MICROBIOLOGICAL METHODS

Carrier Tests to Assess Microbicidal Activities of Chemical Disinfectants for Use on Medical Devices and Environmental Surfaces

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For well over a decade, many deficiencies have been identified in current AOAC methods used to assess the microbicidal activities of chemical disinfectants on medical devices and environmental surfaces. This report discusses the development of quantitative carrier tests (QCT) designed to address these concerns. Decontamination of surfaces with dried inocula is invariably more difficult than when microorganisms are in suspension. For medical device as well as environmental decontamination, microbicides are used on contaminated surfaces, thus making it necessary to evaluate their microbicidal action on representative carrier materials contaminated with a dried challenge microorganism(s). Our approach is a 2-tiered QCT. The first tier (QCT-1) uses relatively ideal conditions to assess performance of the microbicide for screening purposes; the test uses smooth glass surfaces and quantities of disinfectant in excess of those likely to be experienced in the field. The second tier of testing (QCT-2) is more stringent because it uses (*1*) disks of brushed stainless steel as carriers, (*2*) only 50 μL of the test formulation on each carrier as compared to 1 mL in QCT-1, and (*3*) an added soil load to simulate the presence of residual body fluids or accumulated surface dirt. This review also discusses the factors that affect disinfection of medical devices and environmental surfaces in the context of the methodology used to evaluate the potency of microbicides. Specific recommendations for discussion are included, and performance criteria are suggested based on a risk-reduction approach for different classes of disinfectants. The focus is on improving the relevance of the test methodology to actual field use of disinfectants for devices and facilities in health care, and potentially in other settings. It is hoped that this review and its recommendations will initiate needed discussion and resolution of the many issues identified.

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The efficacy of disinfectants prior to registration has been examined by 2 methods. The first, generally recognized as the simplest and least demanding on the disinfectant, uses microorganisms in suspension that are mixed with a known concentration of disinfectant and held for a defined contact time before disinfectant action is terminated by dilution and/or neutralization; these are referred to as suspension tests. In the field, however, disinfectants are almost always used to decontaminate internal and/or external surfaces of medical devices, equipment, furniture, room surfaces, and other articles. Thus, the second type of test uses surfaces contaminated with microorganisms that are dried onto them as a challenge to the disinfectants. These are held with a known concentration of disinfectant for a defined contact time before disinfectant action is terminated by dilution and/or neutralization. The surrogate surfaces used are called carriers, and the tests are referred to as carrier tests.

For registration of chemicals as microbicides in North America, the carrier tests used have been qualitative end point assays that are Official Methods of AOAC INTERNATIONAL (1) summarized in Table 1. Over a number of years, many researchers (2–12) have identified deficiencies in these methods and, shown in Table 1, many of these methods have never been finalized and others have been repealed or revised. Similar concerns were highlighted by the U.S. General Accounting Office (GAO; 13) and, in response, the Antimicrobials Division of the U.S. Environmental Protection Agency (EPA) initiated efforts to explore alternative and improved test methods through extramural research. Critical assessment of the AOAC methods suggests that the tests are attempting to answer too many questions through a single design; we therefore suggest that a quantitative test using a tiered approach would be more logical and more informative. At the same time, we have recognized that each of the current AOAC test methods for different classes of disinfectants has used a separate approach, making it very difficult to compare efficacy across microbial classes. In accepting this challenge, we designed a 2-tiered quantitative carrier test (QCT) initially for sporicidal activity,

Table 1. AOAC Official Methods for examining disinfectant activity

Test method title	AOAC Method	Year finalized
Testing disinfectants against *Salmonella typhi*—phenol coefficient method	**955.11**	1964
Testing disinfectants against *Staphylococcus aureus*—phenol coefficient method	**955.12**	None given
Testing disinfectant against *Pseudomonas aeruginosa—phenol coefficient method*	**955.13**	None given
Sporicidal activity of disinfectants	**966.04**	1967
Germicidal and detergent sanitizing action of disinfectants	**960.09**	None given
Tuberculocidal activity of disinfectants—presumptive in vitro screening test	**965.12**	1967-Rev. 1988
Use dilution test—testing disinfectants against *Salmonella choleraesuis*	**955.14**	1959-Repeal initiated 1988
Hard surface carrier test—testing disinfectants against *Salmonella choleraesuis*	**991.47**	None given
Use dilution test—testing disinfectants against *Staphylococcus aureus*	**955.15**	1959-Repeal initiated 1988
Hard surface carrier test—testing disinfectants against *Staphylococcus aureus*	**991.48**	None given
Use dilution test—testing disinfectants against *Pseudomonas aeruginosa*	**955.16**	None given
Hard surface carrier test method—testing disinfectants against *Pseudomonas aeruginosa*	**991.49**	None given
Fungicidal activity of disinfectants using *Trichophyton mentagrophytes*	**955.17**	None given
Bacteriostatic activity of laundry additive disinfectants	**972.04**	None given
Germicidal spray products as disinfectants	**961.02**	1964
Disinfectants (water) for swimming pools	**965.13**	1970
Germicidal equivalent concentration—chlorine (available) in disinfectants	**955.16**	None given

that was later expanded to include all classes of microorganisms. This has an immediate benefit in permitting direct comparisons among organisms for their relative sensitivity to an applied product.

The first tier of the QCT (QCT-1) provides the simplest test for the product, and is designed to answer the question as to whether the disinfectant can kill the microorganisms under the ideal conditions for the disinfectant—an obvious prerequisite during product development. However, this test would not be suitable for registration of products that are required to be effective in the field. No test can be designed that will replicate all possible sets of field conditions. However, the second tier of the QCT (QCT-2) has more stringent requirements that bring it closer to field conditions; specifically, these are (*1*) a reduced volume of disinfectant, (*2*) a soil load to simulate residuals of body substances or dirt present on the surface, and (*3*) a carrier surface that is more difficult to wet and which can conceal microorganisms within its crevices. It is difficult to require data be generated for large numbers of tests, each showing different aspects of product performance, but manufacturers may wish to use the separate components of the test challenge (volume, soil load, and surface) to inform their product development.

Although the only method included in any detail here is the QCT method we developed for the EPA, the exact details of the method itself are of lesser importance than the principles it represents in evaluating microbicidal products. We have therefore not included all the detailed steps in the test method, as these have been published elsewhere (14). Instead, we focus on discussion of the rationales adopted during the development of the QCT methods and on how the same general principles should apply to any carrier test. We discuss the interactions that take place among the required test elements: the microbial target, the surface on which it is deposited or grown, and the putative microbicidal formulation. Recommendations for improving the general area of disinfectant testing and registration are also included. To anyone venturing into the field of microbicide evaluation, there is a clear lack of systematic and scientific approach that needs to be corrected. We hope that this review and the recommendations it contains will initiate needed discussion and resolution of the many issues mentioned. That some of the recommendations are based on personal opinion is inevitable; however, this opinion is informed by more than 20 years of developing tests to evaluate microbial control products and of contact with those who produce, use, and regulate such chemicals.

Disparity in Current Test Methods

To make comparisons among microorganisms and among products, the tests must be made quantitative with fixed performance criteria. There need be no requirement for a disinfectant to kill every organism on a carrier because there is no indication that will happen in the field. Permitting small numbers of survivors allows statistical analyses for assessment of the relative risks to producers and consumers of the microbicide products being incorrectly assessed.

Thus ideal tests use carriers and are quantitative. QCT tests are both, and are performed so that test microorganisms are

not lost by wash-off but are contained within the carrier system. Although certain current AOAC methods are carrier tests, the fungicidal test uses fungal spores in suspension (1). Also, since the official status of the AOAC tuberculocidal test was rescinded, the EPA has accepted data against mycobacteria from a quantitative suspension test (15). Even for sporicidal and bactericidal tests, there is no requirement in the AOAC end point test methodologies for quantitation of initial numbers on carriers, but clearly it is desirable to have approximately the same number of microorganisms on each carrier. The simplest method to ensure such equivalence is to deposit known numbers of microorganisms on the carrier surfaces as is done in the QCT. However, in the AOAC tests, carriers are inoculated by dipping, and several factors can influence the numbers and distribution of microorganisms acquired in this manner. Moreover, in the protocols for the AOAC tests, challenge microorganisms can be washed off during the procedures of disinfectant contact and neutralization. AOAC tests have been operated as end point assays, where success or failure is measured by no growth or growth, respectively. Modification of most AOAC tests is possible by enumerating challenge microorganisms recovered from control carriers, but the nature of the carriers in some tests makes it difficult to recover all the challenge organisms consistently, which could make the results questionable.

Around the world, microbicide efficacy tests have been quite varied, but a global effort for their harmonization is now being spearheaded through the Organization for Economic Cooperation and Development based in Paris, France (16). It is anticipated that this will facilitate international trade, making products that can pass the test(s) universally acceptable.

Decontamination of surfaces with dried inocula is invariably more difficult than when the microorganisms are in suspension. Moreover, when disinfectants are used in the field, the targets are unknown mixtures of microorganisms of different species and classes. There is, therefore, every reason to consider an approach to testing disinfectants where efficacy against all classes of microorganisms is evaluated in a similar manner and under similar or identical test conditions. Previous studies in our laboratory showed the feasibility of this approach (17, 18). However, in most jurisdictions, disinfectant testing methods have evolved over many years with a completely separate and highly prescriptive test method for each class of microorganism. This review will present a rational and unified approach to disinfectant testing that makes even more sense when international imperatives towards global harmonization are considered. An approach for harmonization of testing within a jurisdiction is a logical first step (14, 19). Further advantages are also apparent; for the manufacturer—clear criteria, simplified testing approach, increased assurance that false positives will not occur, and a level playing field; for the user—increased assurance that the product will perform according to its label claims, clear criteria, and ability to directly compare performance across products and within products against a range of microbial classes; for the independent testing laboratories—having only one basic test method to deal with; and, finally, for the regulatory agency—simplifying product evaluation.

Proper disinfection will only be achieved by selecting a suitable microbicide and by applying it regularly and in a manner that will promote its effective action. Each of these elements is essential for satisfactory disinfection. If an ineffective product is used, then its proper and regular application cannot compensate to inactivate the microbial challenge.

In developing an efficacy test, it must be clear what question(s) the test is designed to answer. Whether a chemical formulation is sufficiently potent to inactivate a particular organism under ideal conditions is a very different question, with necessarily different test design, than whether efficacy can be obtained in the field under use conditions.

Ideally, field efficacy would be determined in the field, much like clinical trials for drugs. However, this would be impractical, too costly, and impossible to conduct in a truly scientific fashion in most clinical settings. Nevertheless, regulation of commercial disinfectants must include a means of assessing their potential for effectiveness in the field; thus, the best that can be done is a laboratory test that, as far as is practical, simulates field conditions. It must be emphasized, though, that no laboratory-based evaluation will ever guarantee field performance.

As mentioned earlier, QCT-1 simply assesses performance of the microbicide under ideal conditions. This could be described as a potency test, and should be considered as a prerequisite or screening test before proceeding to further tiers. QCT-2 includes additional levels of stringency with a standard and representative rougher test surface, and potentially other test surface(s) depending on the projected use(s) of the product. QCT-2, with the additional levels of stringency, is more suited to generate data for product registration.

