MICROBIOLOGICAL METHODS

Hierarchy of Susceptibility of Viruses to Environmental Surface Disinfectants: A Predictor of Activity Against New and Emerging Viral Pathogens

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Proper disinfection is crucial to interrupt the environmental spread of many viruses. In the case of new and emerging viruses still awaiting culture and full characterization, it is proposed that any official recommendations for disinfectant use be based on the well-established hierarchy of susceptibility to such chemicals as related to virus particle size and structure.

Viruses represent nearly 15% of all infectious agents catalogued as human pathogens (1). Viruses also predominate in the list of nearly 40 human pathogens discovered in the past 4 decades (2). Successful control of many bacterial diseases, together with the discoveries of links between viruses and chronic conditions, further enhance the relative significance of viruses (3).

Although vaccination, insect vector control, screening of blood and tissues, and barriers such as condoms can effectively interrupt the spread of many viruses, others require environmental control instead (4, 5); hand hygiene and chemical disinfection of semicritical medical devices and environmental surfaces constitute the backbone of such control in healthcare settings. Hand hygiene and disinfection of environmental surfaces are also crucial where foods are handled.

In the United States, antiseptics are regulated by the U.S. Food and Drug Administration (FDA), but that agency currently has no system to review and register label claims of such formulations against viruses (6). The FDA, which also regulates chemi-sterilants and high-level disinfectants for use on medical devices (7), requires data for efficacy against viruses but simply refers to testing for virucidal activity as specified by the U.S. Environmental Protection Agency (EPA). The EPA deals mainly with environmental surface disinfectants through specific rules on testing and approval of label claims against human pathogens (*see* http://www.epa.gov/opp00001/factsheets/antimic.htm). It also accepts the use of surrogate organisms for claims of activity against vegetative and spore-forming bacteria, mycobacteria, and fungi (8). Apart from certain recently granted exceptions, this does not apply to viruses, and testing is required against each virus type to be listed on the product label. However, a disinfectant can call itself a 'virucide' even with activity against one or more enveloped, thus easier to kill, virus. This is also in sharp contrast to regulations elsewhere. In Canada, an environmental surface disinfectant can call itself a 'general virucide' only upon demonstrated activity against the Sabin vaccine strain of poliovirus type 1 (9), and in Europe, in addition to the poliovirus, human adenovirus type 5 must be used to make a similar claim (10). A general virucidal claim in Australia requires testing against a poliovirus, an adenovirus, and a herpesvirus; a parvovirus can be substituted for the poliovirus (11). This paper highlights the implications of the current EPA policy (http://www. google.ca/search?hl=en&q=EPA+%2B+virus+surrogates&btnG) with particular reference to new and emerging viral pathogens and proposes an alternative.

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Virus Particle Size and Structure and Activity of Disinfectants

The differences in the lipophilicity of enveloped (hydrophobic) and nonenveloped (hydrophilic) viruses were clearly delineated in the late 1950s (12, 13). Klein and Deforest (14) subsequently showed that hydrophobic viruses were considerably more susceptible to microbicidal chemicals due to the presence of essential lipids in their envelope. Among the hydrophilic ones, those with a smaller particle diameter (25–35 nm) proved to be comparatively less susceptible than those of a larger size (40–75 nm). Those initial observations have been repeatedly confirmed and extended over the years. The Spaulding classification (15) is also frequently referred to in this context; however, it relates exclusively to chemical disinfectants to be used on medical and surgical devices, which come under the purview of the FDA.

