Hand Hygiene in Controlled Environments

Studies with topical antiseptics against bacteria and viruses inform the choice of rapid kill agents for hand sanitization.

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The United States Pharmacopeia describes the use of antiseptics and disinfectants in chapter 1072 as important components of a contamination control program, especially for aseptic processing.[1] In a section entitled, "Selection of an Antiseptic for Hand and Surgical Site Disinfection," the chapter lists the following antiseptic agents for use in a program of hand hygiene as well as by cleanroom operators in the pharmaceutical industry: isopropyl alcohol, hexachlorophene, chlorhexidine, povidone-iodine, and chlorhexidine alcohol. Of these, only isopropyl alcohol and povidone iodine have universal acceptance for safety and efficacy for use in industry (pharmaceutical, vaccine, tissue bank) or healthcare practice.[2,3,4] Ethyl alcohol solution, the most frequently used hand antiseptic,[5] is not mentioned by the USP in this connection.

We have performed a series of comparative experiments on the speed and range of activity of antiseptics likely to be used in areas requiring aseptic or sanitary practice, including the less frequently studied effects against viruses. The fact that these agents are "narrow spectrum" (and not "broad spectrum" as with bacteria) is important information that will be discussed in this paper. This knowledge may well discourage their use when there is a transmission danger regarding certain respiratory or enteric viruses.

Even though it is recognized that antiseptics can decrease cross-contamination in pharmaceutical production areas, R & D labs, cleanrooms, and support areas,[5] it is the purpose of this paper to analyze the laboratory methods that researchers use in the development of such agents.

METHODS

Methods for the kill time experiments and procedures were based on modified ASTM E-2135[6] and ASTM E-1052[7] methods for bacteria and viruses, respectively, as well as by methods developed at Gibraltar Laboratories, Inc. The basic technique, known as the plate count log reduction method, was introduced in USP 18 (1970).

Antiseptics

(PVP-I) 10%, ethanol 62% w/w [EtOH], isopropanol 62% w/w [IPA], Triclosan liquid soap 0.15% and Benzethonium chloride [BC] 0.3% were tested. Tween-lecithin and casein-soy bean digest broth were used as neutralizers. For the viruses, 20% fetal bovine serum (FBS) and dilution were the neutralization entities for viral recovery (recorded as dilutions beyond the toxic doses). Chlorhexidine and hexachlorophene, although mentioned in USP 31, were not tested since

they are deemed by FDA as inappropriate for OTC use either for efficacy or safety issues.[2]

Challenge Microorganisms (Resident and Transient Hand Flora)

Bacteria and Yeast: Suspension kill-time assays were performed selected from the list of bacteria required by FDA[2] for topical antisepsis, with ATCC designations: *Staphylococcus aureus* 6538, *Staphylococcus aureus* (MRSA-CA strain) 33591, *Escherichia coli* 11229, *Salmonella typhi* 6539, *Pseudomonas aeruginosa* 15442, *Proteus mirabilis* 43071, *Klebsiella pneumoniae* 4352, *Streptococcus pyogenes* 19615, *Staphylococcus epidermidis* 14990, *Acinetobacter baumannii* 19606, *Enterococcus faecium* (VRE) 51559, yeast, *Candida albicans* 10231.

Viruses: *Influenza A Hong Kong 8/68(H3N2)* (VR-544), *Herpes simplex virus type 1* (VR-260), *Adenovirus type 2* (VR-846), *Rhinovirus type 42* (VR-1112), *Poliovirus type 1* (VR-1562 and VR-192), *Hepatitis A virus* (HAV) (HM-175), and the *Feline Calicivirus* (FCV) (USDA challenge strain), surrogate for the Norwalk virus. Viruses were propagated in monkey kidney cells fed with Eagle's medium tissue culture fluid supplemented with 5% fetal calf serum under 5% CO_2 with standard cytopathic endpoint (CPE). Influenza virus was propagated in 9-10 day chick embryos and the presence or absence of virus determined by the hemagglutination reaction (HA) with chick RBC; the readings were elicited from infected chorioallantoic fluid. All virus testing was performed in a BSL-3 facility built to ATCC/BEI/Government specifications with restricted access and fingerprint recognition.

