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Dear Colleague:

We are pleased to provide you with copies of some slides and poster materials presented in the Virology Sessions at the ASM International Symposium on Chemical Germicides, July 22-29, 1990, Atlanta, Georgia.

VIRAL INFECTIVITY, GERMICIDE TOXICITY AND VIRAL INACTIVATION

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ABSTRACT

Virulence titrations in cell cultures, chick embryos and weanling mice were performed with encephalomyocarditis, Coxsackie B-1, ECHO-9, Rhino-39, Influenza A_2 and HSV type 1, Vaccinia, and Adeno virus type 7. Cell cultures were permissive to high titers for 8/8 viruses; mice and chick embryos for 5/8 and 3/8, respectively. Only Vaccinia, Influenza and HSV-1 had virulence sufficient to allow all three host systems to be useful in virucide testing. Mice displayed no carry-over toxicity from various germicides; embryos had a moderate tolerance and cell cultures displayed high cytotoxicity. Embryos and mice can be considered as replacements for cell cultures in those cases where neutralization detoxification is difficult. Previous studies showed that the same antiviral agent can inactivate in vitro and in vivo by different mechanisms emphasizing differences between disinfectants and chemotherapeutics. Quaternary ammonium compounds at germicidal concentrations displayed no toxicity in mice relative to cytotoxicity. Phenol-phenate potentiated the toxicity of glutaraldehyde without widening the antiviral spectrum.

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The importance of animal research in combating viral infections

Virus infections are more difficult to treat than are infections due to bacteria and fungi. One explanation is that the symptoms of a virus infection, unlike a bacterial infection such as a strep throat or TB, appear after growth has peaked and virus concentration in the body is declining by normal means. Thus, prevention, such as with the use of disinfectants (germicides) and vaccines has always been and remain paramount. We still can not cure the common cold or viral diarrhea or influenza, much less AIDS. The importance of developing improved techniques for testing against viruses is at cross purposes with current demands for reduced animal testing, reduced toxicity by secondary exposure to man and animals, and compatibility with the environment (biodegradation).

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antiviral effects than either cell cultures or mice. Finally, although cell cultures showed great versatility in predicting the effectiveness of chemical agents as viral disinfectants in the home, environment, and in the hospital, certain potential chemotherapeutic agents or drugs for the use against viral infection in man were falsely ranked when tested by the in vitro (non-animal) systems. In one series of experiments a group of compounds produced cures of viral infections in mice in 8/15 instances ranging from herpes to influenza. However, when tested in cell cultures (non-animal alternative replacements) not one of the eight activities would have been predicted and the compounds would have fallen out of the screen. Scientists and non-scientists should be wary of over-zealous movements to entirely eliminate the use of animals in toxicity or in drug discovery.

