DISINFECTANT ACTIVITY AGAINST BACTERIA AND VIRUSES: A HOSPITAL GUIDE

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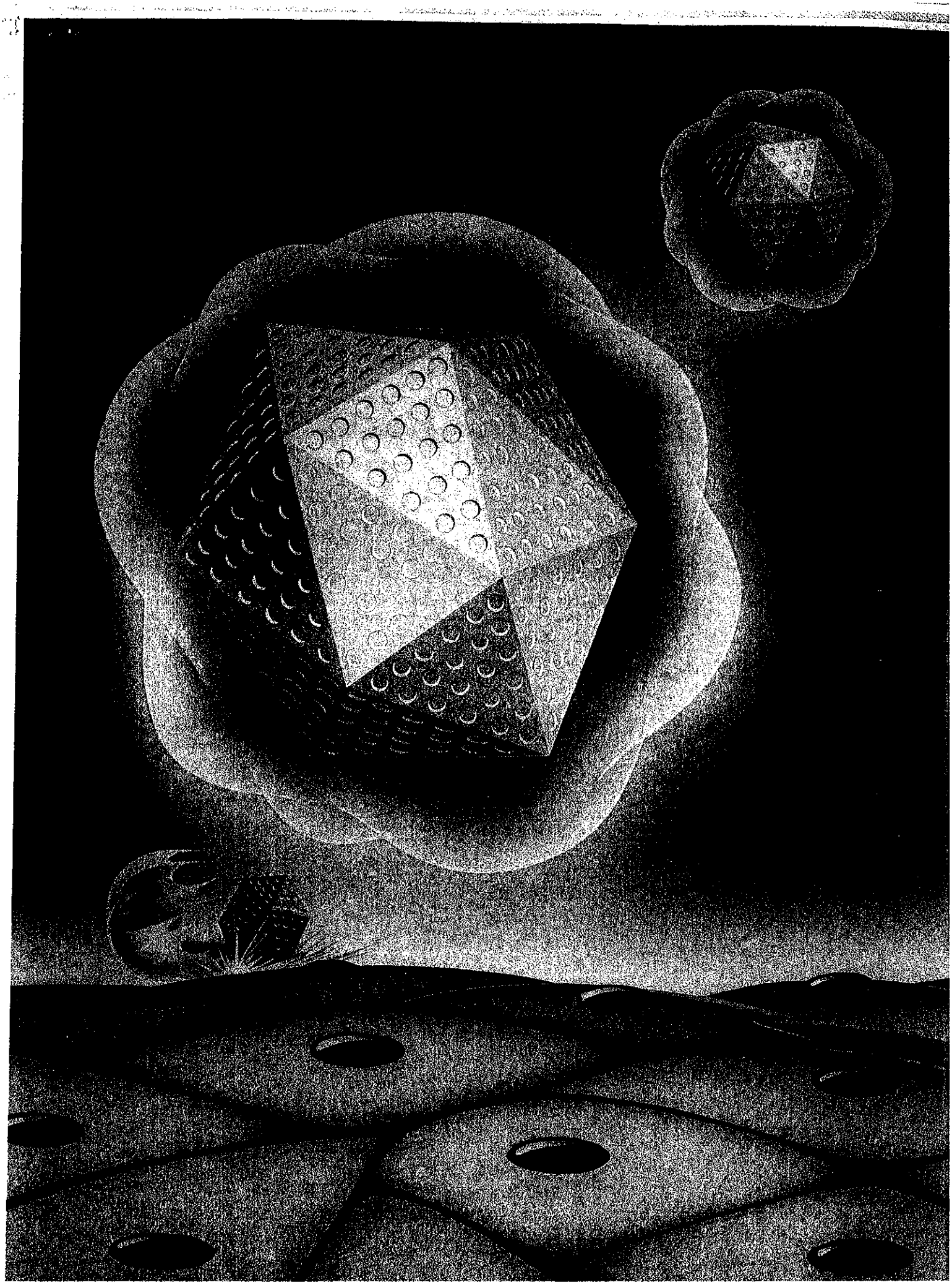
REPRINTED FROM PARTICULATE & MICROBIAL CONTROL, MARCH/APRIL 1983