Recovery Endpoints for Survivors

For the bacteria and yeast, log reduction values were obtained by standard plate counts as colony-forming units in Trypticase Soy Agar with neutralizers as cited. For the viruses, survivors were titrated for recovery in monkey kidney cell cultures or chick embryos by the quantal response TCID-50 (or EID-5) calculation of Reed and Muench.

Pass-Fail Criteria

No official values exist for this type of *in vitro* analysis. However, pass-fail criteria for *in vivo* tests have been given in certain cases by the FDA,[2] e.g. 2-$\log_{10}$ and 3-$\log_{10}$ reductions of the indicated organism

Organism	Antiseptic – LR Value			
	EtOH, IPA, BC*, PVP-I Active Ingredient		Triclosan 0.15% Liquid Soap	
	15 s	60 s	15 s	60 s
Gram-Positive Bacteria				
1. *Staphylococcus aureus* (MRSA) (CA)	5	5	3	4
2. *Enterococcus faecium* (VRE)	5	5	4	5
3. *Streptococcus pyogenes*	5	5	5	5
4. *Staphylococcus aureus*	5	5	0	2
5. *Staphylococcus epidermidis*	5	5	0	2
Gram-Negative Bacteria				
6. *Escherichia coli*	5	5	0	0
7. *Proteus mirabilis*	5	5	0	0
8. *Klebsiella pneumoniae*	5	5	0	0
9. *Pseudomonas aeruginosa*	5	5	0	0
10. *Salmonella typhi*	5	5	0	0
11. *Acinetobacter baumannii*	5	5	0	2
Yeast				
12. *Candida albicans*	5	5	0	0
Spectrum	12/12 = 100%		3/12 ≥ 25%	

LR
5 = complete kill ≥ (99.999% kill)
4 = 99.99% kill
3 = 99.9% kill
2 = 99% kill
0 = Inactive, No Reduction
MRSA = Methicillin-resistant Staphylococcus aureus
CA = Community Associated
VRE = Vancomycin-resistant enterococcus

*Effects seen with Benzethonium chloride in this study probably would apply to other quaternary ammonium compounds, e.g. Benzalkonium chloride, etc.

Table 1: Antibacterial Suspension Tests In Vitro Rapid Kill Times < After ASTM E-2315 > (Log reduction (LR) values as pertain to hand sanitizing)

after certain intervals in clinical testing of healthcare personnel hand wash products. In the present study, we have arbitrarily taken a 3.0 to 4.0 log reduction at 15 seconds as a significant result.

Results of the antibacterial and antiviral tests are given in Tables 1 and 2.

RESULTS AND DISCUSSION

As seen in Table 1, EtOH, IPA, BC and PVP-I were equally effective against all of the bacteria and yeast tested producing a broad spectrum score of 12/12 = 100% at contact time of 15 seconds. Triclosan 0.15% liquid soap produced a narrow spectrum score of 3/12 = 25% at a contact time of 15 seconds. Better effects were seen at 60 seconds. We point out that Triclosan and other liquid-type soaps with various concentrations of active with rub and wash directions are not labeled as to rapid percent kill; rather, they are formulated as a means of potentiating the normal de-germing effect of soap and water. The rapid kills shown for the quaternary (BC) and Iodophor (PVP-I) compounds can be diminished in the presence of organic matter. Alcohols have a unique and valuable advantage in skin sanitizing or skin de-germing as under normal conditions,they are not inactivated in this manner. Alcohols, however, lack substantivity as can be shown for the other agents in Table 1.

A battery of rapid-kill antiviral tests with pathogenic RNA and DNA viruses is shown in Table 2.

The data in Table 2 show that EtOH, IPA, and Benzethonium chloride were equally effective against the enveloped viruses *Herpes* and *Influenza A*, but were inactive against the *Adenovirus* and the non-enveloped viruses, *Rhino, Polio, FCV* and *HAV* producing a narrow spectrum score of 2/7 = 28%. Triclosan liquid soap produced a score of 14% (see also comments Table 1). The results in Table 2 are striking in that we see the failure of all the antiseptics to inactivate the non-enveloped virus in these short-term experiments. It can be pointed out that longer contact times for PVP-I and the alcohols may produce a different result, but it is the rapid short-term results that are the matter of interest in this report. The literature on naked viruses shows inconsistent results with alcohol against polio virus.[3] This virus is a key indicator of effectiveness against other enteric viruses of enormous public health significance (*ECHO, Coxsackie A, Coxsackie B, Hepatitis A* and the *Norwalk* agent), as well as against rhinoviruses (common cold).