Standards developed for European countries use a similar tiered approach but with a suspension test as a preliminary screen before proceeding to 2 levels of carrier tests. Our rationale for QCT was that suspension tests would be the first level of screen used in-house by the manufacturer screening a range of formulations, but did not need to be included as a regulatory test. We consider, therefore, that 2 levels of testing are sufficient to qualify a chemical formulation as a microbicide against whichever class(es) of microorganisms is included in the test inoculum. However, for field performance of products with particular claims, such as high level disinfection of reusable medical devices, additional simulated or actual field tests may be required by regulatory agencies before registration.

Many disinfectants are applied with some mechanical action. We chose to make our tests static rather than dynamic because the testing itself is simpler and more reproducible, and not all products are applied with mechanical activity. Even with mechanical action, contact cannot be guaranteed for all surfaces, and a standardized mechanical action for a test might never be carried out in the field in the same manner. We have also chosen to make the tests fixed time rather than

kinetic, and suggest the inclusion of different product concentrations that will provide more information relevant to field application (*see* discussion below).

There are a number of other considerations in designing a carrier test(s) to measure the microbicidal potential of a given product in the field. For example, what should the level of the soil load be, and what should be the required reduction in the level of microbial challenge? None of these questions are simple, and indeed the current AOAC tests address these factors inadequately. Before discussing our rationales for the approaches taken in QCT-1 and QCT-2, it is important to understand all the factors that can affect microbicidal efficacy, and to have some discussion of how one can establish performance criteria.

Factors Affecting Microbicide Efficacy

As a part of the design for a microbicidal test and its potential impact on current disinfectant regulation, it is important to examine critically the range of factors that can affect disinfectant action; these are summarized in Table 2.

Certain factors listed below have already been incorporated into the design of our tests; others are specific to a given product category, are a part of labeling requirements, or are market drivers determined by circumstance or practicality.

One issue, however, is particularly important to emphasize. Disinfection models have usually been developed with data obtained under demand-free conditions (20), and are often aimed at producing dose level (product of disinfectant concentration and time of exposure or Ct) values used in water treatment plants or elsewhere where breakpoint disinfection is routinely practiced, and the disinfectant is present in very large excess. Some recent advances in disinfection models (21, 22) examine the result of tailing survivor curves (23) and of interfering substances (24), but even these models only address disinfection in fluids. For surfaces, the implications of surface interactions and a limited disinfectant volume have not been satisfactorily addressed.

In the field, products used on surfaces face an unknown disinfectant demand and are rarely present in large, or perhaps any, excess. The demand can therefore greatly weaken the inactivating potential of the product and sometimes render it totally ineffective. Although no data are presented here on this issue, it can readily be demonstrated in the laboratory (unpublished results; manuscript in preparation). This is particularly exacerbated when the quantity of product is small in relation to the surface area to be treated. The reader is asked to bear that in mind during the subsequent discussion.

Disinfectant Formulation

The tested disinfectant formulation is specific to the manufactured product, but products with the same listed active ingredients frequently perform quite differently in a test. Such differences can often be ascribed to differences in the inert ingredients. That these are not always inert is self-evident, but commercial interests usually prevent their disclosure. The user is unable, therefore, to select knowledgeably from among products with identical active ingredients.

The concentration(s) of disinfectant active(s) is part of the labeling requirements, and the concentration(s) at which the product is used is manufacturer-determined. Some product labels are packed with information but can be confusing and difficult to interpret, requiring a judgment call and knowledge of target microbial contaminants. Because the target organisms are rarely known, labeling to use different disinfectant concentrations for different target organisms should not be permitted; it would be confusing, and the wise user will use the longest time listed on the label. Recommendations for different concentrations should be acceptable only for different product uses. The main reason for this is clarity and simplicity. Both the AOAC tests and QCT-1 use a single manufacturer-specified use concentration. QCT-2 can be performed with a single concentration, but most information can be obtained from QCT-2 by using multiple concentrations as suggested and described below.

Target Organisms (Surrogates)

In general, test organisms are selected to be representative surrogates. In the United States, this does not apply to viruses, except for the recently introduced acceptance of the duck hepatitis B virus and the bovine viral diarrhea virus as surrogates for the human hepatitis B and C viruses, respectively (25). There is a good argument (19) to be made that viruses should not be treated differently; enveloped viruses are much more sensitive to disinfectants than nonenveloped viruses. Many specific virucidal claims create confusion for users, and often only enveloped viruses are tested, which, for the poorly informed user, can lead to a false sense of security and potential risk. Handling certain types of enveloped or nonenveloped viruses in disinfectant testing facilities can also be potentially dangerous. We consider a rational approach to virucidal claims with appropriately selected surrogate(s) to be an essential part of reforming disinfectant testing (14, 19).

The sensitivity or resistance of many microorganisms to the disinfectant can be significantly affected by the prior growth history (26). Growth and/or survival under low nutrient or other stress conditions can lead to significantly more microbial resistance to disinfection. Prior exposure to sublethal levels of the same or another disinfectant may also increase resistance. Altered phenotypes with different sensitivities to applied microbicides can be the result of differential gene expression triggering intrinsic microbial defense systems, or may be from selection of mutants with altered resistance. These phenomena are well recognized for sensitivity to oxidant disinfectants but less well defined for some other disinfectant classes.

Currently, most microorganisms used for disinfectant testing are grown under relatively ideal conditions in nutrient-rich liquid or semi-solid media. However, many of the real target organisms in the field are the stress survivors that may have been grown under nutrient-poor conditions, or in biofilms, or have become dried onto surfaces subsequent to

Table 2. Summary of factors affecting microbicidal activity of chemicals

Disinfectant formulation	There is a minimum concentration for potency against particular pathogens in suspension; when contaminants are dried onto a surface, the concentration required is invariably higher. For further assurance of field effectiveness, a safety margin is necessary to overcome disinfectant demand of surfaces, surface soiling, and applicators. Even when nominal concentrations of active ingredients are the same in 2 products marketed for the same use, differences in inert components can affect their microbicidal effectiveness. Compatibility with precleaning solutions is also desirable.
Target organism(s)	Pathogens have varying degrees of resistance to disinfectants; the order of resistance is generally considered to be: bacterial spores > nonenveloped viruses = mycobacteria > fungi> enveloped viruses = vegetative bacteria, although there is definite overlap between classes, depending on organism and product. Pathogens should be represented by appropriately chosen surrogates for testing of label claims. *Note*: Under field use, the target microorganisms are often not known. Also, they may be attached to or imbedded in body fluids, tissues, or other particulates.
Temperature	Disinfection efficacy generally increases with air and liquid temperature; therefore, it is important to observe minimum temperatures on label instructions.
Product diluent (if needed) and use of hard water in testing	Disinfection efficacy can be affected by the nature of the diluent. If no diluent is specified, distilled water is expected to be used. However, this is usually impractical in a clinical setting, and so the default diluent should be tap water with distilled water specified if needed. Water hardness can diminish product efficacy; the EPA's Scientific Advisory Panel has recommended using water with a standard level of hardness of 400 ppm in testing formulations that require dilution before use.
Contact time	As a consequence of the design of current commonly used official methods, the contact time listed on many products labels is frequently too long for its expected use in the field. Therefore, unless the product is applied for at least the time specified on the product label, it may not work under field conditions.
Soil load	Inorganic or organic soil remaining on surfaces, even on precleaned ones, can (*1*) partially neutralize applied disinfectants by interaction, and (*2*) help to shield microbial contaminants from disinfectant contact. Such soiling can include biofilms on wet/damp surfaces that are not always readily removed by cleaning.
Carrier surface	Disinfectants must work on many kinds and ages of surfaces in the field. Surface irregularities and imperfection caused by wear are unavoidable. Even new surfaces that appear visually flat to the naked eye contain many irregularities at a microscopic level that can shield microorganisms from proper disinfectant contact; formulations often contain wetting agents to facilitate such contact. Test carriers should simulate the surfaces to be disinfected. One of the major considerations in the formulation and use of disinfectants is their compatibility with the wide variety of materials they may contact in the field. QCT-2 can use carriers of a variety of materials to assess such incompatibilities.
Precleaning	Many disinfectants are meant for use on precleaned surfaces and objects. However, it is necessary to ensure that such cleaners and disinfectants are compatible. For example, this can be a special problem for quaternary ammonium compounds that are readily neutralized by anionic detergents.
Method of microbicide application	Whether a disinfectant is applied by immersion, flooding, brushing, or wiping, a potentially different amount of chemical is delivered to the target. Moreover, the nature and cleanliness of any applicator is important so as not to neutralize the applied disinfectant.
Microbicide application rate	The amount of disinfectant applied to a contaminated surface is important, especially when soiling is present; the amount to be applied per unit area is not specified by manufacturer.
Storage and shelf life	The conditions of storage can influence the shelf life of a disinfectant. Shelf life of undiluted product is part of label requirements, but, once diluted, product aging can occur at a very different rate, and this can affect disinfection outcomes. Aging of diluted product can also be influenced by the cleanliness of the reservoir used.
pH	Disinfectants are usually designed to work optimally at a specified pH; for some products, the pH of storage and stability differs from that where the most microbicidal activity occurs. This may require an activation step.
Relative humidity	Relative humidity indoors affects not only the level of moisture on the contaminated object and, therefore, the penetration kinetics of the disinfectant, but also the rate with which the applied product evaporates.

a contamination event. Most emphasis has been on the use of standardization of growth media as a means to eliminate observed variability in the test. While standardized growth media are clearly desirable, the media used should, as far as possible, yield microorganisms that are representative of those found in the field. Thus, although it is neither possible nor desirable to mimic all stress conditions in a laboratory test, use of standardized media with lower nutrient levels can help to address this issue. This has been partially incorporated into our test design with *Pseudomonas aeruginosa* and *Bacillus subtilis*, but further work is needed to define suitable growth media for some of the other test microorganisms. Some species may grow only poorly under low nutrient conditions and may not tolerate much reduction in the level of nutrients in the media. Such an approach may also minimize the impact of perceived differences in the disinfectant susceptibility of microorganisms grown on semi-solid media as opposed to those from broth cultures.

We believe this is an important issue, but the intent is not to achieve the most resistant organism possible; rather, the objective is to give a realistic evaluation. In general, QCT-1 and QCT-2 were developed for use with inocula deposited on the surface of the carrier and dried, but they could be adapted for wet or dried biofilms. Although it is recognized that dried microbial inocula and biofilms are each more resistant than the same organisms in suspension, no adequate comparison between biofilms in their moist or dried state and dried inocula has been performed.

