Readers' Forum

Registration of Disinfectants Based on Relative Microbicidal Activity

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Along with proper hand hygiene, disinfection of contaminated surfaces and medical instruments has been a key method of preventing patient-to-environment-to-patient transmission of infectious agents via the hands of healthcare workers.[1-3] However, there is growing concern regarding the increase in antibiotic-resistant pathogens for which environmental and device contamination may play a role in disease transmission, such as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus* (VRE), *Clostridium difficile*, and multidrug-resistant aerobic gram-negative bacilli (eg, *Pseudomonas aeruginosa* and *Acinetobacter*).[1] Proper use of disinfectants plays an important role in reducing person-to-person transmission of these pathogens.

For decades, the medical community in the United States has relied on the federal government's disinfectant testing and registration program for assurance that registered disinfectants meet their label claims. However, recognized flaws in test methodologies could result in registration of ineffective disinfectants.[4] Control measures should be instituted at the federal level to improve the test methodology and reduce the frequency of contaminated or ineffective disinfectants and the threat of serious healthcare-associated infections related to disinfectant use.

We have previously reviewed the issues surrounding the selection and registration of high-level disinfectants and chemical sterilants.[5] However, there are several unique aspects of testing and registration of low-level and intermediate-level disinfectants (eg, microbicidal testing methods) that warrant separate discussion. This article proposes a scheme for testing and registration of low-level and intermediate-level disinfectants that could be used by the U.S. Environmental Protection Agency (EPA).

BACKGROUND

Chemicals formulated as disinfectants in the United States are registered and regulated in interstate commerce by the Antimicrobial Division, Office of Pesticides Program, EPA. The authority for this activity was mandated by the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) in 1947. In June 1993, the U.S. Food and Drug Administration (FDA) and the EPA issued a "Memorandum of Understanding" that divided responsibility for review and surveillance of chemical disinfectants between the two agencies. Under the agreement, the FDA regulates disinfectants used on critical or semicritical medical devices and antiseptics and the EPA regulates disinfectants used on noncritical surfaces. In 1996, Congress passed the Food Quality Protection Act (FQPA). The Act amended FIFRA regarding several products regulated by both the EPA and the FDA. One provision of FQPA is that regulation of disinfectants used on critical and semicritical medical devices (the EPA continues to regulate non-medical disinfectants) was removed from the jurisdiction of the EPA and now rests solely with the FDA.[1,6]

Examples of disinfectants that are registered by the EPA with the intent of providing a public health benefit, therefore requiring efficacy data as a condition of their registration, include disinfectants used in hospitals and other healthcare settings on floors, walls, and medical equipment surfaces and household products claiming to have disinfectant activity. There are three types of disinfectant products that the EPA registers based on submitted efficacy data: limited, general or broad-spectrum, and hospital disinfectants. When a disinfectant is represented in its labeling for use in hospitals, medical clinics, dental offices, or any other medical-related facility, it must show

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TABLE 1
BACTERIAL TARGETS FOR GERMICIDES*

Target	Mechanism	Biocide
Outer layer		
Cell wall	Cross-linking	GTA, OPA, FMA (?)
Outer member†	Increased permeability	CHA, QACs, CRAs, mercury (II) salts, PHE
Cytoplasmic membrane	Increased permeability	ACD, alcohols, anilides, CHA, QACs, PHE, HCP
	Membrane potential and electron transport chain	ACD, anilides, QACs, PHE, HCP
	Adenosine triphosphate synthesis	CHA, copper (II) salts, ETO
	Inhibition of enzyme activity	CHA, QACs, PHE
Cytoplasmic constituents	General coagulation	CHA, QACs, GTA, HCP, metallic salts,‡ PHE
	Nucleic acids	ACD, ACR, ETO, FMA, GTA, CRAs, POP
	Ribosomes	HOP, mercury (II) salts, organomercurials
Interaction with specific groups	Thiol groups	BOP, ETO, GTA, HOP, CRAs, IOD, POP, metallic salts, IST
	Amino groups	ETO, FMA, GTA, OPA
	Sulfhydryl groups	BOP, ETO, GTA, HOP, CRAs, metallic salts, IST
Biocide-induced autocidal activity	Accumulation of free radicals	BOP, IST, HOP, membrane active agents§

