ANTIBACTERIAL AND ANTIVIRAL STUDIES WITH TOPICAL ANTISEPTICS AS RELATES TO HAND HYGIENE AND SKIN DEGERMING

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Introduction and Purpose

Recovery and processing teams employ antiseptics and surgical scrubs prior to gloving and gowning for tissue recovery and aseptic processing. Procedures can vary from company-to-company. Recently, WHO has reviewed the antibacterial effects of alcohol, Polyvinyl Pyrrolidone Iodine (PVP-I), and other topical antiseptics with respect to spectrum and speed of activity against bacteria but the report included only limited information on against viruses. WHO notes wide variation in hand washing techniques worldwide as to choice of agent, dose, and contact time. We know of no single study comparing these factors as to antibacterial and antiviral speed of kill. Accordingly, since tissue bank workers are vigilant in maintaining aseptic techniques, we thought it important to provide current laboratory data so as to assist in the ranking of the various techniques and reagents that are available. Contact times of 15 and 60 seconds were studied as examples of kill time ranges reported by WHO and as examples of kill times at Gibraltar Laboratories in studies conducted for various antiseptic manufacturers according to FDA and ASTM methods.

Methods

Methods for the kill time experiments and procedures were based on ASTM E-2135 and ASTM E-1052 for bacteria and viruses respectively.

ANTISEPTICS: (PVP-I) 10% Ethanol 62% w/w [EtOH], Isopropanol 62% w/w [IPA], Triclosan 0.15% and Benzethonium (QAC) chloride[BC] 0.3%. Tween-lecithin and casein digest 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.

CHALLENGE MICROORGANISMS: [Bacteria, Yeast, and Viruses] Suspension kill time assays were performed selected from the list of bacteria required by FDA for topical antisepsis, with ATCC designations: Staphylococcus aureus 6538, MRSA 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 and the viruses: Influenza A Hong Kong 8/68, Herpes simplex virus type 1, Adenovirus type 2, Rhinovirus type 42, Poliovirus type 1, Hepatitis A virus (HAV) and the Feline Calicivirus (FCV), surrogate for the Norwalk virus.

Methods (Cont'd)

RECOVERY ENPOINTS FOR SURVIVORS: For the bacteria and yeast log-reduction values were obtained by standard plate counts as colony-forming units in Trypticase Soy Agar. For the viruses survivors were titrated for recovery in Monkey Kidney cell cultures (CPE) or chick embryos (HAggl) 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 analysis with antiseptic agents. We have taken a 3.0 log reduction as an indication of efficacy. The results reported from this study are for the contact times and concentrations described and are not intended to suggest that results are not dose-related.
All virus testing was performed in a BSL-3 facility built to CDC specifications with restricted access and finger-print recognition.

Results

Table 1: Antibacterial kill times – 15 and 60 seconds
Table 2: Antiviral kill times – 15 and 60 seconds
Table 3: FDA guidance on antiseptics and de-germing
Table 4: Dose-Time Relations

BACTERIAL RESULTS: BROAD SPECTRUM

Table 1: Antibacterial Kill Times: Bacteria and Candida (based on ASTM E-2315) Log-Reductions[1]
At 15 and 60 Seconds Contact in Suspension

	EtOH, IPA, BC, PVP-I		TRICLOSAN	
ORGANISM	15s	60s	15s	60s
Staph. aureus (MRSA) (GP)	5[2]	5	3	4
VRE (GP)	5	5	4	5
Streptococcus pyogenes (GP)	5	5	5	5
MRSA (GP)	5	5	0	2
Staph. epidermidis (GP)	5	5	0	2
Acinetobacter (GN)	5	5	0	2
Escherichia coli (GN)	5	5	0	0
Salmonella typhi (GN)	5	5	0	0
Pseudomonas aeruginosa (GN)	5	5	0	0
Proteus Mirabilis (GN)	5	5	0	0
Klebsiella pneumoniae (GN)	5	5	0	0
Candida albicans (GP-Y)	5	5	0	0
Spectrum	12/12 = 100%		3/12 = 25%	

[1] - Log-Reduction of 3.0 taken as "active" (99.9% kill)
[2] - 5.0= 99.999% kill, maximum obtainable in the assay
Shaded areas represent Rapid Kill
Un-shaded areas represent Inactive

GP = Gram Positive, GN = Gram Negative, Y = Yeast
0 = "Inactive"

- 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.
- The rapid kills shown for the quaternary (BC) and Iodophor (PVP-I) compounds can be diminished in the presence of organic matter.
- Alcohols are not inactivated in this manner, a unique and valuable advantage in skin sanitizing or skin degerming.

Results (Cont'd)

VIRAL RESULTS: NARROW SPECTRUM

Table 2: Antiviral Kill Times: Enveloped and Non-Enveloped Viruses: Log-Reductions[1] (Based on ASTM E-1052)

	EtOH, IPA, BC		PVP-I		TRICLOSAN	
VIRUS	15sec	60sec	15sec	60sec	15sec	60sec
Herpes -1(DNA)	5[2]	5	5	5	4	4
Influenza A(RNA)	5	5	3	3[3]	0□	0
Adeno (DNA)	0	0	0	0	0	0
Rhino (RNA)	0	0	0	0	0	0
Polio (RNA)	0	0	0	0	0	0
FCV (RNA)□	0	0	0	0	0	0
HAV (RNA)	0	0	0	0	0	0
SPECTRUM	2/7 = 28%		2/7 =28%			
1/7 = 14%						