Problems have also been identified with the surrogate organisms used in the AOAC tests. *Mycobacterium bovis,* used for confirmatory testing against *M. tuberculosis,* is relatively slow-growing and tends to form clumps that are difficult to dissociate. In addition to *M. tuberculosis,* many other mycobacteria are being incriminated in human disease, including those acquired from improperly decontaminated items, thus emphasizing the need for label claims for mycobactericidal (i.e., kills all mycobacteria) rather than just tuberculocidal activity. Our own testing (27), and that of others (28, 29), using a variety of mycobacteria, suggests *M. terrae* as a replacement for *M. bovis. M. terrae* is also recognized as a surrogate for mycobacteria in the ISO standard 14937 on the general criteria for sterilization, and in the draft standard from CEN TC216–*Quantitative Carrier Test for Evaluation of Mycobactericidal Activity of Chemical Disinfectants for Instruments Used in Medical Area*. However, all mycobacteria are highly hydrophobic and tend to clump together to different degrees under the influence of a variety of stimuli. Requirement for mycobactericidal testing only against monodispersed mycobacteria would be unreasonable, but such clumping should not be so severe that it skews the results of the test(s). The disinfectant resistance of *M. smegmatis*, a fast-grower that is used as a screen in the AOAC tuberculocidal test, is generally lower than that of slower-growing mycobacteria. Even as a screen, we do not believe that *M. smegmatis* is sufficiently predictive of a given formulation's potential as mycobactericide to warrant its inclusion.

In spite of the demonstrated suitability of *M. terrae* in most cases, it may be possible, as with any other surrogate, to find one or more products that will work better against the selected surrogate than against a related organism with more serious clinical implications. Similarly, it will always be possible to find or develop a tougher microorganism. This should not be used as an argument against using surrogates, but for using a rational approach to disinfectant evaluation where the test method is sufficiently stringent that minor differences among related microorganisms may be less important than other factors that determine test outcome. A well-designed test needs to encompass an overall microbial challenge that is suited to the degree of risk from disinfection failure, and to combine that with performance criteria to ensure that real risk(s) to the user/consumer are reduced to a minimum based on currently available knowledge (*see* discussion below on performance criteria).

The AOAC test for sporicidal activity uses both *B. subtilis* and *Clostridium sporogenes* as surrogates. Originally we were proposing to drop *C. sporogenes* as a test organism from the sporicide test because our data showed that it is almost always more sensitive to chemical disinfectants than *B. subtilis*. An experimental formula that inactivated *B. subtilis* more easily than *C. sporogenes* caused us to reconsider in view of the criticality of high-level disinfection.

At the present time, claims for fungicidal activity based on the AOAC test require testing against *Trichophyton mentagrophytes* only, also using a suspension test. We have proposed retaining this filamentous fungus, while adding a 'yeast-type' fungus. *Candida albicans* is an obvious choice based on its importance as a nosocomial pathogen, and we have some preliminary data in this regard (30).

In the AOAC test for bactericides, 3 surrogates are used: *P. aeruginosa, Staphylococcus aureus*, and *Salmonella choleraesuis.* While recognizing the importance of the first 2 of these surrogates, we also recommend that *S. choleraesuis* be dropped as a test organism, because data obtained suggest that its sensitivity to disinfectants is higher than that of either *P. aeruginosa* or *S. aureus*. We have not proposed any replacement bacterial species at this time, but this option should be reconsidered as more knowledge becomes available. However, *Salmonella* spp. are prominent foodborne pathogens, as are certain *Escherichia coli* strains. Enterococci are also important nosocomial pathogens that may be considered.

For a number of reasons, we believe it is desirable to limit the number of microorganisms that can be listed on a product label to those required by regulation. Long lists of organisms on product labels are confusing, making labels difficult to read because of the small typeface needed for all the information, and therefore the label is less likely to be read. This may also create the erroneous impression that such products are better than those with shorter lists of tested organisms. Moreover, the additional test organisms may not have been examined in similar test procedures as no standard methods exist for them. Also, different concentrations or contact times further

confuses the user, who may not know the target organisms present.

Selection of microorganisms for regulatory testing has been determined predominantly by the need for disinfection in clinical settings. Such organisms may be irrelevant for environmental control in many industrial settings. Therefore, it may be desirable to allow substitutions by organisms that are recognized to be particular problems in the targeted industrial market. For example, particular bacteria, viruses, or fungi may be a problem in specific industries where appropriate substrates or hosts are used in manufacturing processes. In other cases, such as the clean rooms of pharmaceutical manufacturers, the environmental and/or bioburden organisms against which products are required to be effective may be mainly oligophilic, or site-specific (31).

No test for virucides currently exists within AOAC, and current U.S. legislation requires testing against each virus for which an efficacy claim is made. With few exceptions for phages of industrially important microorganisms, viruses are only of concern in settings where diseases of humans and animals can be transmitted readily. However, we consider it important to make appropriate selections of surrogates for testing virucides, and considerations for their selection have been presented (19).

When the proposal for test development was originally made to the EPA, one of the suggestions made about spores was that the test microorganisms should be purchased from a single source rather than grown in the individual laboratories in order to reduce one potentially major source of interlaboratory variation. During a collaborative study for QCT-1, all spores were supplied from a single batch grown and titrated in our laboratory. Differences of microorganism among locations and over time, with slight differences in growth conditions, continue to be an issue that has been well recognized, for example, in applying the phenol coefficient as a measure of consistency in microbial challenge. Some changes in microorganisms over time may be inevitable, but in a facility that is set up to provide the conditions least likely to result in changes, and which can routinely perform all the necessary quality controls, this should be kept to a minimum, with all recipients using similar and relatively well defined challenge organisms. Originally, this concept was to apply only to spores and perhaps to mycobacteria, but with the ready availability of overnight courier services, all test microorganisms could come from one national central supply house. It would clearly be better not to have test microorganisms transported across international boundaries because of import-export restrictions and potential delays in processing at the border(s). If any subsequent passaging is needed in the testing laboratory, it could then be limited to a single passage.

Temperature

Temperature is a recognized factor affecting the rate of microbicide action, and there is a direct relationship between temperature and speed of microbicidal action. Current hard surface carrier tests, including QCT-1 and QCT-2, specify the temperature of 20°C, which is perfectly adequate for products to be used at room temperature. However, there are a number of uses for which the disinfectant product must be able to cope with lower temperatures. If a product is targeting one or more such uses, there should be an option for the product to make a label claim if it can pass the test conducted at a lower, specified temperature. No specific recommendation is made here, but the point is drawn to the reader's attention. In fact, this may be an extremely important factor in food processing establishments, on farms, and in major trauma situations in cold climates.

Product Diluent

In clinical settings, distilled water is not always readily available. At the present time, the assumption in the AOAC series of tests is that, if no diluent is specified on the product label, then distilled water should be used as the diluent. This assumption is largely unrecognized by health care and other users and, as a result, the products are almost always diluted in tap water. The assumption should be reversed, and tap water should be considered to be the norm. Water with varying degrees of hardness is a fact of life in many hospitals and elsewhere; therefore, disinfectants requiring dilution in water by the user should be tested for their microbicidal activity using hard water as a diluent. We have included this requirement as a part of the QCT protocols. EPA's Scientific Advisory Panel on Germicide Test Methodology has recommended the use of 400 ppm $CaCO_3$ as the level of water hardness (32). If a product cannot meet the test requirements at 400 ppm, for example, but can do so at 100 or 200 ppm, then such a claim should be allowed providing it is clearly stated on the product label. Similarly, if distilled water, or another diluent, has been used to generate the test data for registration, this should be clearly stated on the product label. However, the default should be water with 400 ppm hardness.

Contact Time

The contact time is included in the test procedure as a manufacturer-specified parameter. In large part, contact time is a matter of practicality, but is also partially controlled by market forces. However, contact times and conditions of use specified on the product label may not be compatible with routine practices in infection control. This needs to be examined carefully because flooding of surfaces, for example, is only realistic under certain conditions. It is impossible to flood vertical surfaces or important high-contact items such as door handles and telephones, and it is also impractical in many situations even to flood horizontal surfaces and then wait 10 min before proceeding.

Environmental disinfection is most frequently practiced by wiping for a very brief period with moistened absorbent applicators. These may hold relatively little disinfectant per unit area unless they are dripping wet. We have measured liquid volumes in hand-squeezed but damp hospital cloths of approximately 5–15 $\mu L/cm^2$, dependent on type of applicator and how much of the absorbed water is expelled during squeezing; only a small fraction of this volume

(approximately 0.2–2.0 μL/cm^2) may be transferred to the surface during wiping and thus be available for direct contact with microorganisms on the surface (unpublished data; manuscript in preparation). Because each contacted surface with its associated soil load will have its own demand on the applied product, the disinfectant obviously experiences anything but ideal conditions in the field. Depending on the specified use and means of application of the disinfectant, should a maximum contact period be specified on the label? This is certainly a matter of debate and should be considered along with other recommendations made below. In its 1991 version of the standard for disinfectants to be used on environmental surfaces, the Canadian General Standards Board's standard (33) addressed this matter by stating that the contact time for environmental surface disinfectants "should not be longer than 10 min." Much shorter test contact times are desirable and, given the practicalities of disinfection, obviously reduce the risk more rapidly.

Soil Load

The soil load (a mixture of organic and inorganic materials as a surrogate for residual body substances) for disinfectants is a difficult issue because its level will vary greatly in the environment where the disinfectant is required to perform. Where published methods have deliberately added additional material to simulate residual soiling, this has been traditionally 5–10% serum. In the field it is quite possible that, although surface soiling is inapparent, there is actually a much greater mass of soil than microorganisms. Such soil plays 2 potential roles in protecting microorganisms from disinfectants. Because disinfectants are relatively nonspecific in their interactions, they can readily interact with many surface soils. This can significantly reduce the concentration of active ingredient(s) available for disinfection. They can also hinder the penetration of the disinfectant to microorganisms that may be physically protected by organic or inorganic debris.

Currently, the AOAC tests do not require a specific soil load, but review of the method of preparing the inoculum suggests that a certain unspecified level of nutrients from the growth medium may be present. However, this unspecified soil load is dependent on the growth medium used to a significant extent and may provide an unequal challenge between different microorganisms that does not reflect their intrinsic resistance to the applied product(s). For example, we have noted above that *C. sporogenes* spores are almost always more sensitive to microbicides than are *B. subtilis* spores when both are prepared in a similar fashion. In the media used to grow these 2 organisms in the AOAC test, the reverse is often observed, which may reflect the physical and chemical shielding afforded to the clostridial spores in residual particles from the cooked meat medium.

The approach we have taken is to use a soil load approximately equivalent to 5–10% serum. Because the uniformity of bovine serum cannot be guaranteed, and serum components can inhibit some microorganisms, the composition of the soil load used in QCT is designed to provide an approximately equivalent protein load with low and high molecular weight materials as well as mucoprotein. Full details of the soil load composition are given in the complete methods in the experimental manual (14). A more stringent challenge would be untenable for routine disinfection procedures but, at the same time, if manufacturers wish to have claims for use of their products directly on spills, an alternate and realistic soil load could be used (e.g., whole blood, fecal matter). However, it would be important in such cases to demonstrate proper recovery of the test organism(s) from the soil matrix in "no treatment" controls. The issue of soil load may be one of the more contentious issues for international negotiation, because several countries have national soil loads and no adequate comparisons among these have been made.

