



Factors Affecting the Efficacy of Disinfection and Sterilization

DISINFECTION AND STERILIZATION GUIDELINE
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Guideline for Disinfection and Sterilization in Healthcare Facilities (2008)

WHAT TO KNOW

Factors Affecting the Efficacy of Disinfection and Sterilization from the Guideline for Disinfection and Sterilization in Healthcare Facilities (2008).

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Overview

The activity of germicides against microorganisms depends on a number of factors, some of which are intrinsic qualities of the organism, others of which are the chemical and external physical environment. Awareness of these factors should lead to better use of disinfection and sterilization processes and will be briefly reviewed. More extensive consideration of these and other factors is available elsewhere ^{13, 14, 16, 411-413}.

Number and Location of Microorganisms

All other conditions remaining constant, the larger the number of microbes, the more time a germicide needs to destroy all of them. Spaulding illustrated this relation when he employed identical test conditions and demonstrated that it took 30 minutes to kill 10 *B. atrophaeus* (formerly *Bacillus subtilis*) spores but 3 hours to kill 100,000 *Bacillus atrophaeus* spores. This reinforces the need for scrupulous cleaning of medical instruments before disinfection and sterilization. Reducing the number of microorganisms that must be inactivated through meticulous cleaning, increases the margin of safety when the germicide is used according to the labeling and shortens the exposure time required to kill the entire microbial load. Researchers also have shown that aggregated or clumped cells are more difficult to inactivate than monodispersed cells ⁴¹⁴.

The location of microorganisms also must be considered when factors affecting the efficacy of germicides are assessed. Medical instruments with multiple pieces must be disassembled and equipment such as endoscopes that have crevices, joints, and channels are more difficult to disinfect than are flat- surface equipment because penetration of the disinfectant of all parts of the equipment is more difficult. Only surfaces that directly contact the germicide will be disinfected, so there must be no air pockets and the equipment must be completely immersed for the entire exposure period. Manufacturers should be encouraged to produce equipment engineered for ease of cleaning and disinfection.

Innate Resistance of Microorganisms

Microorganisms vary greatly in their resistance to chemical germicides and sterilization processes (Figure 1) ³⁴² Intrinsic resistance mechanisms in microorganisms to disinfectants vary. For example, spores are resistant to disinfectants because the spore coat and cortex act as a barrier, mycobacteria have a waxy cell wall that prevents disinfectant entry, and gram-negative bacteria possess an outer membrane that acts as a barrier to the uptake of disinfectants ^{341, 343-345}. Implicit in all disinfection strategies is the consideration that the most resistant microbial subpopulation controls the sterilization or disinfection time. That is, to destroy the most resistant types of microorganisms (i.e., bacterial spores), the user needs to employ exposure times and a concentration of germicide needed to achieve complete destruction. Except for prions, bacterial spores possess the highest innate resistance to chemical germicides, followed by coccidia (e.g., *Cryptosporidium*), mycobacteria (e.g., *M. tuberculosis*), nonlipid or small viruses (e.g., poliovirus, and coxsackievirus), fungi (e.g., *Aspergillus*, and *Candida*), vegetative bacteria (e.g., *Staphylococcus*, and *Pseudomonas*) and lipid or medium-size viruses (e.g., herpes, and HIV). The germicidal resistance exhibited by the gram-positive and gram-negative bacteria is similar with some exceptions (e.g., *P. aeruginosa* which shows greater resistance to some disinfectants) ^{369, 415, 416}. *P. aeruginosa* also is significantly more resistant to a variety of disinfectants in its "naturally occurring" state than are cells subcultured on laboratory media ^{415, 417}. *Rickettsiae*, *Chlamydiae*, and mycoplasma cannot be placed in this scale of relative resistance because information about the efficacy of germicides against these agents is limited ⁴¹⁸. Because these microorganisms contain lipid and are similar in structure and composition to other bacteria, they can be predicted to be inactivated by the same germicides that destroy lipid viruses and vegetative bacteria. A known exception to this supposition is *Coxiella burnetii*, which has demonstrated resistance to disinfectants ⁴¹⁹.

Concentration and Potency of Disinfectants

With other variables constant, and with one exception (iodophors), the more concentrated the disinfectant, the greater its efficacy and the shorter the time necessary to achieve microbial kill. Generally not recognized, however, is that all disinfectants are not similarly affected by concentration adjustments. For example, quaternary ammonium compounds and phenol have a concentration exponent of 1 and 6, respectively; thus, halving the concentration of a quaternary ammonium compound requires doubling its disinfecting time, but halving the concentration of a phenol solution requires a 64-fold (i.e., 2^6) increase in its disinfecting time ^{365, 413, 420}.