ACD = organic acids and parabens; ACR = acridines; BOP = bronopol; CHA = chlorhexidine; CRAs = chlorine-releasing agents; ETO = ethylene oxide; FMA = formaldehyde; GTA = glutaraldehyde; HCP = hexachlorophene; HOP = hydrogen peroxide; IOD = iodine and iodophors; IST = isothiazolines; OPA = ortho-phthalaldehyde; PHE = phenolics; POP = beta-propiolactone; QACs = quaternary ammonium compounds.
*Adapted with permission from Maillard J-Y. Bacterial target sites for biocide action. *J Appl Microbiol* 2002;92(suppl):S16-S27, Blackwell Publishing.
†Gram-negative bacilli.
‡Copper (II) salts, mercury (II) salts and organomercurials, and silver (I) salts.
§Agents causing damage to the cytoplasmic membrane.

effectiveness against both gram-negative and gram-positive microorganisms in addition to efficacy against *Pseudomonas aeruginosa.* In addition to the efficacy data for a public health claim, the applicant is required to submit supporting data pertaining to product chemistry and toxicologic hazards.[6]

The EPA reviews efficacy data for disinfectants for two reasons. First, if these products are ineffective, individuals may become ill secondary to a contaminated surface, potentially leading to an avoidable public health problem. Second, microorganisms are small and not visible, and unlike with larger pests such as weeds or termites, users cannot determine whether products are working.[6]

DEFINITIONS

Germicidal agents inactivate microorganisms and include disinfectants, antiseptics, and preservatives. Germicides designated by words with the suffix "-cide" (eg, virucide, fungicide, bactericide, sporicide, and tuberculocide) destroy the microorganisms identified by the prefix. Disinfectants are substances that are applied to inanimate surfaces and objects to destroy harmful microorganisms, although they may not kill bacterial spores. Antiseptics are antimicrobial substances that are applied to the skin or mucous membranes to reduce transient microbial flora, resident microbial flora, or both. Preservatives are agents added to products, including medications, to prevent microbial growth.

Disinfectants are categorized by their spectrum of microbicidal activity. High-level disinfectants inactivate all microorganisms with the exception of high numbers of bacterial spores. High-level disinfection is used for semicritical medical devices that contact mucous membranes (eg, bronchoscopes) or non-intact skin. Intermediate-level disinfectants inactivate mycobacteria (eg, *Mycobacterium tuberculosis*), vegetative bacteria, and most viruses and fungi, but do not necessarily kill bacterial spores. Low-level disinfectants kill most vegetative bacteria, and some viruses (eg, enveloped) and fungi, but cannot be relied on to kill more resistant microorganisms such as mycobacteria, nonenveloped viruses, or bacterial spores. Intermediate-level and low-level disinfectants are used on environmental surfaces or equipment that comes into contact with intact skin (eg, blood pressure cuffs).[1,2]

MECHANISMS OF ACTIVITY OF DISINFECTANTS

The mechanisms by which germicides inactivate microorganisms remain incompletely understood. Two excellent articles review this topic in detail.[7,8] Unlike antibiotics, most disinfectants have multiple sites of action (Table 1).

MECHANISMS OF RESISTANCE TO DISINFECTANTS

Microbes may exhibit resistance to antibiotics[9-11] via several broad mechanisms including drug inactivation or modification,[12-14] target site alteration,[15-19] development of bypass pathways,[20] and altered intracellular concentration due to decreased permeability or enhanced efflux.[17,18,21-23]

TABLE 2
MECHANISMS OF BACTERIAL RESISTANCE TO GERMICIDES AND ANTIBIOTICS*

Mechanism of Resistance	Examples
Intrinsic	
Impermeability	Gram-negative bacilli; several biocides or antibiotics
Efflux	Multidrug-resistant gram-negative bacilli; several biocides or antibiotics
Inactivation	Beta-lactams, triclosan (?), chorhexidine (?)
Acquired	
Inactivation or modification	Beta-lactams, chloramphenicol, AGACs, formaldehyde
Insensitive target site	Several antibiotics, triclosan
Decreased accumulation (plasmid-mediated efflux)	Several antibiotics; *qac* genes and biocides
Bypass of sensitive step	Sulfonamides, trimethoprim
Overproduction of target	Trimethoprim, triclosan (?)
Absence of enzyme-metabolic pathway	Isoniazid

AGACs = aminoglycosides–aminocyclitols.
*Adapted with permission from Russell AD. Introduction of biocides into clinical practice and the impact on antibiotic-resistant bacteria. *J Appl Microbiol* 2002;92(suppl):S121-S135, Blackwell Publishing.