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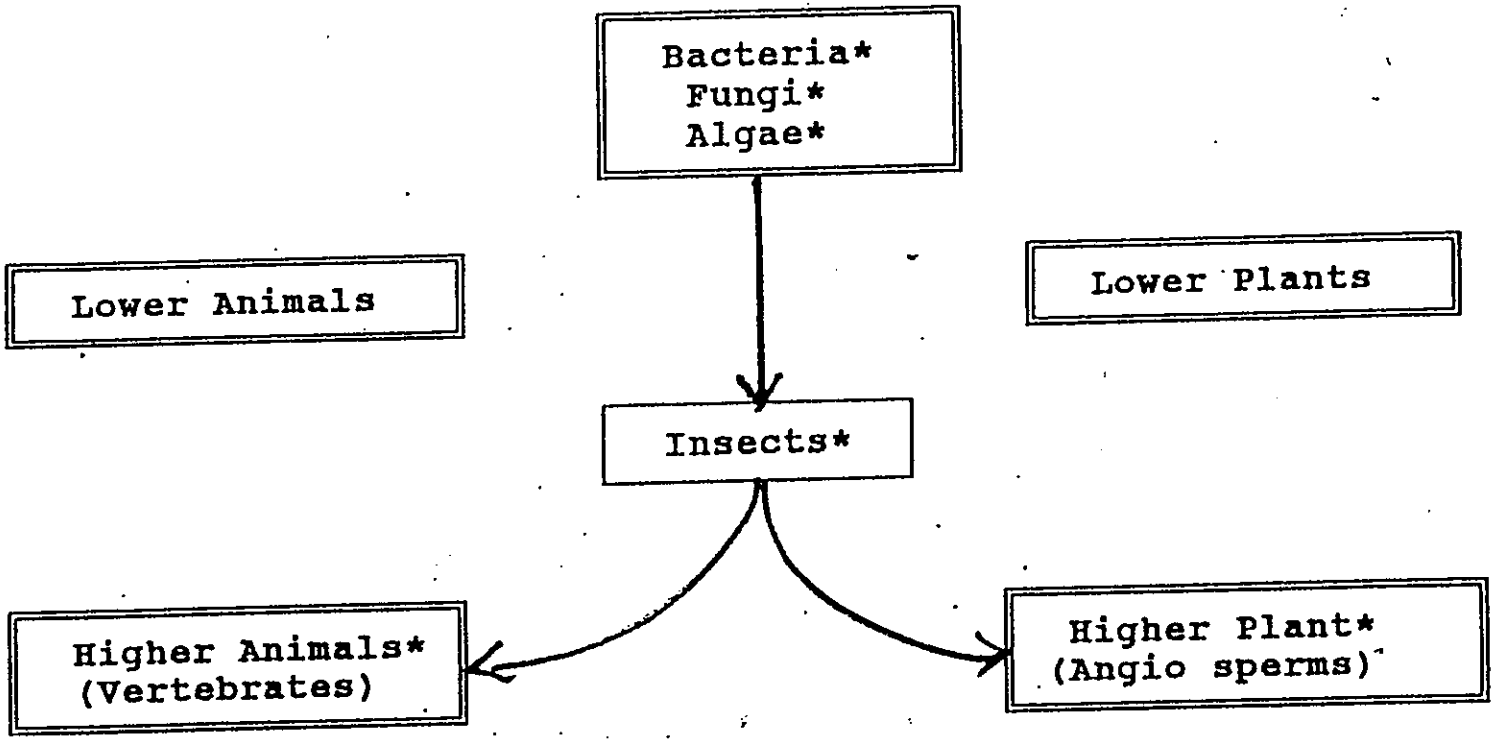
Studies have showed that non-animal testing methods such as cell cultures had the greatest utility on determining if chemical germicides could kill viruses in the environment but that animal tests were also required with certain viruses e.g. EMC virus, an agent that produces cardiac and brain infections in man and animals. In all those cases where cell cultures and mice produced equally effective test systems to judge the extent to which a chemical germicide produces an antiviral effect, the use of mice produced valuable "first instance" or "early access" information on the secondary issue of toxicity to man. It was shown that the use of cell cultures produced false predictions on how toxic certain chemicals derived from carbolic acid (phenols) and ammonia (quaternary salts) might be to a living host. Chick embryos, often espoused as replacements for whole animals, were less useful in detecting



* Viral Disease

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Germicides Are Applicable:

Site	Odors	Mildew	Slime	Infections
Home/Office	(X)	(X)	0	X (a) Respiratory fungi (SBS) (b) Home Care (AIDS) (c) Feces (d) Dermatophytes (e) Legionella
Public Facilities (restrooms gyms)	(X)	(X)	(X)	X (a) Herpes (b) Fecal Contamination (c) Dermatophytes
Restaurants	(X)	(X)	(X)	(X) (a) Salmonella (b) Staph (c) Camphylobacter (d) Enteroviruses (e) Hepatitis
Food	(X)	(X)	(X)	(X) (a) Salmonella (b) Staph (c) Campy (d) Enteroviruses (e) Hepatitis (f) Botulism
Pharmaceutical Devices	(X)	(X)	(X)	(X) (a) Sterility Contamination (b) USP Limits (c) GMP Limits (d) Pyrogens (e) Nosocomial Infections
Hospitals Laboratories	(X)	(X)	0	(X) Nosocomial (a) (environmental) (b) devices and instruments (c) hands, etc.