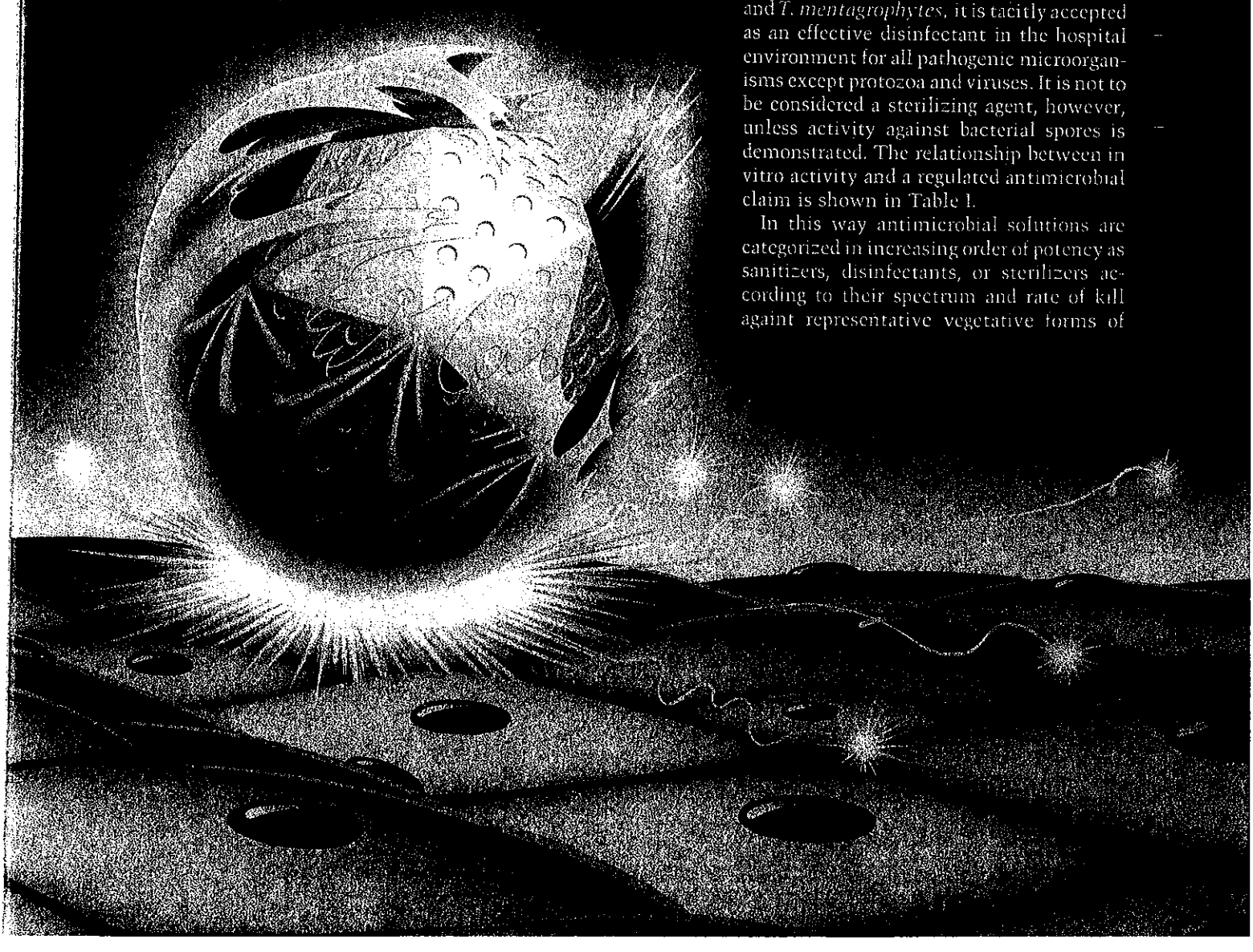
DISINFECTANT ACTIVITY AGAINST BACTERIA AND VIRUSES: A HOSPITAL GUIDE

Herbert N. Prince

Disinfectant solutions that are used in hospitals to decontaminate surfaces, respiratory equipment, dialyzers, and surgical instruments are originally tested for efficacy by the use of a set of rigidly controlled in vitro tests that are described in the *Federal Register*,[1] the manual of the Association of Official Analytical Chemists (AOAC),[2] and various EPA guidelines.[3,4] The tests specify that the product must kill dried films or suspensions of the following organisms within 10 minutes at room temperature: *Staphylococcus aureus* ATCC 6538, *Salmonella choleraesuis* ATCC 10708, and *Pseudomonas aeruginosa* ATCC 15442. Mere reduction in numbers is inadequate.