Resistance may be intrinsic (ie, innate to the species) or an acquired characteristic (ie, resistance that arises by mutation or acquisition of plasmids or transposons) of the organism. Resistance genes may reside on the chromosome, on a plasmid, or on a transposon. Multiple mechanisms may mediate resistance to a specific antibiotic such as trimethoprim–sulfamethoxazole[24] or quinolones.[25] Resistance to disinfectants has been reviewed.[8,26-34]

As with antibiotic resistance, resistance to disinfectants may be an intrinsic or an acquired property (Table 2).[35,36] Disinfectant resistance is mediated by mechanisms similar to those of antibiotic resistance including drug inactivation or modification, target site alteration, and altered intracellular concentration due to decreased permeability or enhanced efflux. In parallel with antibiotic resistance, resistance to disinfectants may be encoded on plasmids.[37-39] Heinzel has noted that most cases that are attributed by the user to disinfectant resistance turn out to be misapplications of the disinfectant including use of an inappropriate product (ie, pathogen exhibits intrinsic resistance); application of the product without regard to proper duration, concentration, pH, or temperature; failure to remove organic debris (ie, cleaning) prior to disinfection; insufficient contact of the disinfectant with the surface or object to be treated; or insufficient availability of the active product (eg, failure to use a proper dilution of an iodophor as free iodine may be present in lower concentration in more concentrated products).[37,40]

HIERARCHY OF INTRINSIC DISINFECTANT RESISTANCE

Microbes exhibit a wide variation in intrinsic resistance to disinfectants (Figure). Intrinsic resistance may be associated with constitutive degradative enzymes but is more commonly linked to cellular impermeability. Both mechanisms limit the concentration of the disinfectant to reach the target site(s) in microbes. Prions are the agents most resistant to disinfectants and are not inactivated by any of the commonly used high-level disinfectants except sodium hypochlorite.[41] Coccidial cysts such as those of *Cryptosporidium parvum* are also resistant to most high-level disinfectants.[42] Spore-forming bacteria are resistant to many disinfectants (eg, phenolics, quaternary ammonium compounds, and alcohols), but other disinfectants inactivate spores with extended exposure times (eg, glutaraldehyde and hydrogen peroxide). Mycobacteria are the most resistant vegetative cells and owe their resistance, in large part, to permeability barriers posed by the cell wall. In general, non-enveloped viruses are more resistant than either vegetative bacteria or enveloped viruses.[43]

The hierarchy of microbes presented in the figure is a general scheme. The relative resistance of individual microbes and potentially classes of microbes may vary depending on the specific class of disinfectants (ie, phenolics, alcohols, or chlorine compounds). For example, alcohols inactivate mycobacteria but are less active against non-enveloped viruses such as poliovirus. The test methodology may also affect the relative ranking of pathogens regarding ease of inactivation. For example, alcohols and chlorine are relatively less effective in the presence of protein than is glutaraldehyde.

A SCHEME FOR EVALUATING DISINFECTANT ACTIVITY: RATIONALE

Based on the above hierarchy, a logical method for assessing the efficacy of a disinfectant would be to test the disinfectant's activity against an appropriate member of each group of microbes in the hierarchy. An appropriate representative for each group should have the following characteristics: (1) microbiologically well characterized; (2) a clinically important human pathogen or a validated surrogate for a human pathogen; (3) standardized stock strains available from commercial sources; (4) require only