It is sometimes argued that a soil load is not needed in a disinfectant test because the label of a given product specifies its use on precleaned surfaces or objects. However, it is well recognized that routine precleaning often only reduces the soil load and does not eliminate it completely. It may just spread it around to a wider area. Therefore, the addition of a reasonable and standardized level of soil in disinfectant test protocols is also meant to ensure that products will not fail under field conditions. By the same token, the use of certain soils, such as clotted blood, may be difficult to justify when precleaning is to be applied before disinfection.

The presence of biofilms can add an additional level of complexity and stringency to disinfection (34). Although not specifically examined during development of our test methods, biofilms, grown on coupons/carriers similar to those used here, could be incorporated to address this issue within the same basic protocol. AOAC currently has no test that applies to disinfection of biofilms, though one (34) may be under consideration by the AOAC Task Force on disinfectant test methodology (35).

The Carrier

The nature of the surface, both material of composition and microtopography of the surface(s), can significantly impact the efficiency of disinfection. Microbicides tend to contain very reactive chemicals as active ingredients. Such chemicals are relatively nonspecific in their interactions, and most disinfectants interact directly with most surfaces that they contact. This surface demand will reduce disinfectant efficacy, and the microtopography and nature of the surface will influence the degree of surface wetting by the agent and the protection which microbial contaminants can receive.

The surface selected for QCT-1 (specifically the internal bottom surface of the glass test vial) is relatively smooth and inert, and thus is among the easiest for disinfectants to act on. QCT-2 uses disks from brushed stainless steel of the type normally used for manufacturing countertops and sinks. Its many surface striations can shelter portions of the microbial load and present the disinfectant with a stronger challenge and greater need for penetration of soil materials (14). An additional advantage of the QCT-2 carrier design is that disks of a similar size can be made out of virtually any material,

including porous ones. Thus, although a specific type of stainless steel would be the standard or reference surface, manufacturers should be free to examine, and the EPA or the U.S. Food and Drug Administration (FDA) to require, additional test surfaces that may be relevant to the intended use of the product. For example, vegetable disks would be appropriate to evaluate vegetable washes, or rubber disks to evaluate disinfection of rubber surfaces. However, for tests using alternate surfaces, validation of microbial recovery would be required for mock-treated controls. Unfortunately, without a physical label intrinsic to the test microorganism, kill and failure to recover will always be confounded. Particular care needs to be taken in this regard for irregular or porous surfaces.

A variety of carrier surfaces are used in the current AOAC tests, including stainless steel and glass penicylinders as well as the more stringent carriers (porous porcelain penicylinders and silk suture loops) used for examining products for their sporicidal activity. In spite of their stringency, we consider that porous penicylinders and silk suture loops are inherently unsuited to qualify liquid chemicals as sporicides because they impose unrealistic conditions for disinfectant evaluation. Nowadays, any wholly porous product used as a medical device and requiring treatment to inactivate spores remaining on or within its structure would almost certainly use gaseous products such as ethylene oxide. The chief exceptions to this would be rubbers or synthetic polymers that may be incorporated into complex medical devices as gaskets, o-rings, and tubing. Thus, we do not oppose the inclusion of porous surfaces per se, but we believe that such surfaces need to be realistic to the intended product use, and they can certainly be used as additional carriers in QCT-2.

The porous porcelain penicylinders used in the AOAC tests are inoculated by immersion in the test organism suspension and acquire spores on the surface and many that are held within the pores of the porcelain. When the inoculated porcelain penicylinders are subsequently placed in the test disinfectant solution, some of these spores may be protected from contact with the disinfectant by small air bubbles. This presents an unfair challenge to the product under test, and a real opportunity for variability within a test where the result is simply growth or no growth. This is supported by a study demonstrating the subjectivity of the results of the AOAC sporicide test if the carriers were not previously selected based on prior treatment (6). Suture material is hardly ever, if at all, subjected to decontamination by soaking in liquid chemicals. Silk sutures, as protein, react quite dramatically with oxidizing chemicals and can break down during the testing. Further, the silk suture material requires considerable preparation to remove waxes and other coatings from them, and this is not likely to be consistently applied among laboratories. The knots that are generated in the making of the loops can hide the target spores and prevent access of the disinfectant to them; they also tend to float in some test disinfectants, giving uneven exposure of the carrier surface. When a disinfectant cannot get to the target, it simply cannot be expected to inactivate it. Recently, it has been suggested that Dacron be permitted as a replacement for silk for preparing the loops (36). We believe that such a change may be a good temporary measure, but would not address the problem of the inappropriateness of the carrier design itself.

The size or surface area of the carriers may be considered as a factor of only minor importance to a carrier test design. However, in developing the QCT tests, we wanted to provide a test protocol that can readily be used to examine all microbial classes by the same methods and principles. Work with viruses has unique considerations in the volumes of inocula and eluants that are practical, and this suggested maintaining a small carrier size to permit the same test to be used for all classes of microorganisms. Further, maintaining a small carrier size in QCT-2 made it possible to place entire inoculated carriers inside vials for elution of control and test microorganisms. Thus, QCT-2 is also a completely closed test system avoiding wash-off of the challenge microorganisms.

The concentration of the test organism per unit surface area of the carrier has a significant influence on the level of stringency of the test. The size and shape of penicylinders used in many AOAC tests make it necessary to dip them in the microbial test suspension and, for the nonporous cylinders, this results in spreading the inoculum relatively thinly over the entire surface of the carrier. Even when these inocula are enumerated, considerable variation in numbers can occur among penicylinders, depending on the microbial suspension. In contrast, the test microbial suspension (10 μL) in both QCT-1 and QCT-2 is deposited at the center of the horizontal carrier and allowed to dry at that spot, thus creating a more realistic challenge similar to the uneven contamination to be encountered in the field. Moreover, direct deposition with a calibrated positive displacement micropipet allows relatively little carrier-to-carrier variation, and this can be further minimized by inoculating an entire batch of carriers with a single pipet tip.

Precleaning

Precleaning can enhance the effectiveness of the subsequent decontamination step, and is usually practiced in more critical disinfection events. However, in routine use of microbicides, precleaning is often missed, partly from ignorance, partly from time and personnel pressures, and partly because certain products claim to be one-step cleaner-disinfectants. Many users are also unaware that residues of a cleaner can interfere with the activity of the subsequently applied disinfectant, e.g., between anionic detergents and quaternary ammonium-based products. Some disinfectants are required to provide label notification of precleaning requirements. The wording of such notifications needs to be carefully adjusted to take account of potential incompatibilities. Manufacturers may also be required to suggest one or more precleaning solutions with which their products are compatible.

Method of Microbicide Application

Interaction of microbicides and the surfaces upon which they are to act, or residues of precleaners on those surfaces,

have already been mentioned above. However, such compatibility must be mentioned again in association with how a given disinfectant is applied to the contaminated surface or object. Microbicides are often applied by wiping, and porous applicators tend to have very large surface areas that can trap and hold relatively large amounts of soil on the applicator surface(s). Such soil will interact with the applied chemicals and act to partially, or in some cases completely, neutralize microbicide activity. Thus, both the nature and the cleanliness of the wipers/applicators are extremely crucial in determining the final level of active chemicals that reach the contaminated surface and target microorganisms. There may also be differences in the interaction between the disinfectant and various types of applicator material, even when soiling is absent. Labeling requirements related to product application are relatively minimal in North America, but because of the importance of the application method to the outcome when the product is used according to its manufacturer's instructions, it is suggested that this matter be revisited by the regulatory authorities to ensure that sufficient active ingredients actually reach the contaminated surface. A diligent manufacturer should be able to recommend an applicator or means of application that does not neutralize the product on contact. This issue may be one where education rather than regulation can achieve significant change. It is particularly important that users are educated on the importance of using clean applicators.

Rate of Microbicide Application on the Surface to Be Treated

The dose of active ingredients (concentration × contact time) is the most usually recognized measure of product efficacy. As stated earlier, where the microbicide is present in large excess, and the demand of the surface and its soiling is overcome, this is an acceptable approach. However, for disinfectants that are applied in small volumes and/or by wiping, the demand on the disinfectant is directly proportional to the surface area contacted. The concentration of active chemical(s) is decreased for each unit area in proportion to the relative chemical demand of the surface, and one has a continuously variable disinfectant concentration. Thus, the rate of application of a disinfectant can greatly affect its efficacy because it determines how much disinfectant chemical actually contacts and interacts with the contaminating microorganism(s), and for surface disinfection, the dose should be considered in terms of the (concentration × volume × time)/area.

Although product concentration is the focus in current microbicide testing, the important parameter of the volume used at that concentration has not been adequately addressed in relation to actual practices in the field. In the AOAC tests, and in many other published methods, the volume of disinfectant used is an arbitrary defined volume that is often relatively large in relation to the unit surface area being decontaminated. It is usually much greater than could be applied in most field situations. In the field, the quantity of disinfectant that can be placed into contact with the contaminated target often may be very small depending on what is being decontaminated and constraints that may be the result of surface orientation, design (e.g., narrow orifices/lumens), and means of application.

It is impossible to design one test that can adequately represent an infinite range of field contact conditions, but this issue has been addressed to some degree in the design of our test protocols. In QCT-1, the conditions are relatively ideal for the disinfectant, and the contaminated carrier surface is in contact with a relatively large volume of the test formulation (1 mL). In QCT-2, the volume of the test formulation (50 μL) is greatly reduced in relation to the same contaminated area on the carrier disk. This volume was selected as being appropriate for the narrow channels of endoscopes. However, based on our data discussed above, even this volume is much larger than can generally be applied by wiping. It would be ideal to have the manufacturer recommend an application rate/dose per surface area, but in order to do so certain assumptions would have to be made about the surface type, applicator type, and the cleanliness and compatibility of both of these with the product. Because such issues are beyond the control of the manufacturer, it would be impossible to regulate. Nevertheless, some understanding of this, and recommendations for typical application rates from the manufacturer, should be a matter for education of both users and producers.

Storage and Shelf Life of Disinfectant

The length and conditions of storage of a given product can affect its microbicidal potential. These factors are handled partly by labeling and partly by requiring testing of formulations that have been subjected to accelerated aging. Such requirements are independent of the method used to assess microbicidal activity. If, for safety or other reasons, disinfectants are stored at temperatures below that at which they are to be used, it is important that they are equilibrated to ambient temperature before use. Statements to that effect should be placed in manufacturer's instructions for use if storage of product at cold temperatures is recommended.

pH

Many disinfectants have an optimum pH for efficacy, which is not always the same pH at which they are stored and are more stable. Some need activation immediately prior to use to adjust the working pH to an appropriate level. This factor is taken into account in the product formulation and instructions for use and should be verified during product testing.

Relative Humidity (RH)

The effect of RH on disinfectant action is indirect and only of relevance for drying or dried contaminants. RH itself can have a beneficial or detrimental effect on microbial survival, depending on the microorganism and the ambient RH. In some cases, therefore, a detrimental RH can augment the action of the disinfectant. Also, if the contamination is already dried onto the surface, a relatively high RH that helps to

rehydrate the surface contamination can facilitate penetration of the disinfectant.