Testing is extensive: 180 tests on slides or cylinders against each of these organisms for a total of 540 assays. (Claims against additional bacteria are satisfied by 20 additional assays per organism.) If this battery of tests demonstrates a consistent kill at the 95% confidence level, the product can be registered with the EPA as a hospital disinfectant. These tests also must demonstrate that the disinfectant acts in the presence of organic matter and that this activity is not diminished in the presence of hard water. If activity against tuberculosis is claimed, additional tests employing the BCG strain of *Mycobacterium tuberculosis* var. *bovis* must be performed. If activity against pathogenic fungi is claimed, an additional test against *Trichophyton mentagrophytes (interdigitale)* is required. Once a product passes the mandated AOAC tests against *S. aureus*, *S. choleraesuis*, *P. aeruginosa*, *M. tuberculosis*, and *T. mentagrophytes*, it is tacitly accepted as an effective disinfectant in the hospital environment for all pathogenic microorganisms except protozoa and viruses. It is not to be considered a sterilizing agent, however, unless activity against bacterial spores is demonstrated. The relationship between in vitro activity and a regulated antimicrobial claim is shown in Table 1.

In this way antimicrobial solutions are categorized in increasing order of potency as sanitizers, disinfectants, or sterilizers according to their spectrum and rate of kill against representative vegetative forms of



Organism Tested[a] (EPA Prototype Agents)	Action	EPA Designation
S. aureus ATCC 6538[b] *S. choleraesuis* ATCC 10708[b] *P. aeruginosa* ATCC 15442[b]	Reduction in count without complete kill	Sanitizer
M. bovis BCG *T. mentagrophytes* ATCC 9533	Complete kill in 10 min	Disinfectant
Above plus spores of *B. subtilis* ATCC 19659 and *C. sporogenes* ATCC 3584	Complete kill in stated time (see product label)	Chemosterilizer

[a]Propagated under controlled conditions. [b]Relatively desiccation-resistant strains.

Table I: Guide to categories of disinfectants based on reduction of microbes.

gram-positive and gram-negative bacteria, a filamentous fungus, and endospores of aerobic and anaerobic bacteria.

PROTOTYPE ORGANISMS

The EPA has chosen a strain of *S. aureus* as the prototype gram-positive organism because it is one of the more difficult of this group to kill when dried on an inanimate surface. Effectiveness against this organism tacitly presupposes effectiveness against other gram-positive bacteria, such as streptococci, micrococci, other staphylococci, and coryneform bacilli.

Organism Tested
S. aureus (penicillin-resistant)
S. aureus (toxic shock strain)
S. pyogenes
S. pneumoniae
E. coli
P. mirabilis
P. cepacia (CDC iodophor strain)
P. stutzeri
S. typhimurium (ampicillin-resistant)
C. albicans
Campylobacter
Yersinia
M. tuberculosis (streptomycin-resistant)
E. coli (tetracycline- and sulfonamide-resistant)
A. fischerii

Table II: Clinical isolates killed in 10 minutes at room temperature by representative types of hospital disinfectants containing active ingredients such as iodophors, phenolics, and quats and registered with EPA on the basis of activity against *Staphylococcus*, *Salmonella*, and *Pseudomonas* (contaminated—10^4 to 10^6 colony forming units—stainless-steel carriers immersed in use-dilutions and transferred to recovery broth according to AOAC methods). Killing predicted by EPA prototype agents listed in Table I.

A strain of *S. choleraesuis* is accepted as the prototype enteric bacillus, and activity against it is assumed to predict effectiveness against members of the genera *Escherichia, Proteus, Salmonella, Shigella, Serratia, Enterobacter, Citrobacter, Arizona, Providencia, Morganella,* and other members of the family Enterobacteriaceae, i.e., all gram-negative rods that ferment glucose, do not produce spores, and are negative in the cytochrome oxidase test.

A strain of *P. aeruginosa* is accepted as the prototype aerobic bacillus. Action against it is assumed to predict effectiveness against oxidizing and nonoxidizing members of the large group collectively cited as nonfermenting bacilli. These include pseudomonads such as *P. cepacia, P. putida, P. stutzeri, P. alcaligenes, P. paucimobilis, P. maltophilia, P. pseudoalcaligenes,* as well as members of the genera *Achromobacter, Flavobacterium, Acinetobacter, Moraxella,* and a variety of unnamed CDC strains.

It is to be stressed that broth-dilution tests for minimum inhibitory concentration—such as those used for drugs and antiseptics[5]—are inadequate to prove disinfectant efficacy. Furthermore, because there is an almost endless list of bacteria that are likely to be encountered in the hospital, including drug-resistant forms, the EPA has chosen these prototype strains as guides to efficacy. Our own studies have shown that tests with representative iodophor, phenolic, and quaternary ammonium-based hospital disinfectants, materials originally screened with the organisms listed in Table I, did indeed reveal effectiveness against the hospital isolates cited in Table II.