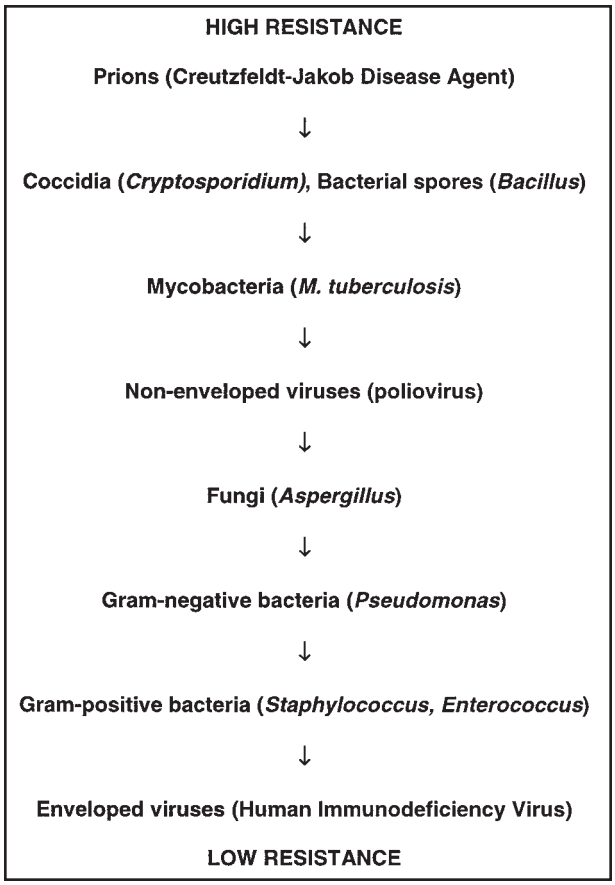

FIGURE. Classification of microorganisms according to their sensitivity to disinfectants.

TABLE 3
EXAMPLES OF SURROGATE MICROBES FOR THE DISINFECTANT TESTS

Vegetative bacteria
Staphylococcus aureus
Pseudomonas aeruginosa
Fungi
Aspergillus niger
Trichophyton mentagrophytes
Candida albicans
Mycobacteria
Mycobacteria terrae
Bacterial spores
Bacillus subtilis
Clostridium sporogenes
Viruses
Rotavirus
Poliovirus
Calicivirus
Adenovirus
Rhinovirus
Hepatitis A virus
Small, non-enveloped bacteriophage

biosafety level 1 or 2 for propagation and evaluation; (5) more resistant to disinfectants than other members of the group or comparable resistance; and (6) standard methods available for propagation (sufficiently high numbers to allow a 6-$\log_{10}$ reduction), assay, and storage. Some candidate microbes for disinfectant testing are listed in Table 3. Demonstration of activity against the test organism of any group should allow the manufacturer to claim activity against all members of the group.

This claim of group activity clearly differs from the requirements for registration of a human antibiotic that should be pathogen specific. We believe that the registration process for antibiotics and disinfectants is fundamentally different for several reasons. First, antibiotic therapy is ideally based on laboratory identification of the causative pathogen, whereas disinfectant use is based on the likely class of pathogens (eg, bacteria). Second, resistance to antibiotic therapy may evolve during therapy and antibiotic resistance among important healthcare-associated pathogens and community-acquired pathogens is an increasing threat. In contradistinction, the development of clinically relevant disinfectant resistance is extremely rare and disinfectant resistance to currently recommended disinfectant agents is not an important problem. Third, antibiotics have a low toxic–therapeutic ratio, whereas disinfectants are generally employed at concentrations vastly in excess of microbicidal levels. This is a major factor in limiting the development of disinfectant resistance. Finally, antibiotics prevent microbial growth via specific targets, whereas disinfectants, in general, inactivate microbes via multiple targets. This is another important difference that limits the development of disinfectant resistance.

Use of Antibiotics Versus Disinfectants

Antibiotics may be administered in three different circumstances—prophylactic therapy, empiric therapy, and definitive therapy. Prophylactic therapy follows a defined exposure to a contagious individual by a susceptible individual. In empiric therapy, antibiotics are chosen based on the likely organ system involved and epidemiologic features such as age, gender, occupational exposures, environmental exposures, and host defense abnormalities that suggest specific pathogens. Definitive therapy is based on the laboratory identification, usually via culture, of a specific pathogen(s). Antibiotic therapy, in general, is guided by susceptibility tests and patient factors such as drug allergies, presence of renal or hepatic dysfunction, pregnancy status, and the possibility of drug–drug interactions. Ideally, all antibiotic therapy should be guided by appropriate laboratory results and the agents with the narrowest spectrum should be used. However, we rarely if ever know the specific pathogens contaminating an environmental surface or a medical instrument. Rather, we base the

choice of disinfectant on the likely class of pathogens, the type of surface or object, and the risk that the contaminated surface might lead to human infection. Thus, in bathrooms one should use an agent active against fecal bacteria. In kitchens, the disinfectant chosen should be active against food-borne and water-borne human pathogens. In healthcare, the disinfectant should be active against both gram-positive and gram-negative bacteria. Given that disinfectants are chosen because of their activity against a class or classes of pathogens, it is logical to register such agents based on activity against the entire class.

