Each vial was vortexed 2–3 times for 30 s each to recover the inoculum from the disks. The eluates and any 10-fold dilutions needed were separately passed through a membrane filter, 47 mm diameter, 0.2 m pore size (Millipore Corp., Billerica, MA). Each carrier vial was washed with about 60 mL normal saline + 0.1% (w/v) Saline-T; the washes were also passed through the filter to capture any possible remaining organisms. The membrane filters were then placed on a suitable agar recovery medium and the plates were incubated at 36 1 C for 2 days. Growth was recorded as CFU, and $\log_{10}$ reductions in viability were calculated in relation to the values on the control carriers. Each test included three control carriers and at least three test carriers.

Neutralizers

For sodium hypochlorite solutions, the neutralizer was 1.0% (w/v) sodium thiosulfate in normal saline, with 0.1% of Tween-80. The remaining formulations were neutralized with Letheen Broth (Difco) with 1.0% (w/v) sodium thiosulfate. The process of neutralization was validated prior to the actual testing.

Results

Bactericidal Activity

Tables 3 and 4 summarize the findings of the bactericidal activity based on the drying times for different chemistries. All surfaces tested with all products dried in <4 min, with the alcohol-based products drying significantly faster. Although the alcohol-free quat and phenolic claim a contact time of 10 min, they dried in <4 min after a single application and failed to achieve the arbitrarily set level (6 $\log_{10}$) of bacterial kill. While accelerated hydrogen peroxide (AHP) also dried in about 3 min, it met its label claim even at 1 min of contact. The two quat-alcohol formulations and the phenolic alcohol dried in <1 min and were ineffective. Even though bleach 500 ppm and quat 3000 ppm in 17% isopropyl alcohol (IPA) dried faster than the specified label contact time, these disinfectants were able to achieve a 6 log reduction in *P. aeruginosa*, as well as a 5.94 log reduction in *S. aureus*. It should be noted that most guidelines recommend a bleach concentration of 5000 ppm, which may be more effective than the 500 ppm concentration tested in this study.

Table 3. Microbicidal activity of selected disinfectants at their drying time against *Staphylococcus aureus*[a]

Product	Time	Dilution	Initial titer	Final titer	LR
AHP 0.5%	1 min	RTU	1×10^6	0	6
Concentrated quat (75000 ppm)	3 min	1:128	1×10^6	2.45×10^4	1.65
Quat/alcohol [quat (3000) in 17% IPA]	1 min	RTU	8.7×10^5	0	5.94
Bleach, 500 ppm	3 min	RTU	8.7×10^5	0	5.94
Alcohol/quat/phenol blend	30 s	RTU	1×10^6	1.03×10^5	0.99
Concentrated phenol	3 min	1:128	1×10^6	1.05×10^4	1.99
Quat (3000) in 60% ethanol	30 s	RTU	3.06×10^6	7.77×10^4	1.59

[a] LR = log reduction.

Testing for Wettability

Figure 2 shows the wetting capability of different chemistries on a hydrophobic surface. For solutions with poor wetting capability, the total surface does not get exposed to the solution. Most chlorine bleach formulations do not contain any surfactants and therefore cannot wet the surface completely. Quat and phenol solutions, as tested, also did not contain enough wetting agents to cover the whole surface, while other solutions completely wet the surface, indicating their sufficient wetting capability.

Drying

The results of the drying tests (Figure 3) show that the solutions with high water content dried in 3–4 min, while the alcohol-based ones did so much faster—approximately 1 min for 17% IPA and 30 s for 60–70% ethanol-based formulations—due to their much higher evaporation rates than that of water (14).

Figure 3 also shows the comparison of the drying time for each solution versus its label contact time. In most cases, the drying time is much faster than the label-specified contact time. The results showed that three water-based solutions dried on an average time of 3–4 min, while a 17% IPA dried at about 50 s, and two solutions of about 60 and 70% ethanol dried at about 30 s.