A better understanding of the virucidal mechanism of action of antiseptic attack can be obtained by understanding lipid denaturation (Figure 1). The lipid envelope is essential for attachment of the virus to either the influenza or herpes receptor sites. The non-enveloped viruses lack an easily disrupted surface structure and, therefore, greater resistance to germicidal agents is seen, especially for a claim of rapid inactivation.

Virus	Antiseptic					
	EtOH, IPA, BC Active Ingredient		PVP-I Active Ingredient		Triclosan Liquid Soap	
	15s	60 s	15s	60 s	15s	60 s
Herpes (DNA) (E)	5	5	5	5	4	4
Influenza A (RNA) (E)	5	5	3(1)	3(1)	0	0
Adeno (DNA) (N)	0	0	0	0	0	0
Rhino (RNA) (N)	0	0	0	0	0	0
Polio (RNA) (N)	0	0	0	0	0	0
FCV (RNA) (2) (N)	0	0	0	0	0	0
HAV (RNA) (N)	0	0	0	0	0	0
Spectrum	2/7 = 28%		2/7 = 28%		1/7 = 14%	

E = Enveloped (lipid or lipophilic)
N = Naked (non-lipid or hydrophilic) (non-enveloped)
0 = Inactive, no virus recovered at lowest dilution of virus – antiseptic mixture inoculated into host
5 = Complete inactivation (≥ 99.999%) See Table 1
(1) Lesser effect possibly due to inactivation by allantoic fluid protein
(2) FCV is surrogate for the Norwalk agent (Noroviral Enteritis)

Note: The above strain is H3N2, 1968 pandemic. Influenza A is divided into subtypes either seasonal or novel, e.g. H1N1 (current swine pandemic), H2N2 (1957 human pandemic), H5N1 (1998 bird flu). Humans are infected only with H1, H2, or H3.

Table 2: Narrow Spectrum Antiviral Suspension Tests In Vitro Rapid Inactivation Times Enveloped and Non-Enveloped Viruses < Based on ASTM E-1052[7] > (Log reduction (LR) values as pertain to hand hygiene)

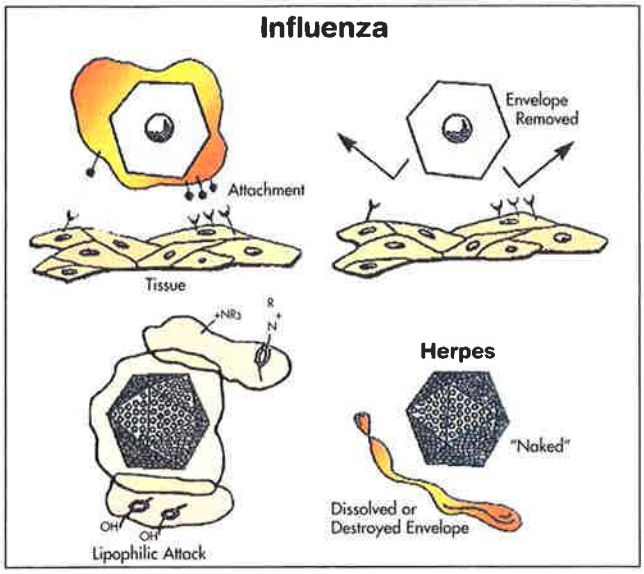

Figure 1: Viral inactivation by disruption of surface structures (alcohol, quat, I2)

TRANSMISSION OF MICROORGANISMS IN HYGIENIC PRACTICE AND ENVIRONMENTAL CONTROL

Transmission of microorganisms in controlled environments or healthcare settings occurs in various ways. The mechanisms are called horizontal, vertical, or zig-zag.[8] It is the purpose of either antiseptics or disinfectants to break such transmission, sometimes called cross-contamination. Examples are:

- *Hand Hygiene*
  Transmission or vectoring can occur in three ways: 1) vertical or autochthonous, as from one body part to another, e.g. fecal-to-mouth, lesion-to-eye, 2) horizontal, as from one person to another, such as handshake or horseplay, and 3) zig-zag as from hand-to-surface-to-product. Transmission is broken by antiseptics, as discussed in this paper.
- *Environmental*
  The following patterns can exist: 1) vertical (airborne) or transfer from air supply to product or test, 2) horizontal, or transmission from surface-to-product, or 3) zig-zag or transfer from environmental surface-to-worker-to-product. Transmission can be broken by disinfectants and antiseptics depending on the amount of human intervention.