Table 1 is based on the hierarchy of susceptibility in relation to the particle size of human pathogenic viruses when tested under similar experimental conditions; other infectious agents are included for contrast only. It should, however, be noted here that nonenveloped viruses in general are often considered more susceptible to chemical disinfectants than are

Table 1. Hierarchy of susceptibility of human pathogens to chemical disinfectants

Level of susceptibility	Microbial class	Virus family	Examples of human pathogenic viruses
Lowest	Bacterial spores		
	Small nonenveloped viruses (25–35 nm)	Astroviridae	Astro
		Caliciviridae	Noro
		Circoviridae	Anellovirus
		Parvoviridae	B19, bocavirus
		Picornaviridae	Entero, hepatitis A, rhino
	Mycobacteria		
↓	Large nonenveloped viruses (40–70 nm)	Adenoviridae	Adeno
		Papillomaviridae	Papilloma
		Polyomaviridae	SV_{40}, Rota
		Reoviridae	
	Fungal conidia		
	Vegetative bacteria, yeast		
Highest	Enveloped viruses	Arenaviridae	Lymphocytic choriomeningitis
		Bornaviridae	Borna
		Bunyaviridae	Hanta, Rift Valley Fever
		Coronaviridae	SARS
		Filoviridae	Marburg, Ebola
		Flaviviridae	Yellow fever, hepatitis C
		Hepadnaviridae	Hepatitis B
		Herpesviridae	Cytomegalo, varicella
		Orthomyxoviridae	Influenza
		Paramyxoviridae	Mumps, measles
		Poxviridae	Smallpox, vaccinia
		Rhabdoviridae	Rabies
		Retroviridae	Human immunodeficiency
		Togaviridae	Rubella

mycobacteria (16). Although this may be true for the larger-sized nonenveloped viruses, many types of smaller-sized nonenveloped viruses show themselves to be more difficult to inactivate than mycobacteria when tested simultaneously (17). Prions are not included in the Table because they are not only substantially different from viruses in size and structure, but their communicability is also much lower. Further, recent studies show them to be much less resistant to chemicals than previously thought (18).

Viruses and Environmental Control

As stated earlier, many viruses have been discovered since 1968 (2, 19) and others have re-emerged (20), together causing substantial numbers of human cases and fatalities (21). Table 2 gives examples of the nonenveloped viruses among those. Several of the listed viruses have the potential to remain viable on nonporous surfaces and thus their spread via such vehicles is potentially interruptible with proper disinfection of the environment. Rotaviruses are a suitable example, as they can survive well on environmental surfaces (22), and their spread in experimental (23) as well as field (24) settings can be prevented through the use of chemical disinfection.

The discovery of a new pathogen elicits an immediate demand for guidance on the selection and use of disinfectants for its environmental control. In the United States, the primary source for any such guidance is the Centers for Disease Control and Prevention (CDC), which normally bases its recommendations on EPA-registered label claims (25). This may not readily apply to a newly discovered virus, particularly a nonenveloped virus in which a greater degree of confidence is essential for its environmental control.

The following additional factors may hinder the ready availability of the information needed to base such recommendations: (*1*) The agent may be refractory to growth in lab animals and cell cultures, thus limiting experimentation with it; human noroviruses illustrate this point well. (*2*) Even if a newly discovered virus is cultivable in the laboratory, its classification at biosafety level 3 or 4 would severely restrict the number of laboratories that could handle it and thus

Table 2. Examples of nonenveloped viruses discovered as new and/or emerging since 1968

Virus	Year of discovery	Virus family (approximate particle size in nm)	Associated disease(s)
Enterovirus 70	1968	Picornaviridae (30)	Acute hemorrhagic conjunctivitis; rare cases of paralysis
Coxsackievirus A24 (variant)	1970	Picornaviridae (30)	Acute hemorrhagic conjunctivitis
Enterovirus 71	1969	Picornaviridae (30)	Aseptic meningitis; hand-foot-mouth disease
Norovirus (Norwalk agent)	1972	Caliciviridae (30)	Acute gastroenteritis
Rotavirus	1973	Reoviridae (70)	Acute gastroenteritis
Parvovirus BI9	1975	Parvoviridae (25)	Aplastic anemia
Hepatitis E	1988	Unclassified (30)	Hepatitis
Anellovirus	1997	Circoviridae (17)	Hepatitis
Bocavirus	2005	Parvoviridae (25)	Respiratory infections

impede the development of information on its susceptibility to chemicals potentially applicable for its environmental control. (*3*) Recently enhanced restrictions on the transportation of infectious agents in general would also limit the availability of the virus to researchers otherwise capable of testing environmental surface disinfectants against it. (*4*) Lack of vaccination and therapy against the virus may put staff at testing labs at undue risk of laboratory-associated infections, as shown by the severe acute respiratory syndrome (SARS) incident in Singapore (26).