RH is also an important factor when the volume of product applied is low or the product is applied by wiping. Low RH will enhance surface evaporation rates. Although this may temporarily increase product concentration directly at the surface, for some products it will also enhance evaporation of the active ingredients. If the surface(s) becomes dry too rapidly, then the product will fail to contact all parts of the surface and inactivate contaminating microorganisms.

Classification of Products and Performance Criteria

If one looks at the role disinfectants play in the field, then currently used classifications spring to mind. That based on Spaulding's scheme of medical devices (37) has some relevance, but really only separates out the high level disinfectants required for semicritical devices from those for all other uses. Because such devices now are under the jurisdiction of the FDA, the relevance of this scheme to the EPA is questionable. Alternatively, one can consider the 3-level (high, intermediate, and low) categories that are often used to describe different types of disinfectant (37). These classifications are based upon the range of types of challenge microorganisms that can be inactivated by a given disinfectant. Unfortunately, sensitivities of categories of microorganisms overlap considerably with one another and can vary depending on a wide range of factors. Moreover, no levels of kill or soil challenges are specified for these categories. Using a single method to evaluate microbicide action against a wide range of microorganisms is expected to enhance our knowledge of their relative sensitivities to particular products or product classes.

Other schemes of microbicide comparison could be implemented, but would require a fundamental shift in thinking from all stakeholders. For example, one could imagine the use of indexes where the efficacy of a single product against each test organism under highly standardized conditions is arbitrarily given a value of unity. The results of all subsequent tests are then compared to this standard and given a fractional or multiple rating. We are not recommending this approach because, not only could it get highly complicated, but one could also envisage considerable disputes about such ratings and differences in ratings depending on where the tests were performed. It has recently been suggested (38) that disinfectants should be evaluated by pathogen class using a range of surrogates not too different from those currently used. Although this scheme does not touch on many of the topics covered here, it deserves further discussion, and proper comparison among products would clearly be favored by using a single test.

The microbial challenge encountered in the field is independent of any required performance and cannot be anticipated accurately. However, an approach to performance criteria selection for microbicides should take into account both the types and numbers of microorganisms using a specified set of challenge conditions. It is equally if not more important to consider also the potential for reduction in risk that would be achieved by successful product use. The challenge conditions need to be relevant to field conditions and the intended product use. Any failure of a disinfection procedure carries a finite but undefined risk that relates to the infectious hazard and other factors. The testing scheme and performance criteria selected can be designed to reflect the relative risks from microbicide failure. However, to ensure maximum benefit from use, and for the confidence of the user, when a label claim is made for a product, the degree of confidence that a product can meet the required criterion(a), and perform according to its label claims, should be the same for all classes of the microbicide. In other words, the label claims do not have to be the same for all classes of product, but each should be clearly able to be met in pre- and post-market evaluation. This has not always been the case, because it has often been assumed that risks from failure of low-level disinfectants are inconsequential, whereas, in fact, they are unknown. This is not just an issue for health care; economic losses from disinfectant failure can also be devastating for individuals, manufacturers, and society as a whole. At the same time, the testing needs to be fair to the manufacturer and provide a level playing field with little risk to the disinfectant manufacturer that the testing will falsely grade the tested formulation as ineffective.

If one assumes that disinfectant performance and classification are tied to the types of microbial challenge as currently accepted, there are 3 possible options for how disinfectant quantitative performance criteria could be established: (*1*) The same specified level of challenge and required reduction is used for each test organism regardless of the class of disinfectant into which the product falls. (*2*) The same specified level of challenge is used but with a varying reduction required depending on the classification and intended use of the disinfectant. (*3*) Varying levels of challenge and reduction are required, depending on the classification and intended use of the disinfectant.

The first option, if applied to all testing tiers, would require the same level of performance for all disinfectants in the field regardless of the level of risk from the targets they are meant to disinfect. This is not a reasonable or a feasible proposition because it would either eliminate many disinfectants from the marketplace or would force higher concentrations and increase both costs and risk to users from exposure to high levels of chemicals. At the same time, there has come to be an acceptance for a level of $\log_{10}$ reduction for challenge microorganisms. Generally this is in the 5–6 $\log_{10}$ range, although for viruses, practicalities may limit this to no higher than 4 $\log_{10}$. We are therefore proposing that, for a screening test such as QCT-1, which strictly measures the potency of the product under ideal conditions, a standard level of $\log_{10}$ reduction be required with a standardized challenge. When conditions more representative of those in the field are used, such as those in QCT-2, where the volume of applied disinfectant may be quite limited, this option would not be suitable.

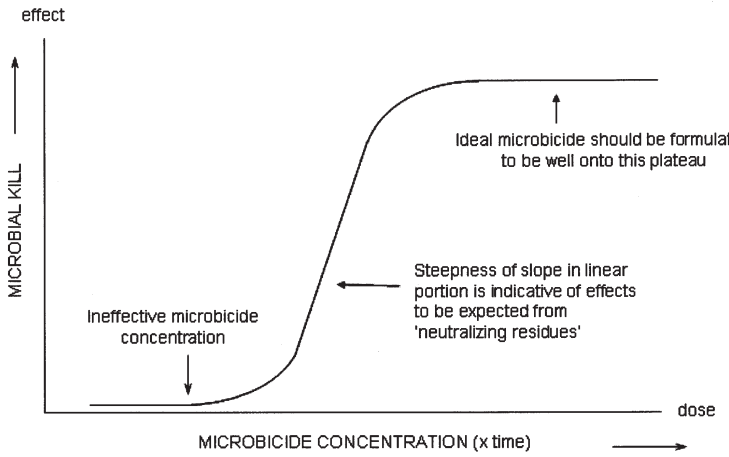

Figure 1. Generalized curve for effects of exposure of a microorganism to a microbicide for any defined contact period.

The second option suggests varying the $\log_{10}$ reduction required from a constant challenge. Our results in examining QCT-1 and QCT-2 with different types of organisms suggest that this approach would be extremely problematic. The following reasons are presented. In developing the QCT tests, we had considered using a kinetic (kill as a function of contact time) approach to evaluating disinfection efficacy. However, we rejected this because (*1*) initial product demand from the carrier and inoculum would reduce effective product concentration and that would then be unknown for the remainder of the contact time; (*2*) products for surface disinfection carry a fixed contact time as a part of their label requirements and, as discussed earlier, this is dictated partly by practicalities in the field and partly by market forces. We therefore preferred to use the manufacturer-specified contact time and examine microbicide activity as a function of concentration of active ingredients in the initial application. This permits an understanding of how efficacy measurement changes in response to the effective concentration of product. We consider this information to be much more relevant to field conditions; the shape of this curve (Figure 1) permits an understanding of how well formulated the products are, and suggests how they may respond to the demands such as soiling that are likely to reduce their concentration and efficacy. Thus, our test methods are fixed-time tests, with the time as specified by the manufacturer. Because the time and surface area (carrier) are constant, concentration becomes the controlling parameter. It is obviously possible and desirable to consider both time and concentration in test design if an elaborate matrix is used. However, we consider that the current uncertainties in disinfectant testing can best be resolved, at least initially, by focusing on varying concentrations.

When QCT-1 was subjected to a collaborative study (15 laboratories, with participants approximately equally divided among disinfectant manufacturers, commercial testing laboratories, government laboratories, and independent or academic laboratories) using *Bacillus* spores as the test organism, 2 important points were revealed. Analysis of the data determined that only a small fraction of the observed variability was attributable to the test directly, and the majority of the variability was among the test sites (39). Secondly, it was clear that the variability among both controls and products that performed well was low, whereas for marginal products or those diluted below their recommended use concentration, it was much higher. This led us to re-examine the theoretical basis for the results we observed in the QCT-1 collaborative, and we now believe that similar issues could have contributed to the variability seen frequently among different laboratories testing the same product by the AOAC methods.

The concept is this: If you examine microbial kill as a function of disinfectant concentration at a fixed (manufacturer-specified) contact time, the response is a sigmoidal curve (Figure 1). At low concentrations of disinfectant, little if any microbial kill occurs; as disinfectant concentration is increased while holding contact time and other experimental conditions constant, a level of kill is gradually increased until inactivation becomes linear as a function of concentration. This remains so over a range of concentrations until full kill of the challenge organisms is approached within the specified contact time. At that point, the curve naturally plateaus because most of the challenge microorganisms are already killed. This plateau is reached at the concentration of disinfectant/product formulation(s) that are optimal for kill under the test conditions. However, in order to allow for the natural attenuation of product concentration when it is used in the field, the use concentration should be well onto the plateau of the kill versus concentration curve. This approach was proposed to the EPA for examination of QCT-2, and the data gathered with a mycobacterium (*M. terrae*; 40) and a nonenveloped virus (a human rotavirus) with a number of disinfectant products fully agree with this model (41).

Thus, it is reasonable to expect that the concentration versus kill curve could be used to assess the potential for field

Table 2. Suggested performance criteria for different classes of disinfectant

QCT tier	Disinfectant class	Microbial challenge level[a]	Performance requirement[b]	Permitted survivors[c]
1	High	$\geq 1 \times 10^7$	1×10^6	10
1	Intermediate	$\geq 1 \times 10^7$	1×10^6	10
1	Low	$\geq 1 \times 10^7$	1×10^6	10
2	High	$\geq 1 \times 10^7$	1×10^6	10
2	Intermediate	$\geq 1 \times 10^6$	1×10^5	10
2	Low	$\geq 1 \times 10^5$	1×10^4	10

[a] Colony-forming units (CFU) or plaque-forming units (PFU). Some modifications may be required to use more practical levels of fungi, vertebrate viruses, and protozoa; statistical requirements for minimum challenge level may vary once degree of variation is known.

[b] Measured as $\log_{10}$ reduction in CFU or PFU.

[c] Based on an inoculum of 10^7.

performance. The shape of the curve obtained, or the difference between the exposure concentration that fails totally (approximately 100% of inoculum survives) in the specified contact time and the one that always succeeds (few or no viable organisms are detected), depends very much on the disinfectant formulation. This translates into a very useful tool that could be used by manufacturers and regulators. If this range of disinfectant concentration is very narrow and the slope of the graph's linear portion is steep, then any formulating or diluting errors resulting in too little disinfectant are at high risk of failure. On the other hand, if the slope of the curve is gradual and much higher concentrations are required to achieve full kill, it may be less likely that formulation/dilution errors would have such drastic effects, although performance would obviously be somewhat impaired at reduced concentrations. These same differences in the shape of curve for this plot of kill against concentration also suggest that any surface or soil interaction effects that tend to reduce the effective disinfectant concentration in the field would have a much greater influence when the curve is steep than when the curve is shallow. Because the data are not available, it is impossible to know whether this has been at least partially responsible for the differences that can occur between laboratories performing the same test on the same batch of the same product (42), but it is one possible explanation. It would, therefore, be of great interest to know whether the most variable test results also correlated with steep response curves obtained for kill against concentration. No specific recommendations are being made on this issue at the present time, but it should be studied as a part of any future collaborative trials.