On the Subject of Soap, Antiseptics, and Sterile Gloves

Another hidden reservoir of transfer organisms are those growing on the skin underneath sterile gloves. Hand washing with soap and water or a medicated soap must include thorough drying of the hands prior to donning gloves. Gloves worn for too long a period of time can release organisms from the moist environment when changed or removed, especially if only bar soap is used. The World Health Organization in a worldwide literature review has ranked the de-germing ability of hand washing agents in decreasing order of effectiveness: 1) alcoholic hand sanitizer (60 to 70%) solution, foam, or gel, 2) medicated soap (solid or liquid), 3) soap and water, with specified wash times of 20 to 30 and 40 to 60 seconds for alcohol and soap and water respectively. From a

formulation and regulatory point of view, solid or liquid soaps are compositions of long chain fatty acids or anionic detergents and water. They are regulated for safety only (acute toxicity) by the Consumer Product Safety Commission (CPSA). When an antimicrobial is added (e.g. Triclosan) the soap becomes a drug and is regulated for both safety and efficacy by the FDA, albeit with rather loose labeling requirements.

CALCULATIONS OF LOG-REDUCTION TESTS

Questions frequently arise as to whether or not one should report germicidal activity as log reduction or percent kill (or percent inactivation for viruses). In regulatory parlance, one frequently sees activity or efficacy end-points reported as "log reduction" as we have done in this report. In over-the-counter antiseptics, activity is commonly referred to as "percent kill." Accordingly, we provide here a step-by-step summary and explanation of the manner in which the observations in the petri dish or cell culture are converted to the endpoints mentioned above.

Understanding and Calculating Data from the Log Reduction Test

The microbiologist notes the extent of killing by counting and recording the number of surviving colonies (bacteria) or cell culture plaques (viruses). The zero-time and survivor counts are converted to values of log reduction (LR) from any table of common logarithms or percent kill by the use of the following expressions:

Step one: The graded number of surviving colonies or plaques (E) are recorded and compared to initial counts (B).

Step two: The values of B and E are entered into the formula:

$$\% \text{ kill} = \frac{B - E}{B} \times 100$$

Step three: The arithmetic counts as exponents are converted to Log 10

e.g. Initial count (B) = 120,000 = 1.2×10^5 log =5.1
Survivor count (E) = 1.5×10^2 log =2.2
Log reduction = 2.9

Step four: Conversion of log reduction (LR to percent kill uses the expression)

Percent kill = $100 \times (1\text{-}10^{-LR})$
[∴LR 2.9 the % kill is 99.9%]

A table of standard values for any combination of arithmetic (plate count) and geometric (log reduction) data can thus be constructed.

SUMMARY

1. A GLP study was performed to compare the antiseptic potency of several common antiseptics as might be used in aseptic or sanitary practice. Both bacteria and viruses were tested and standard ASTM methodology was used.
2. Ethanol, isopropanol, and Benzethonium chloride displayed equally-rapid (15 seconds) broad spectrum against gram positive and gram negative bacteria and Candida, including MRSA, *Salmonella, E. coli,* and *Streptococcus pyogenes.*
3. The same antiseptics tested in a BSL-3 facility displayed only narrow-spectrum activity against viruses, as follows: rapid 15 second inactivation was noted for the enveloped (lipid) viruses Influenza A and Herpes, but not for the non-enveloped viruses (the entero virus and rhinovirus groups).
4. The data in this report can provide for a more uniform use of antiseptics in contamination control as to aseptic and sanitary practice including sterile manufacturing, live virus vaccine production, tissue banking, or other areas of institutional, community or recreational sanitary practice.

References

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