Considering the length of the disinfection time, which depends on the potency of the germicide, also is important. This was illustrated by Spaulding who demonstrated using the mucin-loop test that 70% isopropyl alcohol destroyed 10^4 *M. tuberculosis* in 5 minutes, whereas a simultaneous test with 3% phenolic required 2–3 hours to achieve the same level of microbial kill ¹⁴.

Physical and Chemical Factors

Several physical and chemical factors also influence disinfectant procedures: temperature, pH, relative humidity, and water hardness. For example, the activity of most disinfectants increases as the temperature increases, but some exceptions exist. Furthermore, too great an increase in temperature causes the disinfectant to degrade and weakens its germicidal activity and thus might produce a potential health hazard.

An increase in pH improves the antimicrobial activity of some disinfectants (e.g., glutaraldehyde, quaternary ammonium compounds) but decreases the antimicrobial activity of others (e.g., phenols, hypochlorites, and iodine). The pH influences the antimicrobial activity by altering the disinfectant molecule or the cell surface ⁴¹³.

Relative humidity is the single most important factor influencing the activity of gaseous disinfectants/sterilants, such as EtO, chlorine dioxide, and formaldehyde.

Water hardness (i.e., high concentration of divalent cations) reduces the rate of kill of certain disinfectants because divalent cations (e.g., magnesium, calcium) in the hard water interact with the disinfectant to form insoluble precipitates ^{13, 421}.

Organic and Inorganic Matter

Organic matter in the form of serum, blood, pus, or fecal or lubricant material can interfere with the antimicrobial activity of disinfectants in at least two ways. Most commonly, interference occurs by a chemical reaction between the germicide and the organic matter resulting in a complex that is less germicidal or nongermicidal, leaving less of the active germicide available for attacking microorganisms. Chlorine and iodine disinfectants, in particular, are prone to such interaction. Alternatively, organic material can protect microorganisms from attack by acting as a physical barrier ^{422, 423}.

The effects of inorganic contaminants on the sterilization process were studied during the 1950s and 1960s ^{424, 425}. These and other studies show the protection by inorganic contaminants of microorganisms to all sterilization processes results from occlusion in salt crystals ^{426, 427}. This further emphasizes the importance of meticulous cleaning of medical devices before any sterilization or disinfection procedure because both organic and inorganic soils are easily removed by washing ⁴²⁶.

Items must be exposed to the germicide for the appropriate minimum contact time. Multiple investigators have demonstrated the effectiveness of low-level disinfectants against vegetative bacteria (e.g., *Listeria*, *E. coli*, *Salmonella*, VRE, MRSA), yeasts (e.g., *Candida*), mycobacteria (e.g., *M. tuberculosis*), and viruses (e.g., poliovirus) at exposure times of 30–60 seconds ⁴⁶⁻⁶⁴. By law, all applicable label instructions on EPA-registered products must be followed. If the user selects exposure conditions that differ from those on the EPA-registered product label, the user assumes liability for any injuries resulting from off-label use and is potentially subject to enforcement action under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA).

Microorganisms may be protected from disinfectants by production of thick masses of cells ⁴²⁸ and extracellular materials, or biofilms ⁴²⁹⁻⁴³⁵. Biofilms are microbial communities that are tightly attached to surfaces and cannot be easily removed. Once these masses form, microbes within them can be resistant to disinfectants by multiple mechanisms, including physical characteristics of older biofilms, genotypic variation of the bacteria, microbial production of neutralizing enzymes, and physiologic gradients within the biofilm (e.g., pH). Bacteria within biofilms are up to 1,000 times more resistant to antimicrobials than are the same bacteria in suspension ⁴³⁶. Although new decontamination methods ⁴³⁷ are being investigated for removing biofilms, chlorine and monochloramines can effectively inactivate biofilm bacteria ^{431 438}. Investigators have hypothesized that the glycocalyx-like cellular masses on the interior walls of polyvinyl chloride pipe would protect embedded organisms from some disinfectants and be a reservoir for continuous contamination ^{429, 430, 439}. Biofilms have been found in whirlpools ⁴⁴⁰, dental unit waterlines⁴⁴¹, and numerous medical devices (e.g., contact lenses, pacemakers, hemodialysis systems, urinary catheters, central venous catheters, endoscopes) ^{434, 436, 438, 442}. Their presence can have serious implications for immunocompromised patients and patients who have indwelling medical devices. Some enzymes ^{436, 443, 444} and detergents ⁴³⁶ can degrade biofilms or reduce numbers of viable bacteria within a biofilm, but no products are EPA-registered or FDA-cleared for this purpose.



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