The discussion above further emphasizes the need for disinfectants to be firmly on the plateau of this concentration response curve rather than on the rapidly changing linear portion or at the transition to the plateau where slight to moderate disinfectant demand from a variety of causes could cause performance to fall off the edge, particularly in a field situation. Competition within the disinfectant marketplace can be intense, and active ingredients for disinfectants costly. Consistent with the conclusions of the GAO report (13), our data (40) and even EPA post-market assessment (38) suggest that poor or marginal formulations may have been approved when examined in some of the current AOAC tests. In the case of the AOAC qualitative sporicide test, however, we consider that an occasional failure that may be due to a single surviving spore may unduly punish an otherwise reliable product.

How could this information be useful from a regulatory standpoint? It is suggested that valuable information about potential product performance in the field could be obtained by requiring either a complete response curve at the manufacturer-specified contact time, or by requiring that the test(s) be done at the use dilution and at defined fractional concentrations to ensure that product performance would not be dramatically impaired by, say, a reduction in concentration to half the levels specified on the label.

If option 2 were adopted to set performance criteria, however, it is clear that for only a partial kill of the inoculated microorganisms, most data would fall on the rapidly changing part of the curve. Because this part of the inactivation data is extremely vulnerable to small changes in conditions, the laboratory-to-laboratory variations observed in collaborative data for Tier 1 would be exaggerated and the data may be as variable as they are under the current testing regime. The controversy regarding the variability in testing of disinfectants would remain, and user confidence would not improve.

The third option seems much more attractive for setting performance criteria for examining microbicide activity under conditions relevant to the field. Here, the level of challenge would be selected based on the perceived level of risk from the types and extent of microbial contamination likely to be present and presenting a risk for the intended use of the product. Whether such selection should be based on surface location and exposed population (similar to Spaulding), categories of pathogen (39), or some other criterion should be a matter of debate.

In general, we have adopted a pragmatic approach to the tentative test criteria that are recommended in (Table 3). They are based on principles of fairness to manufacturers and users

and take into account the usually observed performance of disinfectants tested. Although nominally arbitrary, because clinical data are not available to support one particular level of performance over another, these recommendations take into account past practices as well as informed guesses with regard to currently marketed types of disinfectant products. They also use levels of risk reduction that we consider reasonable for the different classes of product. There is no intention to change the structure of products in the marketplace, or to cause disruption to manufacturers and users. However, it is impossible to make fundamental changes in the approach to product testing and guarantee that all existing products will be able to meet the new performance criteria; some may need reformulation.

The performance criteria suggested here have been restricted to categories similar to the normally used high, intermediate, and low levels of disinfectants in health care. These categories are defined by the classes of target microorganisms against which they would be expected to be effective (38). There are other uses of antimicrobial products in industry and agriculture that may require lower, or potentially even higher, criteria in relation to untreated controls. To make arbitrary recommendations without an in-depth review of the field would be unwise. However, decisions on a performance standard for each projected class of use should be carefully considered and based on both risk and performance of potential products. In the same vein, the design of the QCT described in more detail below was based on examining the efficacy of products for use in health care. Certain modifications may be appropriate for some other microbicide uses.

Summary of the Rationales behind the QCT

QCT are designed for a 2-tiered approach. QCT-1 uses an idealized system to evaluate the *potency* of the disinfectant, under manufacturer-specified conditions of concentration and contact time. QCT-2 uses the following additional levels of *stringency* to simulate field conditions that a disinfectant may encounter: required use of a soil load to mimic surface soiling with or without precleaning; use of a deliberately ridged surface to hide some microorganisms, hinder wetting, and mimic used and worn surfaces; and use of a more realistic and smaller quantity of disinfectant. The actual amount was derived from the approximate surface area-to-volume ratio in certain endoscope channels.

The tests are designed to be fully quantitative and support quantitative performance criteria. The tiered approach facilitates manufacturer evaluation of product performance in a logical stepwise progression rather than using a test that cannot separate out the different properties of a product. This is intended to promote better product development by understanding product performance during development and the most difficult hurdles for any particular formulation to overcome. Although it is recognized that both tiers may not be required for regulatory approval of disinfectants, it is recommended that this tiered approach to product development and testing be supported by the EPA as a means to promote understanding of product activity under different sets of conditions and to improve rational product formulation.

Furthermore, previous measures of microbicidal potency, including the AOAC tests, have required a different test for activity against each class of microorganisms, making it difficult to show relative efficacy of a microbicide for different classes of microorganisms. The tiered quantitative carrier tests developed use the same basic test for all classes of pathogens; test 2, in particular, can be used even with viruses and, potentially, with protozoa as well.

QCTs use levels of challenge microorganisms that, in each case, exceed the interim performance criteria proposed by approximately 1 order of magnitude, thus allowing for a small number of potential survivors and the performance of statistical risk calculations. The challenge for the producer is to perform the tests described to demonstrate that the disinfectants can perform reliably during his specified contact time. The performance criteria proposed in Table 3 could be suitable for high-, intermediate-, and low-level disinfectants, respectively, in QCT-2. In QCT-1, it is suggested that potency be demonstrated to the same level for all product classes. However, only limited data are available to justify these suggestions, and they are based as much on knowledge of performance expectations as opposed to hard data. Part of the problem in setting any performance criteria in the absence of reliable outcome data is that they become policy rather than scientific decisions. Nevertheless, such decisions need to be made if the proper quantitation of disinfection efficacy is to be achieved. There is no guarantee that, with acceptance of any formalized quantitative performance criteria, the current product mix can be maintained, but knowledge of the limitations of current disinfectants has been a factor in the suggestions made here. Performance criteria can also be adopted on an interim basis, and as data are collected these can be re-evaluated and changed or confirmed as needed.

A summary flow chart of each of the 2 tiers of the test is shown in Figures 2 and 3. These do not include all necessary and used controls, or issues related to the preparation of test organism suspensions, contamination of the carriers, neutralization of the active(s), numbers of replicates, media to promote recovery of injured test organisms, and quality assurance aspects. These and other experimental details are given in a specially prepared manual (14) and in other publications based on the method (41). During development, each step of the protocol was thoroughly validated in our laboratory to ensure consistency among carriers and recovery of test organisms.

It is not certain that any tests, such as the acid resistance test for spores (1), designed to infer a standardized resistance of a challenge organism, has any real predictive value with regard to the resistance/susceptibility of that organism to other chemicals from the wide variety of disinfectants it may be tested against. However, such internal controls are useful for quality control and should be performed in a quantitative manner.

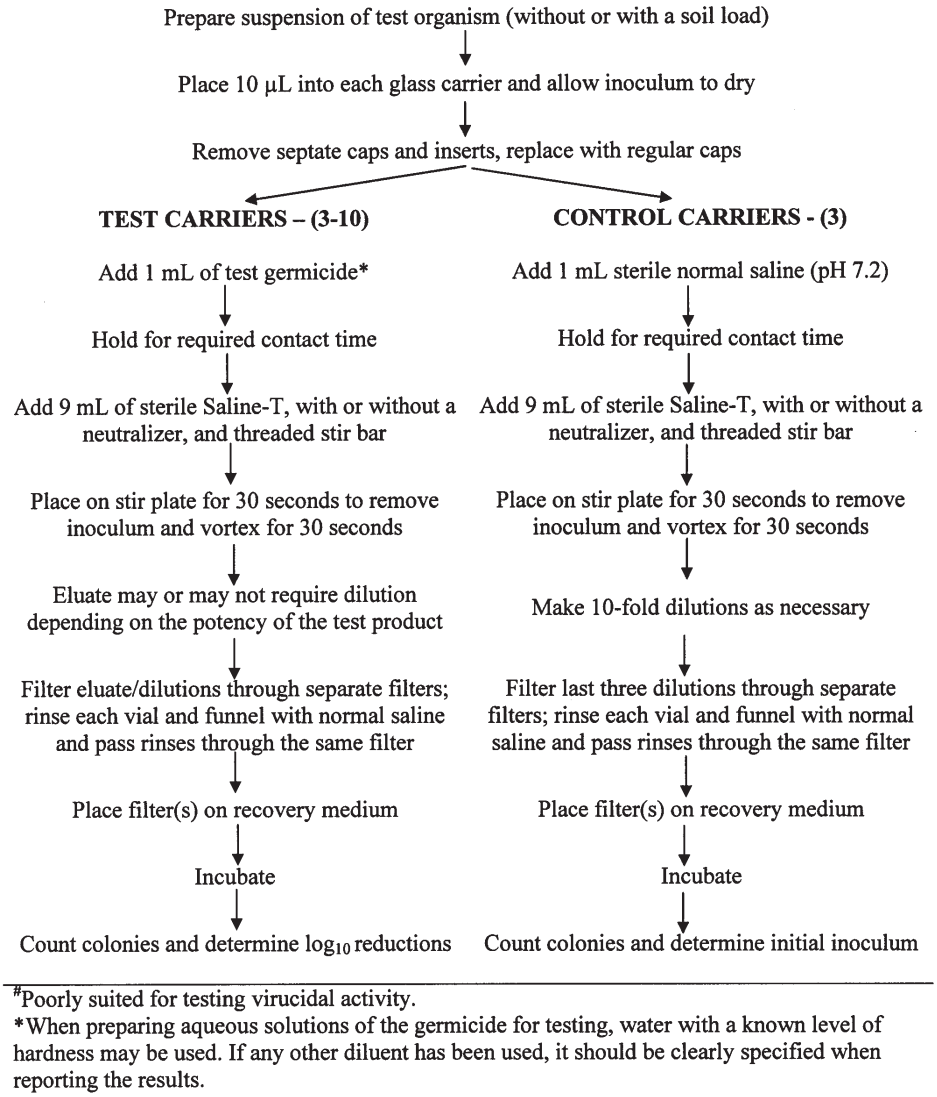

Figure 2. Main steps in Tier 1 of the quantitative carrier test (QCT-1).

Issues of disinfectant quantity have been discussed above. In the field, the quantity of disinfectant in contact with a surface is normally restricted, though to varying amounts. It may even be less than is used in QCT-2 but, in any standardized test, it is essential to ensure complete coverage of the inoculum by the disinfectant; tests using much smaller volumes of product present many practical difficulties. QCT-2 allows manufacturers to show that product(s) can meet the level(s) of microbial reduction required for the class(es) of microorganism targeted. As discussed previously, when tests are conducted at different disinfectant concentrations, this test can also indicate whether the product is properly formulated to achieve the desired $\log_{10}$ reduction in numbers of test microorganisms with potential for field efficacy.

The level of risk reduction by effective disinfection can be defined by establishing testing criteria that must be met by products in a particular use category. Although the risks from inadequate disinfection of surfaces are ill-defined, it is clear that any risks are a function of the number of known or potential pathogens remaining. Therefore, such risks can theoretically be reduced by orders of magnitude by the degree of microbial inactivation, though there is always the caveat that laboratory test conditions never equate exactly to field use.