Acquired Antibiotic Resistance

During the course of antibiotic therapy, drug resistance may evolve. The likelihood of this event is pathogen, antibiotic, and pharmacokinetic dependent. For some pathogen–antibiotic combinations such as ceftriaxone therapy for *Neisseria meningitidis*, the development of resistance may never have been described, whereas for other pathogen–antibiotic combinations such as antipseudomonal therapy for *Pseudomonas aeruginosa*, resistance develops in approximately 10% of treatment courses.[44]

The increasing frequency of resistance among human pathogens to antibiotics has been recognized as a problem of major public health importance.[45-53] Pathogens of major concern predominantly acquired in the community include penicillin-resistant *Streptococcus pneumoniae*,[54-58] multidrug-resistant *M. tuberculosis*,[59-63] penicillin-resistant *Neisseria gonorrhoeae*,[64-66] multidrug-resistant *Salmonella* species,[67-70] and chloroquine-resistant *Plasmodium falciparum*.[71-75] Healthcare-associated pathogens of major concern include MRSA,[76,77] VRE,[78-81] and extended-spectrum beta-lactamase–producing *Escherichia coli* and *Klebsiella pneumoniae*.[12,82,83]

Acquired Disinfectant "Resistance"

Among the reasons that explain the lack of clinically important disinfectant "resistance" developing are the large use effective ratios for disinfectants in common clinical practice and the multiple target sites by which disinfectants act to inactivate microbes. Disinfectants are clinically used at concentrations greatly in excess of the minimum inhibitory concentration for most pathogens. For example, Anderson et al. reported that extended dilutions (eg, 1:45 of quaternary ammonium compound) beyond the recommended use-dilution of quaternary ammonium compounds, phenols, and iodophors still inactivated VRE within 15 seconds.[84] For chlorine, the concentration in drinking water, approximately 1 ppm, demonstrates activity against high concentrations of most vegetative bacteria and viruses.[85] In disinfecting environmental surfaces, chlorine is commonly used at 100 to 5,000 ppm. Such concentrations readily inactivate all microbes including bacterial spores.[1,85]

Acquired tolerance to disinfectants or antiseptics has been reported for only a few agents. The use of chlorhexidine for bladder washes (concentration < 1 mg/mL) has been associated with urinary tract infection due to gram-negative bacilli, especially *Proteus mirabilis*, resistant in some cases to greater than 800 µg/mL of chlorhexidine.[86] However, chlorhexidine is usually used in the hospital at a concentration of 2% to 4% (20,000 to 40,000 mg/mL). Plasmid-mediated resistance to silver,[87] other metals,[88] and organomercurials has been extensively investigated. More recently, there have been reports linking the presence of plasmids in bacteria with increased tolerance to chlorhexidine, quaternary ammonium compounds, and triclosan.

Staphylococci are the only bacteria in which the genetic aspects of plasmid-mediated antiseptic- and disinfectant-resistant mechanisms have been described.[31] Decreased susceptibility to chlorhexidine and quaternary ammonium compounds has been reported to be widespread among MRSA strains. Tolerance is mediated by the *qac* family of genes that code for proton-dependent export proteins involved in an efflux system that actively reduces intracellular accumulation of toxicants such as quaternary ammonium compounds.[89-91] Strains carrying *qac* genes may exhibit reduced susceptibility to aminoglycosides, tetracycline, or both.[91] Coagulase-negative staphylococci frequently also contain *qac* genes.[92] Studies have established that the *qac* genes consist of two gene families, *qac*CD (now referred to as smr) and *qac*AB.

EVALUATING DISINFECTANTS BY PATHOGEN CLASS

Based on the above scientific data, a convincing argument can be made to test only a representative of each microbial class (Figure; Table 3).[93] With the exception of viruses, the EPA has accepted the use of suitable surrogates and for this scheme to succeed, it is critical that the pathogen tested be among the most disinfectant resistant in the class. The other attributes for the ideal pathogen have already been described. Fortunately, there is an extensive literature on disinfectant susceptibility of clinically relevant pathogens. For example, laboratories have used a Sabin vaccine strain of poliovirus type 1 as a representative of small, non-enveloped viruses. The efficacy of disinfectants on poliovirus has been studied because small, non-enveloped viruses are the viruses most resistant to disinfectants.[43] Disinfectants that inactivate the poliovirus could be considered reliably capable of making a general virucidal claim. However, the use of vaccine strains of polioviruses as surrogates for virucidal claims needs reevaluation in view of the anticipated eradication of poliomyelitis. If the use of all types of polioviruses were restricted, a suitable replacement would be needed.[93] For a virucidal claim, a non-enveloped virus such as hepatitis A, rotavirus, animal strains of caliciviruses, or a bacteriophage should be considered.