VIRUSES

The disinfectant solutions and sprays that are used against bacteria are exactly the same as those used against viruses, but there is some confusion about the interpretation of label claims. Some viruses are easier to inactivate than bacteria, others more difficult. Manufacturers are not allowed to screen against prototype strains in order to make the general claim that their products are hospital virucides. If a claim to virucidal activity is made, a duplicate test against the specific strain is required. Figure 1 outlines this test, and Table III provides the general classification of tar-

get viruses. These tests are performed in tissue-culture systems (at least 5% fetal calf serum for pool preparation and 1–2% for maintenance), such as human diploid fibroblasts (WI-38, MRC-5, Flow 2000), malignant epithelial lines (H.Ep2, HeLa) or monkey kidney cells (*Rhesus, Ceropithecus*), chick embryos, or suckling or adult mice.[6] The tests are "overkill" assays, and successful completion is designed to assure effectiveness under worst-case conditions. Failure to pass does not rule out at least a partial inactivation, but a total kill is required when the label claims that the product is a virucide. The presence or absence of virus is determined by microscopic evaluation for cytopathic effect (CPE) in tissue culture or by plaque reduction in monolayers, as well as by the hemagglutination (HA) reaction with chick or guinea pig erythrocytes for myxoviruses in chick embryos and the hemadsorption reaction in cell cultures.

The label of an approved disinfectant will, then, claim virucidal action against a specific virus or an array of viruses—influenza, herpes, and vaccinia, for instance—but cannot make broad claims on the basis of predictive tests, as can the label of an antibacterial agent. A product with activity against rhinovirus will state the exact strain for which data have been submitted. In the case of myxoviruses, a manufacturer cannot claim activity against the myxovirus influenza B if it has submitted data to EPA that document activity against only myxovirus influenza A. These claim restrictions seem justified at present because no published collaborative laboratory trials demonstrate that single viral strains can act as prototypes for other members within their group.

MECHANISM OF ACTION OF VIRUCIDES

It is generally recognized that specific physicochemical groups of viruses behave similarly with respect to sensitivity to chemical agents and that viruses can be ranked in terms of their overall resistance to disinfectants such as phenolics (e.g., orthophenylphenol, phenol), quaternary ammonium substances

Table III: General taxonomic categories of target viruses in the hospital environment.

Nucleic Acid[a] (Symmetry)	Viral Taxon (Size in nm)	Common Name	Disease
ss-RNA (cuboidal)	Enterovirus (hydrophilic) (20–30)	ECHO Coxsackie A, B Polio 1, 2, 3	Diarrhea, exanthems, aseptic meningitis, respiratory disease, paralytic disease
ss-RNA (cuboidal)	Rhinovirus (hydrophilic) (20–30)	At least 95 serotypes related to the common cold and upper respiratory infections	Common cold symptoms
ss-RNA (helical)	Myxovirus and paramyxovirus (lipophilic) (enveloped) (80–300)	Influenza A, B, C Parainfluenza virus Respiratory syncytial virus Measles Mumps	Pharyngitis, tracheitis, bronchiolitis, bronchitis pneumonia
ds-DNA (cuboidal)	Adenovirus (lipophilic) (capsomeric) (70–90)	Swollen glands Common cold Sore throat	Lymphadenopathy Upper respiratory infections Pharyngitis, conjunctivitis
ds-DNA (cuboidal)	Herpesvirus (lipophilic) (enveloped) (120–200)	Herpes simplex type 1 Herpes simplex type 2 Varicella/zoster Epstein-Barr virus Cytomegalovirus	Herpesvirus 1 (buccal/cutaneous) Herpesvirus 2 (progenitalis) Herpesvirus 3 (chicken pox/shingles) Herpesvirus 4 (infectious mononucleosis) Herpesvirus 5
ds-DNA (complex)	Poxvirus (230–300)	Vaccinia Ectromelia	Small pox, vaccinia gangrinosa, cow pox
ss-DNA (cuboidal)	Parvovirus (18–22)	None[b]	None in man, possibly the "Norwalk" gastroenteritis

[a]ss, single stranded; ds, double stranded.
[b]Veterinary pathogen: canine parvovirus (CPV) and feline panleukopenia virus (FPV), inactivated only by hypochlorite solution and, to a certain extent, by iodophor solutions.

Figure 1: An EPA virucide test prototype based on 10-minute contact of use-dilution with at least 10^4 of an infectious dose (50%) per carrier plus organic soil.