Our test protocols have been designed to be fair to manufacturers and to users and to provide a level playing field. It is relatively easy to design a test that no currently marketed products would pass. This would be an unrealistic approach and would not provide the user with any more clarity or choice or the consumer with any more protection than exists currently. It is also possible to design a test that every product currently on the market will pass. Such a test would not take into account the realities of disinfectant use, or protect the user/consumer. The QCTs have kept the realities of the marketplace in mind, but with an approach that the disinfectant users should be assured that the product(s) being used can achieve the required performance criteria and meet their label claims.

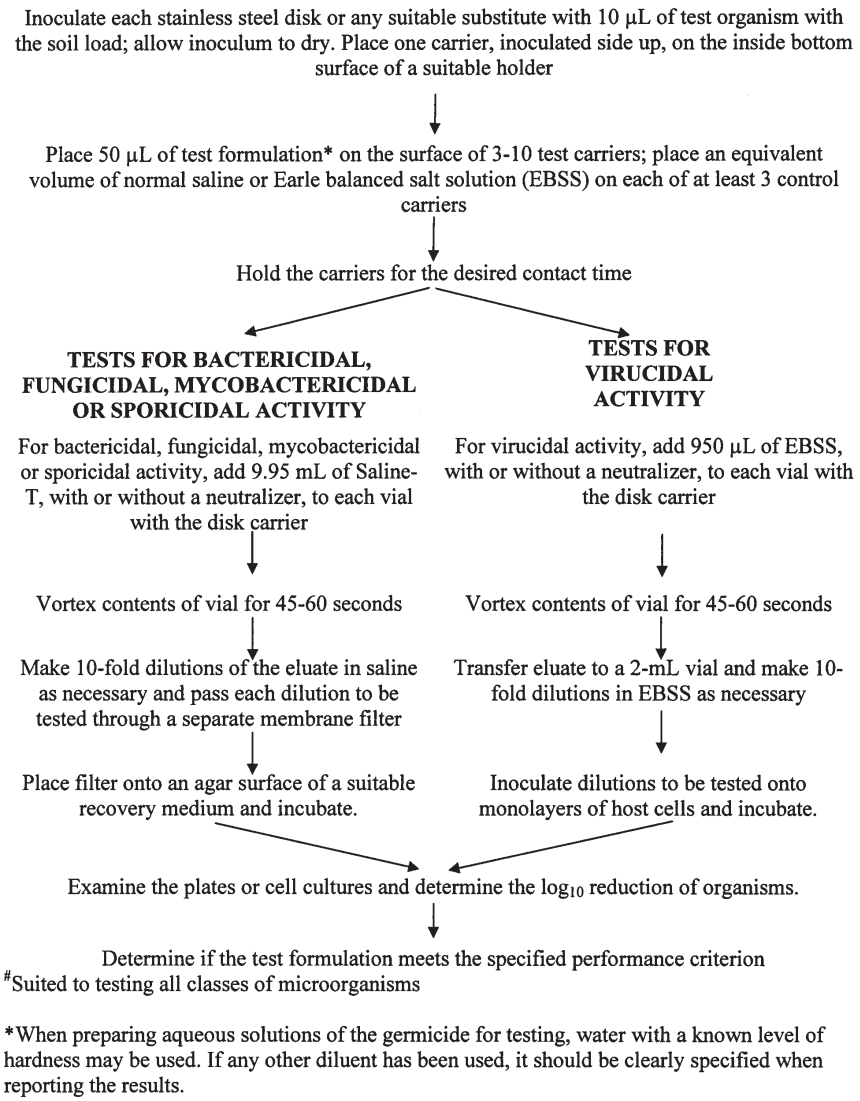

Figure 3. Main steps in Tier 2 of the quantitative carrier test (QCT-2).

It is, of course, never possible to guarantee that there will be no shift in marketed products. However, we see this occurring with individual products that may be poorly formulated rather than with whole classes of disinfectants. Our experience has shown that products permitted to make claims, for example, against mycobacteria in suspension, may no longer be able to make the same claim with a QCT. It would be difficult or impossible, very costly, and undesirable to conduct a study to ensure no changes in the marketplace; if products are not efficacious for the purpose for which they are sold, they should be withdrawn or reformulated.

Killing of microbial contaminants requires contact and chemical interaction with the applied chemical disinfectant. Disinfectants in the field may encounter a variety of challenges in both the types and levels of microbial contamination and associated soiling on a variety of different surfaces. The absolute requirement for an effective disinfectant when used under conditions specified on the product label is to inactivate the microbial challenge in situ, regardless of the type and cleanliness of the surface. In some cases, a precleaning step may be specified as a part of the label requirements.

Proposed Changes in Terminology

Several terms in current use in the area of liquid chemical disinfectants are outdated, inappropriate, or potentially misleading. Any formulation with activity against vegetative bacteria only can legally be called a disinfectant. However, in the minds of many, this term carries a different connotation and is understood to also include activity against classes of microorganisms other than vegetative bacteria. For the most part in this document, we have used the term microbicide simply to remove any confusion with current terminology.

As mentioned above, the term tuberculocide is also outdated in view of the increasing recognition of the role of many other species of mycobacteria as human pathogens and environmental contaminants (43) and their role in iatrogenic

Table 4. Main features and applications of quantitative carrier tests (QCTs)

Item	Features
Carriers	QCT-1: inside flat-bottom surface of glass vials with inserts (specially flattened vials are used).
	QCT-2: Stainless steel disks (1 cm in diameter); if required, other disks of laminates, plastics, rubbers, etc. can also be used.
Number of replicates	Require many fewer test (5–10) and control (3) carriers, thus making the test procedure(s) simpler and operator-friendly.
Culture media	Growth and recovery media for spores, mycobacteria, fungi, and vegetative bacteria are simpler and more standardized, thereby reducing variability in the test.
Suitable for use with (No. of strains already used)	QCT-1: Bacterial spores (2), mycobacteria (7), vegetative bacteria (11), and fungi (4).
	QCT-2: Bacterial spores (2), mycobacteria (5), vegetative bacteria (14), fungi (4), viruses (11), and protozoa (2).
Volume of test inoculum	QCT-1: 10 μL.
	QCT-2: 10 μL.
Volume of test product	QCT-1: 1 mL.
	QCT-2: 50 μL.
Hard water diluent (if used)	QCT-1: 400 ppm or as specified by manufacturer.
	QCT-2: 400 ppm or as specified by manufacturer.
Wash-off test organism	No loss of test organism by wash-off is possible in either test in contrast to the AOAC methods.
Soil load—mixture of bovine mucin, bovine albumin, and peptone	QCT-1: Can be used with or without a soil load in the test suspension.
	QCT-2: Recommended for use only with a soil load.
Removal/neutralization of disinfectant residue	Dilution and/or neutralization of organism-disinfectant mixture immediately at the end of contact time, followed by membrane filtration and thorough rinsing of the filter to wash out disinfectant residue, except when working with viruses and protozoa. In tests for virucidal activity, gel filtration may be needed to eliminate the residual activity of hard-to-neutralize formulations. Procedure for protozoa depends on method used to assess viability.
Log_{10} reductions	Depending on test organism and claim desired, either test can measure from 3 to 8 log_{10} reduction in microbial challenge titer.
Harmonization of test methodology	Basic materials and procedures are common to all test organisms, except culture and recovery media. This makes harmonization of test methodology possible within and between jurisdictions.

infections. This term should therefore be replaced with "mycobactericide" to more accurately reflect the testing of, and the activity of a given disinfectant against, mycobacteria other than *M. tuberculosis* alone.

We also believe that the term cold- or chemisterilant is inappropriate when referring to liquid chemical disinfectants. The reasons for this are as follows: (*1*) Sterilization, as it is usually defined, is impossible to demonstrate. It must be defined in practical terms by the degree of kill obtained by exposure to a sterilizing agent, and by the probability of sterile product being obtained. (*2*) Chemical disinfection, on the other hand, rarely gives completely linear inactivation kinetics, and so extrapolation of kill-to-low numbers is neither feasible nor desirable. The disinfection of contaminated surfaces is further complicated by demands on the disinfectant that continuously reduce its concentration, and this is in contrast to the breakpoint disinfection practiced, for example, in disinfection of water for drinking. (*3*) In the absence of the ability to extrapolate the activity of the process beyond that which is measured, achieving a high degree of sterility assurance requires using very high microbial inocula. Physical limitations on the numbers of microorganisms that can be inoculated preclude achieving the degrees of sterility assurance that are in place for many industrial processes where, for the most part, bioburden is low. In the AOAC sporicidal test, for example, there is a high probability of having at least one survivor starting with an inoculum of, say, 10^6 test spores and, hence, a large number of failures even among the best available products. Some of these products may have been designated as sterilants simply by achieving no surviving spores in a limited number of tests with an undefined inoculum of spores. Even for products routinely inactivating all the inoculated spores, this gives a sterility assurance level of approximately 10^0, and we do not consider this appropriate for a sterilization claim. (*4*) Even if a product was capable of being a chemisterilant under one set of

Table 5. List of major recommendations for discussion for reforming disinfectant testing

1	Data based on only quantitative carrier tests, except for disinfection of liquids.
2	Adoption of appropriate performance criteria, on interim basis, if necessary.
3	Restrict differences in contact times allowed to those for different uses.
4	Adopt appropriate virus surrogates for general virucidal claim.
5	Use concept of working with microorganisms under nutritional stress.
6	Consider using "mycobactericide" rather than "tuberculocide" in disinfectant terminology.
7	Adopt *M. terrae* as surrogate for mycobactericidal tests.
8	Phase-out the term “liquid chemical sterilant” and allow simply sporicidal claims.
9	Consider adding *Candida albicans* as another surrogate for fungicidal claim.
10	Drop the use of *S. choleraesuis* as a test organism in tests for bactericidal activity.
11	Consider limiting microorganisms listed on product labels.
12	Consider permitting alternate surrogates where appropriate for nonhealthcare markets.
13	Consider feasibility and benefits of using national supplier for all challenge microorganisms.
14	Adopt 400 ppm hard water as default diluent for products requiring dilution with water.
15	Adopt tripartite soil load developed for QCT, but consider alternate soil loads, if justified.
16	Adopt mandatory prototypical carrier, but permit additional carriers appropriate to end use
17	Review precleaning requirements and statements on product labels to avoid incompatibilities.
18	Phase out use of carriers of unsuitable design (e.g., suture loops and penicylinders).
19	Engage manufacturers in issues of application rate and compatible applicator material(s).
20	Require disinfectant manufacturer to identify a suitable neutralizer for product testing.
21	Adopt use of a concentration response curve to evaluate disinfectant formulations.
22	Strike task force to review and rationalize disinfectant terminology.

conditions, it would be virtually impossible to extrapolate its activity to any other situation for many of the reasons cited in this document. Thus, to give a sterilant claim to a chemical without having the conditions under which it is used closely defined is unrealistic and misleading for uninformed users. (*5*) Chemicals that are often cited as chemisterilants can only achieve complete kill of exposed microorganisms after prolonged contact times. These are much longer than those normally used in practice and give the uninformed user a false impression. (*6*) Although chemicals can be used for terminal sterilization under certain conditions, the sterilization of an item by soaking it in a disinfectant generally makes no provision for maintaining sterility in its subsequent treatment/handling, nor is such an item used in an aseptic manner.