There are several examples of how this scheme would work for specific pathogens. Norovirus, a non-cultureable, non-enveloped virus, would be placed in the non-enveloped virus class and products would be registered on their ability to inactivate a test non-enveloped virus (such as poliovirus). Human herpes virus 8, a cause of

Kaposi's sarcoma, would be placed in the enveloped virus class and products would be registered on their ability to inactivate a test enveloped virus or a non-enveloped virus (such as poliovirus). *Helicobacter pylori*, a cause of peptic ulcer disease, would be placed in the vegetative bacterial class and disinfectants would be registered on their ability to inactivate test bacteria (eg, *Pseudomonas aeruginosa* and *Staphylococcus aureus*). Products would be registered to inactivate vancomycin-resistant *Staphylococcus aureus* also based on their ability to inactivate the test bacteria, *Pseudomonas aeruginosa* and methicillin-sensitive *Staphylococcus aureus*. *Staphylococcus aureus* and *Pseudomonas aeruginosa* are both clinically relevant pathogens and can be among the more difficult to inactivate bacteria if grown properly (nutrient poor media). Additionally, testing for antibiotic-resistant pathogens (eg, MRSA) is not necessary because they do not have altered susceptibility to disinfectants at the manufacturer's recommended use-dilution. When the anthrax attack occurred in the United States, disinfectants could have been registered based on their ability to inactivate the surrogate, *Bacillus atrophaeus* spores. As another example, one might use *M. terrae* as a representative of mycobacteria.[93] *M. terrae* is a good surrogate for *M. tuberculosis* because it has low virulence and a similar resistance to most disinfectants. In addition to standardized testing of filamentous fungi (eg, *Aspergillus* species and *Trichophyton mentagrophytes*), a non-filamentous and unicellular fungi (yeast) such as *Candida* should be considered (S. Springthorpe, MS, written communication, March 19, 2003). If new data were to suggest a more appropriate representative of the class, then the testing requirements could be altered.

The current EPA approval process has resulted in slow approval of label claims for disinfection of new and emerging pathogens such as the coronavirus causing severe acute respiratory syndrome (SARS) and *B. anthracis*. If our proposed scheme had been in place, a disinfectant could claim activity against the SARS coronavirus or anthrax based on previous studies demonstrating activity against a non-enveloped virus (poliovirus a surrogate for all viruses including SARS coronavirus) or *B. atrophaeus* (a surrogate for *B. anthracis*).

The proposed scheme requires that the microbiologic class of a new microbe be established. The class-specific test organism(s) would serve as a surrogate for evaluating disinfectant efficacy. The label claim (ie, registration) would be based on the use of a validated EPA-approved test that assessed the efficacy of disinfectants against the class-specific test organism. Until a new or emerging microbe could be placed in a microbiologic class, it is suggested that only disinfectants with a mycobactericidal claim be allowed by the EPA. For example, the SARS agent, prior to isolation and characterization as a coronavirus, would necessitate the use of a disinfectant with a mycobactericidal label claim for surface disinfection. Similarly, because noroviruses cannot be tested by a culture method, disinfectants could be registered based on their ability to inactivate poliovirus. Once the agent is characterized and placed into a microbial class (as a coronavirus or virus), all EPA products with a label claim against viruses (test agent, poliovirus) would be acceptable. If there is not a validated test organism in a class, the next most resistant class should be used for purposes of registering disinfectants. For example, if a surrogate for an enveloped virus is not validated, then a non-enveloped virus (eg, poliovirus) could be used instead.

PERIODIC TESTING

A frequent topic of discussion is whether disinfectants should undergo periodic testing by the manufacturer to ensure effectiveness (eg, every 5 years). There are several compelling reasons why periodic testing should not be required. First, as discussed above, the development of disinfectant resistance to current surface disinfectants has not been demonstrated to be a clinically relevant problem. Thus, unlike with antibiotics, we have not been forced to abandon older agents and develop ever more potent or active disinfectants. Second, there is no requirement for periodic testing of antibiotics despite emerging clinically relevant resistance. Finally, given that there is no theoretical benefit from periodic testing, such testing would only serve to increase the cost of these agents.