Discussion

The activity of disinfectants depends on several factors, including air temperature, relative humidity, soil load, concentration of active(s), nature and topography of the target surface, and contact time. Although one is instructed to use a given disinfectant product in accordance with its label claims, unfortunately, the required testing leading to those claims often bears little resemblance to what a given product may encounter in actual field conditions.

One highly relevant example is contact time, which is still quite frequently given as 10 min. The 1991 version of the Canadian General Standards Board's standard on disinfection of environmental surfaces states that the contact time should not be longer than 10 min, thus encouraging manufacturers to test for and make label claims of shorter contact times commensurate with field use (15). For some unexplained reason, that statement was deleted in the 1997 version of the standard (16), essentially nullifying a significant step forward. Nevertheless, Health Canada has been approving label claims with much shorter contact times, and this is now formalized in its recent guidance document (17). The situation with regard

Table 4. Microbicidal activity of selected disinfectants at their drying time against *Pseudomonas aeruginosa*

Product	Time	Dilution	Initial titer	Final titer	LR
AHP 0.5%	1 min	RTU	1.37×10^7	0	7.13
Concentrated quat (75000 ppm)	3 min	1:128	1×10^6	3.64×10^4	1.45
Quat/alcohol [quat (3000) in 17% IPA]	1 min	RTU	1×10^6	0	6
Bleach, 500 ppm	3 min	RTU	1×10^6	0	6
Alcohol/quat/phenol blend	1 min	RTU	1.37×10^7	1.47×10^6	0.96
Concentrated phenol	3 min	1:128	8.7×10^6	2.63×10^3	2.52
Quat (3000) in 60% ethanol	30 s	RTU	8.61×10^5	3×10^2	3.73