Virus in **blue** represents it being enveloped
Virus in red represents it being non-enveloped
0 = "inactive"

Shaded blue designates rapid inactivation

[1] - Log-Reduction of 3.0 taken as "active" (99.9% inactivation)
[2] - 5-logs 99.999% inactivation, maximum obtainable in this assay
[3] - Lesser effect possibly due to inactivation by protein content of chick embryo chorioallantoic fluid
□ - Inactivity not due to organic matter
□ - Surrogate for the Norwalk Agent (Norovirus Enteritis)

- The short-term failure of all of the antiseptics tested against the non-enveloped viruses is noted. The literature shows inconsistent results with alcohol against Polio virus, with longer contact times. (In vitro & in vivo experiments)
- Polio is representative of other enteric viruses such as Coxsackie A, Coxsackie B, the various ECHO strains, hepatitis A and Rota virus.
- Where an "inactive" result is noted, longer contact times may be required that are not in the time-kill range of hand hygiene for tissue bank or health care workers (HCWs).

Table 3: Cadaver Degerming Using FDA Guidance

Tentative Final Monograph (TFM) for OTC Health Care Antiseptic Drug Products; Proposed
Rule 21 CFR Parts 333 and 369, 17 June, 1994 Fed. Reg. 59 (No.116) Categories of Approval

The following categories are based upon in vitro screens and Human in vivo data and are not necessarily judged as to kill time.

Choice of Antiseptic for Cadaver Degerming Employing FDA OTC Clinical Guidelines

Agent	Patient Pre-Op Scrub	Antiseptic Hand Wash (HCW)	Surgical Scrub
EtOH (48-59%)	I	I	I
PVP-I (5-10%)	I	I	I
IPA (70-91%)	I	IIISE	IIISE
I Tincture	I	NA	NA
Benzethonium Chloride	IIISE	IIISE	IIISE
Phenol < 1.5%	IIISE	IIISE	IIISE
Triclosan	IIISE	IIISE	IIISE
Phenol > 1.5%	II	II	II
Hexachlorophene	II	II	II
Chlorhexidine*	II	II	II

*Approved for Rx but not OTC
NA- Not Applicable
I = Efficacy and Safety Established
II = Efficacy and Safety Not Established
III = Efficacy and Safety Not Proven

PVP-I is the predominant clinical pre-op. It has a more rapid kill than Hexachlorophene or Chlorhexidine but is less substantive than these aromatics.

Table 4: Contact Time
Time-To-Dry Dose Volume Experiment
(ETHANOL 62%)

Dose	Rub Time-To-Dry (s)	Rub Time-To-Dry (s)
Dose (mL) to Hand	Emollient Gel	Aqueous Solution
1.0	15*	10
2.0	30	20
3.0	40	30
4.0	60	50
5.0	90	60

*Most common hand-washing time according to WHO

- It is noted that the time-kill contact period is related to the amount of product administered to the hands.
- FDA in the TFM cites 1.5 to 5.0ml to rapid kill
- WHO cites 2-3ml for rapid kill
- For an emollienated alcohol product the volume administered should provide contact at drying of 15 to 60 seconds. (WHO)
- Variations in dose for gels, foams, solutions, and medicated towellettes will product different times-to-dry and therefore different kill rates. Standardization for these practices is not available but is a goal.

Figure 1: Hand Degerming – WHO General Ranking (15-60 Seconds)
Decreasing Effectiveness
1. Alcohol Rub
2. Medicated Liquid
3. Soap and Water
4. Water Rinse

Figure 2: Virucidal Activity

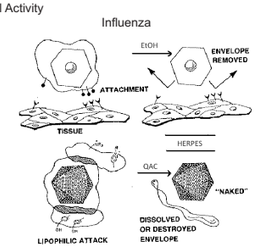

Figure 3: WHO FORMULATION I

Alcohol

ADVANTAGES	DISADVANTAGES
Rapid Kill	Not Substantive
No Inactivation	Flammable
Inexpensive	
Broad Spectrum	

Ethanol 80% v/v H_2O_2 0.125%

- Glycerol 1.45% Apply 2.0mL to palm 1 time and rub back and forth on both sides and fingers, until dry.
- WHO: If drying occurs in less than 15 seconds, volume is too small.

Summary

Bacteria

- EtOH, IPA, Benzethonium Chloride 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 liquid soap was the least effective antibacterial producing a narrow spectrum score of 3/12=25% at a contact time of 15 seconds. Slight effects were seen at 60 seconds.

Virus

- EtOH, IPA, Benzethonium Chloride were equally effective against the enveloped viruses Herpes and influenza A but were inactive against non-enveloped virus Adeno, Rhino, Polio, FCV, and HAV producing a narrow spectrum score of 2/7=28%.
- Triclosan liquid soap was the least effective antiviral producing a narrow spectrum score of 1/7=14%
- The failure of all the antiseptics to inactivate the non-enveloped virus in these short-term experiments is noted and it is pointed out that longer contact times for PVP-I and the alcohol may produce a different result.

General

- EtOH and IPA were equally active in this study and were the most effective agents for hand hygiene in agreement with the WHO review, as regards efficacy and safety (reported elsewhere.)
- When analyzed from both the experimental and regulatory points of view the aliphatic alcohols and PVP-I (Both FDA approved) stand out as the preferred agents, the former for short-term applications (e.g. hand hygiene) and the latter for application not requiring such rapid kill, but producing substantively (e.g.: Surgical pre-op, Cadaver degerming.)