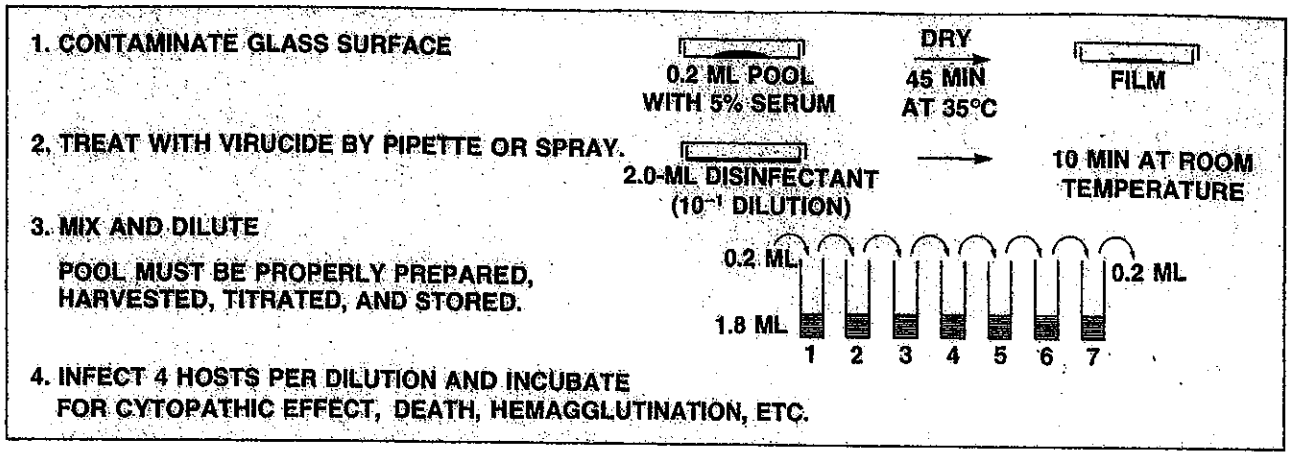


Table IV: Inactivation on contact of viruses in order of increasing resistance.

Virus	Active Agent and Effect
Myxoviruses, herpesviruses, and vaccinia	Glutaraldehyde, formaldehyde, phenols, halogens, alcohols, quats (all equally effective)
Adenoviruses	Halogens, phenols, alcohols, glutaraldehyde, formaldehyde, quats (at high concentrations)
Picornaviruses (ECHO, coxsackie, rhino and polio, similar degrees of resistance)	Formaldehyde, glutaraldehyde, halogens, phenols, alcohols, quats (depending on formulation and concentration)
CHINA viruses; prion or agent of scrapie	10% NaClO and 7 h boiling (partially effective)[a]; 30 min autoclaving (effective)
Parvoviruses (capsid + ss-DNA)	10% NaClO only, phenols, quats, and alcohol (ineffective); iodophors (partially effective)

[a]Infected tissue.

Table V: Chemical guide to viral disinfection at minimum concentration of virucide for 10 min (after Klein and DeForest[7]).

Virus	Sodium Hypo-chlorite	Isopropanol	Ethanol	Benzalkonium Chloride	Betadine Antiseptic Solution	Ortho-phenyl-phenol	Glutaral-dehyde
Polio 1	200 ppm[a]	95% inactive	70% active	10% inactive[b]	150 ppm[a]	12% inactive[b]	2%[a]
Coxsackie B1	200 ppm[a]	95% inactive	60% active	10% inactive[b]	150 ppm[a]	12% inactive[b]	1%[a]
Adeno 2	200 ppm[a]	50% active	50% active	1000 ppm active (400 ppm partial)	150 ppm[a]	0.12%[c]	0.02%[a]
Vaccinia	200 ppm[a]	30% active	40% active	100 ppm[c]	75 ppm[a]	0.12%[c]	0.02%[a]
Herpes simplex	200 ppm[a]	20% active	30% active	100 ppm[c]	75 ppm[a]	0.12%[c]	0.02%[a]
Influenza A	200 ppm[a]	30% active	30% active	100 ppm[c]	75 ppm[a]	0.12%[c]	0.02%[a]
Feline parvovirus	2000 ppm (2.5 log reduction)	30% inactive	50% inactive	5000 ppm inactive	5000 ppm (1 log reduction)	10% inactive	1% (2 log reduction)

[a]Shows that an oxidizing agent or an aldehydic amino-blocking reagent is broadly effective regardless of its affinity for lipids.
[b]Shows the inactivity of lipophilic substances against hydrophilic viruses.
[c]Shows the activity of lipophilic substances against lipophilic viruses, which generally mimics effects against vegetative bacteria.

("quats"; e.g., benzalkonium chloride), halogens (e.g., chlorine and iodine compounds such as hypochlorites and iodophors), basic aliphatic amines (e.g., chlorohexidine), mono- and dialdehydes (e.g., formaldehyde, glutaraldehyde), and lower aliphatic alcohols (e.g., ethanol, isopropanol).[7] Table IV ranks viruses in order of their increasing resistance to chemical agents. CHINA (slow viruses, the acronym for which is based on *ch*ronic *i*nfectious *n*europathic *a*gents) are rarely found in the hospital, and there are as yet no human pathogens known in the parvovirus. CHINA agents and the parvovirus are the most disinfectant-resistant of all mammalian pathogens.