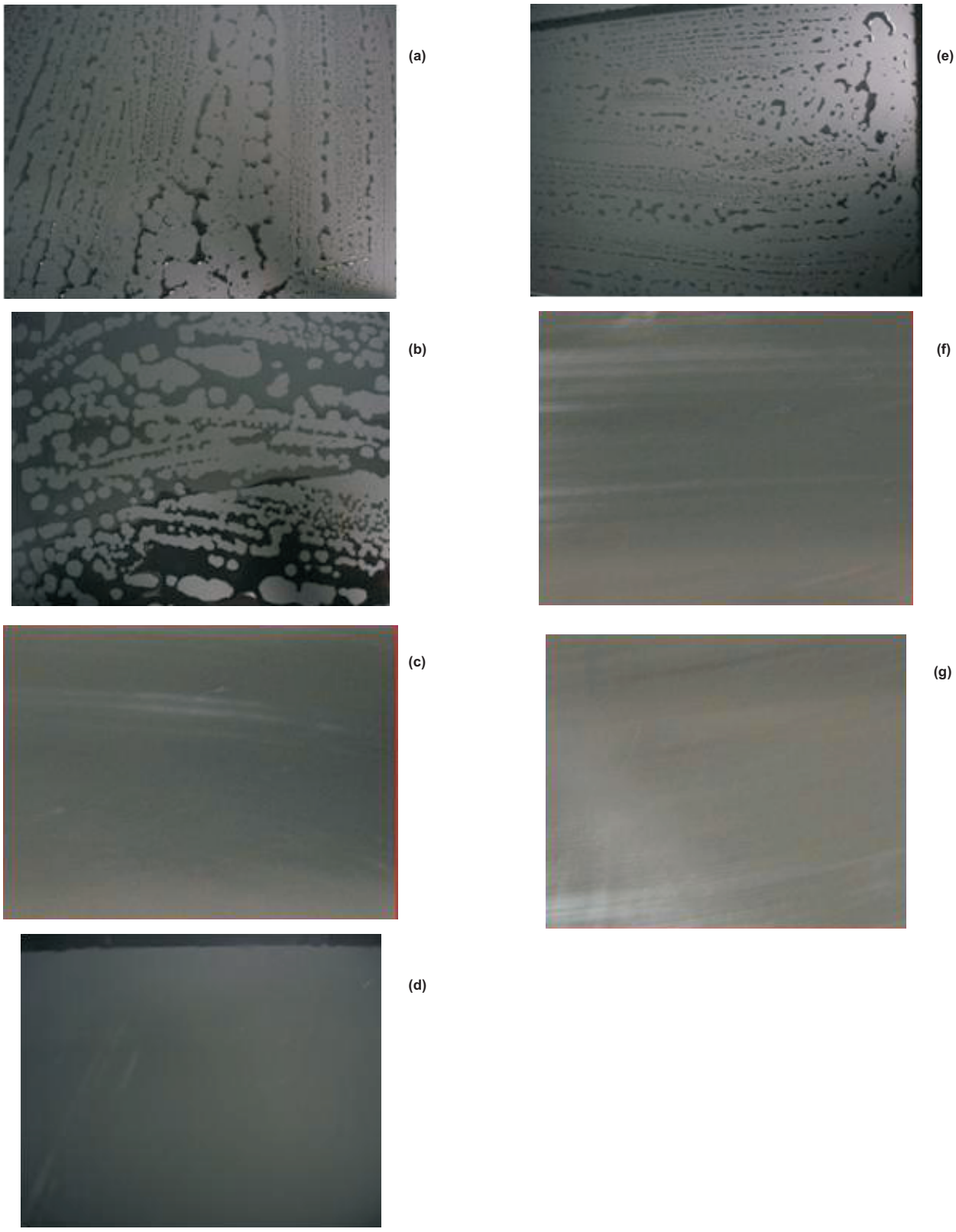

Figure 2. Wettability of different chemistries on hydrophobic surfaces: (a) bleach (500); (b) quat (600); (c) quat (3000) with 17% isopropanol; (d) quat (3000) with 60% ethanol; (e) phenolic (800); (f) quat (2000), phenolic (3000) with 70% ethanol; (g) AHP, 0.5% H_2O_2.