Consideration should, therefore, be given to phasing out the use of the term cold- or chemisterilant when making generic reference to certain liquid chemical disinfectants. It is truly confusing for the user community and should only be used in very specialized applications.

Current Status of QCT

QCT-1 was subjected to a 15-laboratory collaborative, with each participating laboratory being provided with a video and written instructions produced specifically for the purpose. Similar materials are now available for QCT-2. Table 4 presents a summary of QCT features, together with the numbers of test organisms against which it has been used.

In general, products identified as high-level disinfectants have generally performed well, but products claiming mycobactericidal activity based on a suspension test only fared poorly in our carrier tests, and those with virucidal claims based only on testing against enveloped viruses did not necessarily do well when challenged with nonenveloped viruses. Because most previous testing with QCT-1 and QCT-2 used the interim criterion of 6 $\log_{10}$ reduction, it is difficult to assess whether products which failed at that level would have passed the test criterion proposed for QCT-2 (Table 3) for the category of disinfectant into which the product would fall. Data from the collaborative study were included in a statistical review and comparison of quantitative test methods (44); this comparison showed QCT to be at least as good as other test methods examined.

Conclusions

An overview was presented of the rationales we adopted in developing QCTs as potential replacements for the AOAC qualitative tests in use throughout North America. QCTs address many of the identified disparities and deficiencies in the AOAC test methodology. Also provided is a set of recommendations regarding disinfectant evaluation and regulation—many, but not all, of which arise directly from the

research and development funded by the EPA. These are presented to promote discussion and are listed briefly in Table 5.

The first tier (QCT-1) uses as its carrier the relatively smooth surface of a glass vial and can be used for working with bacterial spores, mycobacteria, vegetative bacteria, and filamentous and nonfilamentous fungi. Our second tier test (QCT-2), which uses stainless steel disks as reference carriers, can also be used with viruses and, possibly, protozoa. The tripartite soil load we have developed contains about the same level of protein as in 5% bovine serum and is compatible with all the organisms tested so far (14). The same general method can be used with a variety of test materials made into carriers, and even biofilms rather than dried inocula as a microbial challenge. It can also examine disinfectants that are formulated as foamy or liquid sprays (14).

Our rational and unified approach to disinfectant testing should be extended to requirements for data submission on the activity of disinfectants against a variety of microbial classes, and would greatly assist both regulators and manufacturers in the current market realities and imperatives toward regional and eventual global harmonization. There are some similarities and some differences between the QCTs and those used in Europe and other parts of the world, but we believe that the first logical step in this regard is harmonization of testing within jurisdictions, and this review is therefore focused on tests used or developed in North America. Both QCT-1 (45) and QCT-2 (46) are now standards of ASTM International.

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References

(1) *Official Methods of Analysis* (1998) 16th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, Chapter 6

(2) Alfano, E.M., Cole, E.C., & Rutala, W.A. (1988) *J. Assoc. Off. Anal. Chem.* **71**, 868–871

(3) Ascenzi, J.M., Ezzell, R.J., & Wendt, T.M. (1986) *Appl. Environ. Microbiol.* **51**, 91–94

(4) Ascenzi, J.M., Ezzell, R.J., & Wendt, T.M. (1987) *Appl. Environ. Microbiol.* **53**, 2189–2192

(5) Cole, E.C., Rutala, W.A., Nessen, L., Wannamaker, N.S., & Weber, D.J. (1990) *Appl. Environ. Microbiol.* **56**, 1813–1817

(6) Miner, N.A., Mulberry, G.K., Starks, A.N., Powers-Prather, A., Entrup, M., Armstrong, M., & Maida, B. (1995) *Appl. Environ. Microbiol.* **61**, 1658–1660

(7) Rutala, W.A., & Cole, E.C. (1987) *Infect. Control* **8**, 501–506

(8) Sattar, S.A. (1998) in *Proceedings of the International Symposium on Sterilization, Disinfection and Antisepsis in Health Care, June, 1997*, W.A. Rutala (Ed.), Polyscience, Inc., Champlain, NY, pp 227–240

(9) Sattar, S.A., Best, M., Springthorpe, V.S., & Sanani, G. (1995) *J. Hosp. Infect.* **30**, 372–382

(10) Sattar, S.A., & Springthorpe, V.S. (1995) in *Chemical Germicides in Health Care*, W.A. Rutala (Ed.), Polyscience Publications, Morin Heights, Canada, pp 237–254

(11) Sattar, S.A., & Springthorpe, V.S. (2001) in *Proceedings of the International Symposium on Disinfection, Antisepsis, and Sterilization: Practices and Challenges for the New Millennium*, W.A. Rutala (Ed.), Association of Practitioners in Infection Control and Epidemiology, Washington, DC, pp 173–186

(12) Springthorpe, V.S., & Sattar, S.A (1996) *Infect. Control Sterilization Tech.* **2**, 26–31

(13) U.S. General Accounting Office (1990) Document No. GAO/RCED-90-139, U.S. GAO, Washington, DC

(14) Springthorpe, V.S., & Sattar, S.A. (2003) *Quantitative Carrier Tests (QCT) to Assess the Germicidal Activities of Chemicals: Rationale and Procedures*, Centre for Research on Environmental Microbiology, Ottawa, Canada, available from QCTmanual@webbertraining.com

(15) Ascenzi, J.M. (1991) *J. Hosp. Infect.* **18** (Suppl. A), 256–263

(16) Organization for Economic Cooperation and Development (2002) *OECD Efficacy Workshop On Certain Antimicrobial Biocides Workshop,* Summary Report, Arlington, VA, OECD, Paris, France

(17) Best, M., Springthorpe, V.S., & Sattar, S.A. (1994) *Am. J. Infect. Control* **22**, 152–162

(18) Mbithi, J.N., Springthorpe, V.S., Sattar, S.A., & Paquette, M. (1993) *J. Clin. Microbiol*. **31**, 2988–2995

(19) Sattar, S.A., & Springthorpe, V.S. (2001) in *Disinfection, Sterilization and Preservation*, 5th Ed., S.S. Block (Ed.), Lippincott Williams & Wilkins, Philadelphia, PA, pp 1391–1412

(20) Thurston-Enriquez, J.A., Haas, C.N., Jacangelo, J., & Gerba, C.P. (2003) *Appl. Environ. Microbiol*. **693**, 979–985

(21) Lambert, R.J.W. (2001) *J. Appl. Microbiol.* **91**, 351–363

(22) Corradini, M.G., & Peleg, M. (2003) *J. Appl. Microbiol.* **95**, 1268–1276

(23) Lambert, R.J., & Johnston, M.D. (2000) *J. Appl. Microbiol.* **88**, 907–913

(24) Lambert, R.J., & Johnston, M.D. (2001) *J. Appl. Microbiol.* **91**, 548–555

(25) Pugh, J.C., Ijaz, M.K., & Suchmann, D.B. (1999) *Am. J. Infect. Control.* **27**, 375–376

(26) Stewart, M.H., & Olson, B.H. (1996) in *Modeling Disease Transmission and Its Prevention by Disinfection*, C.J. Hurst (Ed.), Cambridge University Press, Cambridge, UK

(27) Taylor, Y.E., Springthorpe, V.S., & Sattar, S.A. (1996) *Abstract 19-H-002*, Presented at the 110th Annual Meeting of AOAC INTERNATIONAL, Orlando, FL

(28) Gregory, A.W., Schaalje, G.B., Smart, J.D., & Robison, R.A. (1999) *Infect. Control Hosp. Epidemiol.* **20**, 324–330

(29) Griffiths, P.A., Babb, J.R., & Fraise, A.P. (1998) *J. Hosp. Infect.* **38**, 183–192

(30) Traoré, O., Springthorpe, V.S., & Sattar, S.A. (2002) *J. Appl. Microbiol.* **92**, 549–555

(31) Nagarkar, P.P., Ravetkar, S.D., & Watve, M.G. (2001) *Appl. Environ. Microbiol.* **67,** 1371–1374

(32) U.S. Environmental Protection Agency (1993) *Antimicrobial Division, Scientific Advisory Panel on Germicide Test Methodology*, Summary of Proceedings of Meeting, August 24, 1993, EPA, Washington, DC

(33) Canadian General Standards Board (1991) *Assessment of Efficacy of Antimicrobial Agents for Use on Environmental Surfaces and Medical Devices, Document No. CAN/CGSB-2.161-M91*, CGSB, Ottawa, Canada

(34) Hamilton, M.A. (2002) *J. AOAC Int.* **85**, 479–485

(35) Miner, N., Harris, V., Stump, S., Cobb, A., & Oritz, J. (2004) *Inside Laboratory Management*, Jan.-Feb., pp 12–16

(36) McDonnell, G., Amato, R., Malchesky, P.S., Muzic, D.S., & Marchant, R.E. (2000) *J. AOAC Int.* **83**, 269–275

(37) Favero, M.S., & Bond, W.W. (2001) in *Disinfection, Sterilization and Preservation*, 5th Ed., S.S. Block (Ed.), Lippincott Williams & Wilkins, Philadelphia, PA, pp 881–917

(38) Rutala, W.A., & Weber, D.J. (2004) *Infect. Control Hosp. Epidemiol*. **25**, 333–341

(39) Hamilton, M.A., DeVries, T., & Tilt, N. (1997) *Report to the U.S. EPA on Quantitative Carrier Test for Sporicides: Statistical Analysis of the Collaborative Study,* March 31, 1997

(40) Sattar, S.A., Springthorpe, V.S., Zafer, A.A., & Busa, M. (2000) *Poster Presented at the 27th Annual Meeting of the Association of Practitioners in Infection Control and Epidemiology*, Apice, Minneapolis, MN

(41) Sattar, S.A., Springthorpe, V.S., Adegbunrin, O., Zafer, A.A., & Busa, M. (2003) *J. Virol. Methods* **112**, 3–12

(42) Cole, E.C., Rutala, W.A., & Samsa, G.P. (1988) *J. Assoc. Off. Anal. Chem.* **71**, 1187–1194

(43) Falkinham, J.O., III (2002) *Clin. Chest Med.* **23**, 529–551

(44) Tilt, N., & Hamilton, M.A. (1999) *J. AOAC Int.* **82**, 384–389

(45) ASTM International (2003) *Standard Quantitative Carrier Test Method to Evaluate the Bactericidal, Fungicidal, Mycobactericidal and Sporicidal Potencies of Liquid Chemical Germicides*, Document No. E-2111-00, ASTM, West Conshohocken, PA, pp 1526–1533

(46) ASTM International (2003) *Standard Quantitative Disk Carrier Test Method for Determining the Bactericidal, Virucidal, Fungicidal, Mycobactericidal and Sporicidal Activities of Liquid Chemical Germicides*, Document No. E-2197-02, ASTM, West Conshohocken, PA, pp 1607–1616