It is difficult to predict the activity of a formulation because various ratios of excipient ingredients, solvents, and active agents produce somewhat nonspecific and synergistic effects. The presence of alcohol, detergents, and wetting agents will enhance all virucidal claims, for example. Formulations containing quats are generally less active against enteroviruses and adenoviruses (as compared to their antibacterial effect) than are formulations containing phenolics, aldehydes, or halogens. Most hospital disinfectants, whether they contain iodophors, phenolics, or quats, are equally effective against lipophilic viruses such as influenza and herpes, which are the easiest of all common infectious agents to inactivate on contact. Infection-control practitioners in the hospital should have a basic knowledge of the chemical components of various disinfectants and should check labels to determine the following: EPA registration, bacterial and/or viral claims, and the presence of chemical agents singly or in combination. As a practical matter, applications beyond the restricted label claim can be deduced from a knowledge of the agent's spectrum and mode of action (see Tables IV and V). The best readily available virucide in the hospital is 70% ethanol. Isopropanol is completely inactive against hydrophilic RNA viruses such as polio and coxsackie, which can be carried on hands. Sodium hypochlorite is an excellent broad-spectrum virucide/bactericide and is also readily available in the hospital as a 5% NaHClO solution (e.g., Chlorox), which should be diluted 1:10 in tap water before use.

Category	Nature	Structure	Resistance
I	Hydrophilic	Nucleic acid + capsid	Marked (polio)
II	Intermediate (capsomeric lipophilicity)	Nucleic acid + capside	Moderate (adeno)
III	Lipophilic (envelope)	Nucleic acid + capsid +envelope	Slight (myxo)

Table VI: The Klein-DeForest categorization of virions.[7]

Table V, derived from the classic work of Klein and DeForest,[7] depicts the relationship between the hydrophilic or hydrophobic (lipophilic) nature of a virion and chemical inactivation. (The former virion does not dissolve in cholesterol or ether; the latter does.) This relationship is the basis of the Klein-DeForest scheme (Table VI). A summary of the antimicrobial uses of disinfectant agents and their modes of action is given in Table VII. Figures 2 and 3 describe the modes of

Table VII: Effect of virucidal contact disinfectants after 10 minutes.[10]

Agent	Spectrum	Use	Action
Alcohol 70% (EtOH,[a] IPA)	Broad	Skin antiseptics, components of EPA disinfectants[b]	Denature protein
Formaldehyde Glutaraldehyde[a]	Broad	Preservatives, disinfectants, chemosterilizers	React with R-NH_2 groups, protein inactivation
Halogens[a] I_2 Iodophors Hypochlorite	Broad	Disinfectants, topical antiseptics	Oxidizing agents
Phenol and derivatives	Moderate (lipophilic viruses)	Preservatives, skin antiseptics, disinfectants	Solubilize and denature proteins, dissolve lipid envelope
Quats Chlorohexidine	Narrow (lipophilic viruses)	Skin antiseptics, disinfectants, preservatives	Dissolve lipid envelope of ether-sensitive viruses
Hydrogen peroxide[a] or organic peroxides	Broad	Skin antiseptics, disinfectants, chemosterilizers	Oxidizing agents

[a]Most effective.
[b]Disinfectant claims regulated by EPA, antiseptic claims by FDA.

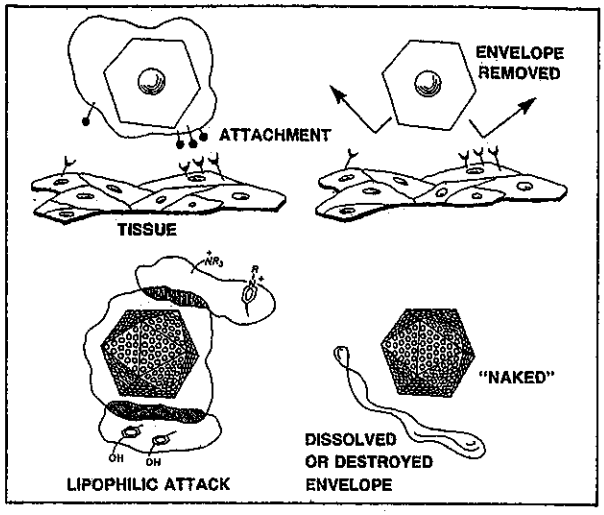


Figure 2: Viral inactivation by disruption of surface structures.