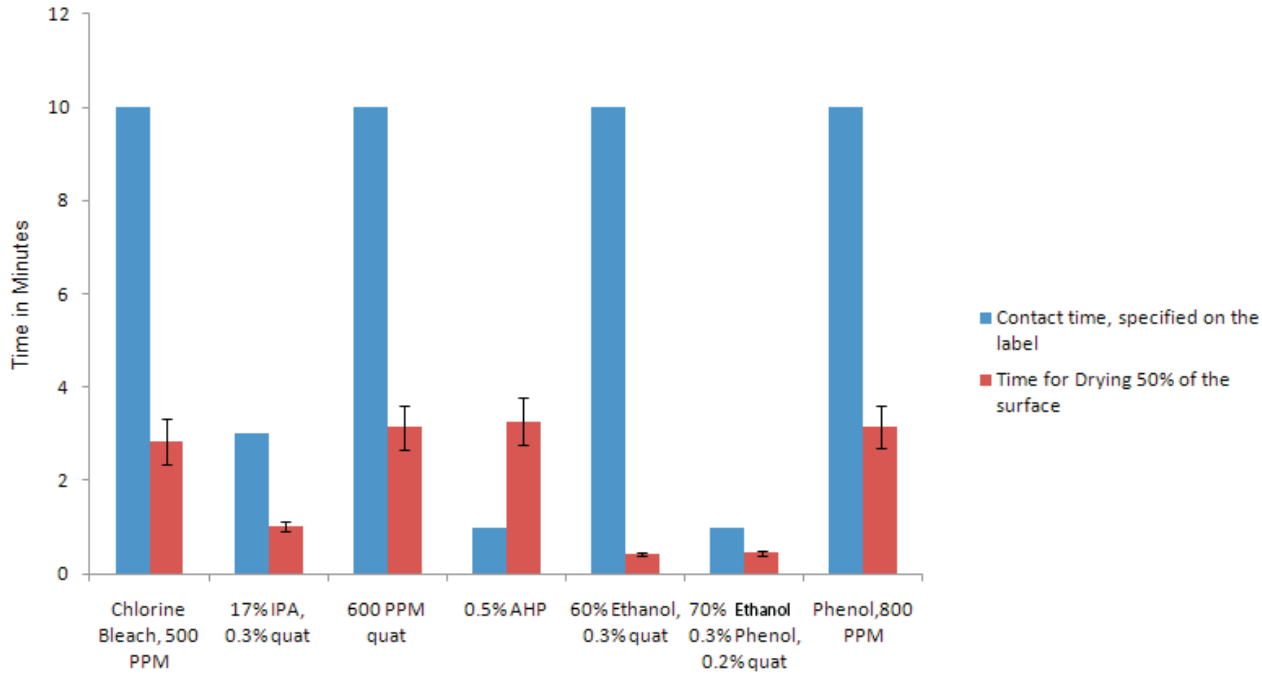

Figure 3. Drying time versus label contact time of different disinfectants.

to contact times is also changing in the United States. The EPA now allows for contact times shorter than 10 min for environmental surface disinfectants (18).

Quite often, disinfectant products are supplied as concentrates for ease of transportation and storage. Unless they are provided with automated and fail-safe means of preparing use-dilutions, mistakes may occur at the site of use, leading to possible overdilution. Also, the use-life of even properly diluted and stored formulations is generally shorter than that of their respective concentrates. These factors can seriously affect the label-claimed microbicidal activity of a given formulation. This issue has been examined with respect to various chemistries.

When dealing with dilutable formulations and those already diluted and stored improperly or longer that recommended, the deviation from label claims may result in linear changes in its in-use levels; for example, 10% dilution more than that recommended on the label may lead to only about 10% drop in the product's microbicidal activity. However, this is not necessarily the case, and as shown here, microbicidal activity does not change linearly with dilution; with many formulations even slight deviations can lead to significant losses in microbicidal activity. As an example, applying an alcohol-based formulation on a wet surface, which results in 10% reduction in concentration, will almost triple the required disinfection time. Also, 10% extra dilution of a phenolic will result in 70–80% slowdown in disinfection rate, while for quats and hydrogen peroxide, the slowdown will be 10 and 4%, respectively. Furthermore, a 1 C decrease in temperature of an ethanol, phenolic, and formaldehyde will result in 46, 15, and 4% increase in disinfection time, respectively.

In carrier testing, the entire contaminated surface of each carrier is exposed to the test formulation either by flooding or dipping. In actual practice though, this may not be the case; hydrophobic surfaces may repel water-based formulations, thereby preventing uniform exposure of the target pathogens during the required contact time. While many plastics are hydrophobic by nature, other surfaces may become so, depending on their prior history of cleaning and waxing. A sufficient level of detergency is, therefore, essential to ensure a more uniform application for effective disinfection.

In efficacy testing of disinfectant products, some critical assumptions do not necessarily hold in practice for all chemistries. As an example, one assumption is that the microbial target is fully exposed to the disinfectant for the entire contact time. The study showed that many products do not stay long enough on the surface and cannot meet their required contact time. Furthermore, it was shown that chemistries with insufficient detergency do not spread uniformly, especially on hydrophobic surfaces, and therefore do not come into contact with microorganisms. This is especially true for products with very high dilution rates which do not have enough wetting agents at the recommended use level, or for products that are only disinfectants and do not contain any detergents in their formulations. Consequently, in decontaminating surfaces and to comply with the label claims, one should make sure that the disinfectant will uniformly spread out on the surface and remain there for the duration of the required contact time, by reapplying the product on the surface more than once.