Reasons for retesting a registered disinfectant might include the following. First, disinfectant testing may be necessary with the discovery of a new pathogen of unclear taxonomic placement. Second, testing would be considered with the development of a superior method for assessing microbicidal activity. In such a case, additional testing might be required to revalidate the correct germicidal activity of a disinfectant. Finally, given that there is no theoretical benefit from periodic testing, the only effect of such testing would be to increase the cost of these agents. For example, it is well known that biofilms may differentially affect the ability of a disinfectant to inactivate microbes. Thus, one might have separate validated test methods for registering surface disinfectants and disinfectants used in environments where biofilms are likely (eg, dental units).

Manufacturers' efficacy claims against microorganisms should be verified using a standardized test by an independent laboratory prior to EPA registration. Post-registration efficacy testing of randomly selected disinfectants by the EPA using a standardized test would continue to provide assurance that registered products are capable of a certain level of antimicrobial activity when used as directed. The EPA has found failures with both registered tuberculocides (11% failure rate) and hospital disinfectants (25% failure rate).[6] For this reason, EPA post-registration testing is important to validate, at least once, the manufacturers' label claims.

Given that development of clinically relevant resistance to registered disinfectants in nature has not been reported, there is no scientific basis for requiring periodic retesting of licensed products by the manufacturer.

However, the EPA should continue to conduct in-house testing of randomly selected registered products to ensure the reliability of label claims.

METHODOLOGY FOR EVALUATING DISINFECTANT ACTIVITY

The methods recognized by the EPA for substantiating a bactericidal claim for a disinfectant are the methods of the Association of Official Analytical Chemists (AOAC) International: the use-dilution method; the hard surface carrier method; and the germicidal spray products method.[94] The former two methods are used to test liquid products applied to surfaces and the third method is used to test spray products.[95] Each method is designed to evaluate the bactericidal activity of the disinfectant against *Salmonella choleraesuis*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. These methods are also used to develop data in support of label claims of efficacy of a disinfectant against specific pathogenic bacteria not listed in the methods. Supplemental claims for activity against pathogenic fungi (*Trichophyton mentagrophytes*) and mycobacteria (*M. tuberculosis*), respectively, are supported by tests performed using the fungicidal method and the tuberculocidal method or quantitative tuberculocidal method.[95] Currently, there is no AOAC International method for testing against protozoa (eg, *Giardia, Cryptosporidium, Cyclospora,* or *Acanthamoeba*) or viruses.

For the past 25 years, these AOAC International methods have undergone extensive examination and collaborative studies, which have revealed an unacceptable level of interlaboratory variability. The major causes of variation in the test are due to wide variability in the number of bacteria on the test cylinders, intrinsic variations in the surfaces of the cylinder itself, wash-off of test organisms, lack of quantitation, and the characteristics of the test pathogen.[96-103] There has been worldwide activity in the development and standardization of improved methods for evaluating the microbicidal activity of disinfectants by organizations such as the American Society for Testing and Materials (ASTM), AOAC International Task Force, Comite Europeen de Normalisation (CEN), and Association Francaise de Normalization (AFNOR).[96] There is also an effort to harmonize disinfectant test methodology between countries to accommodate regional and international trade agreements (eg, Organization for Economic Cooperation and Development).[93,96] A germicidal test must be simple, quantitative, reproducible, and representative of use conditions and the method must be precisely described so that the data are relevant and reliable.[93] There are many benefits for improved disinfectant test protocols to include easier disinfectant registration and minimize the possibility of product recalls and the potential for litigation. If efforts to improve (eg, quantitative carrier test for spores) and unify (ie, one fundamental test protocol that is quantitative and could be used on major classes of test organisms) the test methodology are sustained and coordinated with regulatory agencies such as the EPA, improved test methods and thus the availability of more effective and safer disinfectants should result.[93,96]

We believe that disinfectants should be registered based on the use of a validated test method using one or two appropriate representatives of each class of microbial pathogens. Periodic testing should not be required by the manufacturer, but post-registration testing by the EPA provides some assurance of disinfectant efficacy.

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