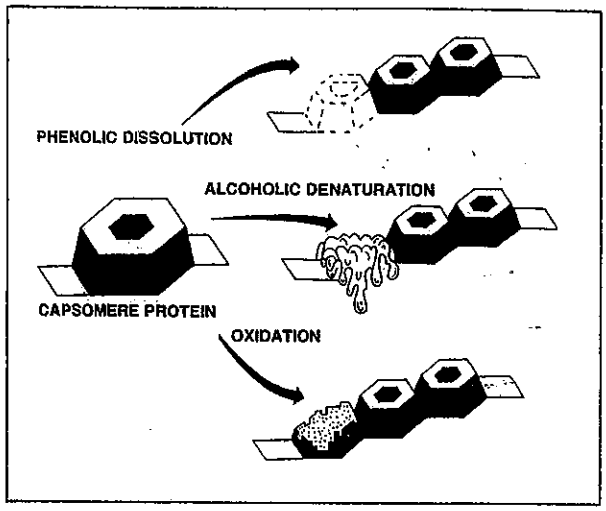


Figure 3: The effects of phenolic dissolution, alcoholic denaturation, and oxidation on viral surface protein.

action of virucides according to the Klein-DeForest concept of surface inactivation.

It should be made clear that disinfectants "inactivate" viruses by disrupting their surface structures. Their antibacterial activity, on the other hand, is frequently associated with intracellular events. Disruption of the viral surface disturbs the essential lock-and-key configuration of attachment and receptor sites on the virion and host cell surfaces. When associated with disinfectants, then, the term "viral inactivation" means that the viral particles are no longer capable of independent attachment to and absorption into the host cell because these critical early events are a function of surface structure. As long as the nucleic acid portion of the virion is intact, however, and if by some extraordinary means the naked nucleic acid should enter the cell, replication is theoretically possible. This possibility has been demonstrated with RNA from phenol-extracted poliovirus.[8] Formaldehyde is a special case: this agent maintains the antigenic nature of the protein coat (capsid) but inactivates the nucleic acid, as in a "killed" vaccine.

Susceptibility Group	Bacterial/Viral Group
A	Myxoviruses and herpesviruses Gram-negative rods and fungi Vaccinia
B	*S. aureus*[a] *M. tuberculosis*
C	Adenoviruses Picornaviruses
D	Bacterial spores (*Bacillus, Clostridium*) CHINA viruses (slow viruses) Parvoviruses[b]

[a]More resistant dried than when in suspension.
[b]Replicate only in dividing cells.

Table VIII: Approximate disinfection scale for common organisms in order of increasing resistance (response to commercial disinfectants).

SCALE OF SUSCEPTIBILITY

Table VIII is an approximate disinfection scale for all of the categories of microorganisms likely to be encountered in medical and veterinary environments. Reference to this chart can help one to make sensible decisions about which disinfectant to use in particular situations. When fecal contamination is likely (possible enteroviruses), for instance, a disinfectant with a rhinovirus claim (common cold) would be superior to one with only a tuberculosis claim. This table makes it clear that a knowledge of the spectra and modes of action of chemical agents can aid in the rational selection of disinfectant agents regardless of label citations. Thus, for example, if a hospital disinfectant kills *S. aureus*, it is clearly capable of inactivating practically all agents in susceptibility group A, i.e., common gram-negative rods as well as lipophilic viruses such as influenza and herpes. Nevertheless, we have in our own experience seen many disinfectant formulations that pass the AOAC-EPA test against vegetative bacteria and fungi and *Mycobacterium* (susceptibility groups A and B), but that are inactive against

adenovirus, enteroviruses (polio), and rhinoviruses (susceptibility group C). Clearly, if a disinfectant is capable of passing the EPA virucide test against polio virus or a rhinovirus (the most difficult of the common infectious disease agents to inactivate by the EPA test within 10 minutes), there is little doubt that it is truly a broad-spectrum disinfectant in terms of susceptibility groups A, B, and C. Sporicidal formulations (or chemosterilizers) such as the glutaraldehyde formulations Cidex and Sporicidin will kill or inactivate all infectious disease agents above them on the disinfection scale. In terms of speed of activity, Sporicidin is probably the most potent against bacterial spores when used full strength, and most potent against viruses and vegetative bacteria when diluted 1:35 and stored for as long as 21 days at room temperature.

MISCONCEPTIONS

It is important to explore two myths concerning antimicrobial agents that have developed over the years. The first concerns the classification of antimicrobial substances as "static" or "cidal." These terms are relative and do not define the intrinsic properties of an antimicrobial molecule. Rather, they define the parameters of *time* and *concentration*. All of the so-called bacteriostatic agents are bactericidal when given sufficient time or tested at sufficient concentration. The only truly bacteriostatic agent is the refrigerator. Properly, we should speak of "static" or "cidal" concentrations or "static" or "cidal" exposures, not of "static" or "cidal" agents. This myth also extends to the physician's armamentarium of chemotherapeutic

GLOSSARY

Antiseptic: A substance or formulation that kills microorganisms in or on the human body to prevent infection. An in vivo effect, regulated by FDA.