Although only one Gram-positive and one Gram-negative bacterial species were tested here, it is reasonable to generalize these finding to other disinfection claims, such as

those for virucidal, fungicidal, and tuberculocidal activities, even though the disinfection kinetics may be different from those for the tested bacteria. While several factors covered here relate to testing of environmental surface disinfectants in general, they do not address the equally important issues surrounding the common practice of wiping surfaces for decontamination. In wiping surfaces, in particular using a presoaked applicator, the volume of the applied disinfectant on a unit surface area is frequently much smaller than that used in currently available tests for microbicidal activity. There is also the influence of the mechanical action of wiping to physically remove contamination and possibly provide better access to microbial targets in dried up areas. However, as mentioned earlier, improper use of a disinfectant may lead to the spread of localized contamination over a wider area during wiping (5). Healthcare facilities need to assess the time it takes for a disinfectant solution to evaporate, and if this is less than the label-recommended contact time, ensure that the solution is reapplied until the label-specified contact time is met.

With the increasing recognition of the role of high-touch environmental surfaces in the spread of many types of nosocomial pathogens (19), manufacturers and regulators must ensure that only the most effective and safest products are put on the market to be applied by end-users with ease and confidence against pathogens that are amenable to environmental control. Any success in meeting this laudable objective must begin with an immediate, thorough, and unbiased re-evaluation of how environmental surface disinfectants are tested for label claims, as well as the regulatory process which grants them permission for sale.

References

(1) Llata, E., Gaynes, R.P., & Fridkin, S. (2009) *Clin. Infect. Dis.* **48**,1434–1440

(2) Rutala, W.A., & Weber, D.J. (2001) *J. Hosp. Infect.* **48** (Suppl. 1), S64–S68

(3) Springthorpe, V.S., & Sattar, S.A. (2005) *J. AOAC Int.* **88**, 182–201

(4) Sattar, S.A. (2006) *J. Appl. Microbiol.* **101**, 743–753

(5) Wilcox, M.H., Fawley, W.N., Wigglesworth, N., Parnell, P., Verity, P., & Freeman, J. (2003) *J. Hosp. Infect.* **54**, 109–114

(6) Jones, L.A., Jr., Hoffman, R.K., & Phillips, C.R. (1967) *Appl. Microbiol.* **15**, 357–362

(7) Jones, L.A., Jr., Hoffman, R.K., & Phillips, C.R. (1968) *Appl. Microbiol.* **16**, 787–791

(8) Russell, A.D., Hugo, W.B., & Ayliffe, J.A. (1999) *Principles and Practice of Disinfection, Preservation and Sterilization*, 3rd Ed., Blackwell Scientific Publications, Oxford, UK

(9) Bean, H.S. (1967) *J. Appl. Microbiol.* **30**, 6–16

(10) Denyer, S.P., Hodges, N.A., & Gorman, S.P. (2004) *Hugo and Russell's Pharmaceutical Microbiology*, 7th Ed., Wiley-Blackwell, Oxford, UK

(11) Rutala, W.A. (1996) *Am. J. Infect. Control* **24**, 313–342

(12) *Quantitative Disk Carrier Test Method for Determining the Bactericidal, Virucidal, Fungicidal, Mycobactericidal, and Sporicidal Activities of Liquid Chemical Germicides* (2002) ASTM E2197-02, ASTM International, West Conshohocken, PA

(13) *Official Methods of Analysis* (2006) 18th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, Chapter 6

(14) Wypych, G. (2008) *Knovel Solvents–A Properties Database*, ChemTec Publishing, Toronto, Canada

(15) Canadian General Standards Board (1991) *Assessment of the Activity of Antimicrobial Agents for Use on Environmental Surfaces and Medical Devices*, Document CAN CGSB-2.161-M91, Ottawa, Canada

(16) Canadian General Standards Board (1997) *Assessment of the Activity of Antimicrobial Agents for Use on Environmental Surfaces and Medical Devices*, Document CAN CGSB-2.161.97, Ottawa, Canada

(17) Health Canada (2007) *Guidance Document, Disinfectant Drugs*, Document 07-119627-108, Ottawa, Canada

(18) U.S. Environmental Protection Agency (2008) *Clostridium difficile* product labeling, http://www.epa.gov/oppad001/clostridium_diff.htm

(19) Boyce, J.M. (2007) *J. Hosp. Infect.* **65** (Suppl. 2), 50–54