An Alternative Approach

An alternative approach in dealing with newly discovered nonenveloped viruses is to apply the hierarchy of disinfectant susceptibility as it relates to their size and structure (Table 1). One plausible scenario is that a newly discovered virus is assigned a tentative grouping based on either direct electron microscopy only or by molecular and immunological means with or without culture in the laboratory. Even this preliminary information has a reasonable predictive value based on the already available data on the relationship between virus particle size and structure and susceptibility to environmental surface disinfectants.

It is recommended that the data to be submitted for consideration by the EPA be based on an approved carrier test using at least one large-sized nonenveloped virus and one small-sized nonenveloped virus. The actual choice of the viruses may be determined depending on the predominant clinical picture of the new or emerging virus under consideration and the testing initiated with the following criteria and conditions in mind: (*1*) The virus in question must first be identified as to its tentative grouping, and the CDC must make a formal announcement to that effect. (*2*) Manufacturers of environmental surface disinfectants must submit data to the EPA supporting claims of activity of the proposed product against a virus expected to be equally or less susceptible than the newly discovered virus. (*3*) Claims of hierarchy-based virucidal activity must be limited only to those products already classified as broad-spectrum and hospital-grade disinfectants. (*4*) Claims for anticipated activity against the target virus will not be allowed on product label or in mass media, but may possibly be allowed on company Web sites and tech bulletins. (*5*) Such a claim based on the hierarchy of susceptibility to disinfectants will be allowed only until actual information on the agent's susceptibility to environmental surface disinfectants becomes available from actual testing which has been accepted by the EPA. (*6*) Limited disinfectants and sanitizers, as defined by the EPA, will be excluded from consideration in this context.

Conclusions

Since the work of Klein and Deforest (14) and Spaulding (15), we know of many more human pathogenic viruses and also better understand their susceptibility to a wider variety of chemical disinfectants using more sophisticated test methods (27). This information, while confirming the original tenets, should allow for greater confidence in the scheme proposed here. It should also be noted that the Spaulding scheme was developed for the decontamination of medical devices and is limited in its relevance to the chemical disinfection of environmental surfaces.

New human pathogenic viruses continue to come to light (28) and it is quite likely that such discoveries will continue well into the future, while those already known may also emerge or re-emerge as a result of ongoing societal changes (2). In view of this, and in the United States in particular, the existing regulations with regard to awarding label claims for virucidal activity need urgent review. This issue is especially pertinent when it comes to new and emerging viral pathogens of humans. The proposed system, based on the already well-recognized hierarchy of disinfectant susceptibility, is not only scientifically valid, but it will not require a major overhaul of the existing regulations.

The concept of using indicator strains for classes of microbial pathogens has been in place for some time now. More recently, the EPA has begun to allow surrogate viruses to be used to substantiate virucidal activity for

difficult-to-culture human pathogenic viruses such as hepatitis B (http://www.epa.gov/oppad001/hbv.htm) and hepatitis C viruses (http://www.epa.gov/oppad001/pdf_files/hepcbvdvpcol.pdf), and norovirus (http://www.epa.gov/oppad001/pdf_files/initial_virucidal_test.pdf). Further, the concept of allowing the use of data based on testing with surrogate viruses has been accepted by the EPA for new and emerging *enveloped* viruses. As a general rule, nonenveloped viruses are more stable outside hosts and have a greater potential to spread by environmental means (29, 30). Therefore, in the interest of human health and proper infection control, the public and professionals alike have an immediate need for recommendations to counter new and emerging viral pathogens based on the best available science. It is scientifically justifiable to substantiate those public health recommendations and determine product efficacy based on prior testing of environmental surface disinfectants with appropriately related surrogate viruses using valid test methods. Such an arrangement would add value to, and place greater confidence in, any guidance issued by agencies such as the CDC and EPA.

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