Capsid: The outer protein coat of a virus, consisting of small structural units of specific geometric design called capsomeres.

Chemosterilizer: A formulation that produces a total kill of aerobic and anaerobic bacterial spores as well as bacteria, fungi, and viruses within a specified time. An in vitro effect.

Disinfectant: A liquid or spray formulation of specific or nonspecific chemical composition that kills at least 10,000 microorganisms on an inanimate surface within 10 minutes at room temperature and in the presence of organic soil equivalent to at least the total protein in 5% serum. An in vitro effect, regulated by EPA.

Envelope: The outer lipid membrane of lipophilic viruses that helps the virus attach to the host cell and that is derived from the host cell during release of the virus.

Hydrophilic: The property or tendency to dissolve in water. Hydrophilic viruses dissolve in water or in polar water-soluble materials, such as hypochlorite, ethyl alcohol, aldehydes, etc. All hydrophilic viruses, such as picornaviruses, are immiscible with lipids.

Iodophor: A category of disinfectants in which the active ingredient is di-atomic iodine loosely bound to a large organic carrier. Kills microorganisms on contact without development of resistance.

Lipophilic: The property or tendency to dissolve in water-insoluble materials such as ether, cholesterol, and certain oils because of an absence of charged polar groups. A lipophilic chemical and lipophilic virus will have an affinity for each other by mutual solubilization of the viral envelope in the nonpolar portion of the chemical (e.g., herpesvirus + orthophenyl-phenol). All lipophilic viruses, such as myxo- and herpesviruses, have an easily disrupted lipid envelope.

Phenolic: A monocyclic or bicyclic halogenated or nonhalogenated substance derived from phenol that is not highly water soluble or surface active. Kills microorganisms on contact.

Quat: The abbreviated form of the term *quaternary ammonium compound*, a surface-active, water-soluble disinfecting substance that has four carbon atoms linked to a nitrogen atom through covalent bonds, with the nitrogen or ammonium moiety of the salt forming a positively charged cation, rendering water solubility to the molecule, and with high-molecular-weight aliphatic or aromatic nonpolar side chains rendering a lipophilic nature to the substance. Kills microorganisms on contact.

Spore: A relatively water-poor round or elliptical resting cell consisting of condensed cytoplasm and nucleus surrounded by an impervious lamellar cell wall or coat. Relatively resistant to disinfectant activity and drying conditions (specifically in the genera *Bacillus* and *Clostridium*).

Vegetative Cell: A water-rich bacillary or coccal metabolizing cell consisting of nucleus, cytoplasmic membrane, and cell wall. It actively multiplies in culture or in tissue and is relatively sensitive to disinfectant activity.

Virion: Another name for *viral particle;* denotes the complete infectious unit. The virus may be enveloped and coated (e.g., influenza, herpes), coated with capsid only (e.g., enteroviruses), or naked nucleic acid (CHINA).

Virucide: A category of disinfectant.

agents, in which penicillin is erroneously said to be "cidal" and sulfonamides are said to be "static". Both kill by different mechanisms, rates, and concentrations.

The second myth relates to the practice of rotating disinfectants in a plant or hospital to prevent resistance. We have studied this problem in its proper arena, the administration of chemotherapeutic agents in systemic disease[9] or antiseptic agents in topical drug therapy.[5] When dealing with diseases, we encounter such high populations of organisms that Mendelian genetics (mutation from rapid growth) and Darwinian selection (the random survivors within a chemically altered environment) are indeed operable. So when a chemical agent is used against millions of organisms without rotation, the probability of selecting resistant forms at the normal 10^{-6} rate of mutation is high. A similar situation exists in restaurants, food-processing plants, dairies, and other places where growth-promoting material resides in the environment. It generally does not exist in relatively clean clinical or pharmaceutical environments, however, where the development of high ambient populations would presage such a breakdown in housekeeping, sanitation, and vigilance that the use of disinfectants would be tardy and futile.

CONCLUSION

The EPA carefully regulates disinfectants used in the hospital because it is concerned with the death of organisms on inanimate surfaces. Specific test organisms are mandated for laboratory screening before hospital registration is allowed, the most important of these being *S. aureus*, *S. choleraesuis*, and *P. aeruginosa*. Additional tests against *M. tuberculosis* and hydrophilic and/or lipophilic viruses are optional. A scale of disinfection exists, and by applying our knowledge of microbial taxonomy and mechanism of kill we can extrapolate from limited label data predictions about which microorganism will or will not be controlled by a specific disinfectant.

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