Bacteria and Fungi

Viruses

(X) = Applicable occurrence is highly probable
X = Applicable, occurrence is probable but not high risk
0 = Not applicable; occurrence is not probable

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E. COLI PHAGE

Recent Data (GBL):

Agent	T-1	T-4
Quaternary Compound	+	+
Phenolic	0	+
HCLO 200 ppm	0	+
50 ppm	0	0

+ = active (100% inactivation of high titered pool)

0 = inactive or incomplete

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ACCEPTED VIROLOGICAL TECHNIQUE

1. Confirmed in Publication By Several Authors And shown to perform in a Germicide Test with Active and Inactive Controls

2. No artificial manipulations to either the:

   (a) virus (alter its avidity)

   (b) cell (alter or modify the receptor apparatus)

   If infection is so difficult that you have to provide special conditions, hormones, enzymes etc. must prove that lack of infection is truly caused by inactivated virus not uncontrolled variable in the artificial conditions.

3. Germicide must inactivate the virus not the receptor apparatus.

TABLE A

TITRATION DATA OBTAINED WITH VACCINIA (Wyeth Strain)*

Host	Result	Comment	Toxicity of Germicides(a)
1. Tissue Culture (WI-38)	TCID50 = $10^{-3.5}$	0	High
2. Tissue Culture (Rhesus MkK)	TCID50 = $10^{-6.0}$	+	High
3. Chick Embryo (dropped CAM)	EID50 = $10^{-6.0}$ or higher	+	Low
4. Mice (Intracerebral)	LD50 = $10^{-6.0}$ or higher	+	Practically None

0 = Viral concentration insufficient to obviate cytotoxicity in EPA assay.

+ = Acceptable for EPA qualification, assuming no more than 3 logs of toxicity (viral concentration sufficient).

(a) General level of toxicity to be expected with most germicides.

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* H.Ep-2 cells in microtiter plates, 35°C, 5% CO_2, MEM with Earle's base, 8 days.

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TABLE B

COMPARATIVE VIRULENCE OF 7 VIRUSES IN 3 HOST SYSTEMS*

Virus	Cell Culture TCID50 (a)	Mouse LD50 mice (b)	Chick embryo EID50 (c)
Col Sk (EMC) (RNA ether-resistant)	10-4.7 10-6 .3 (d) 10-7.5	10-6.7 10-6.8 10-6.5	No Take
Herpes Simplex Type I (DNA ether-sensitive)	10-5.8 10-6.3 10-7.7	10-6.5 10-6.0 10-6.7	10-3.5 pocks 10-3.0 on CAM 10-3.0
Cox B1 RNA (ether-resistant)	10-3.8 10-6.0 10-5.5	10-4.4 10-4.5 10-4.7	No Take
ECHO 9 (Cox A23) (RNA ether-resistant)	10-5.6 10-5.5 10-4.7	No Take	No Take
Rhino (RNA ether resistant)	10-4.7 10-4.0 10-5.5	No Take	No Take
Influenza A2 (J-305-57) (RNA ether-sensitive)	10-4.3 10-5.5 10-4.0	10-4.4 intra- 10-2.8 nasal 10-2.5 infection	10-7.0 10-6.0 10-7.5
Adeno (DNA, ether var. or resistant)	10-55 10-6.0 10-4.8	No Take	No Take
Vaccinia	$10^{-6.5}$	$10^{-6.0}$	$10^{-5.0}$

(a) = CPE in Rhesus Monkey Kidney Cells at 4 to 10 days (or WI-38)
(b) = 9-12 gram weaning albino mice, 0.1 ml ip at 10 days for paralysis and death.
(c) = 9-10 day chick embryos, 0.1 ml IA, 48 hours, 35°C, CAF harvest for HA 0.5%. GPRBC (one hour at room temperature or overnight at 4°C).
(d) = Blind passage (no CPE) of 5 day supernatant via intraperitoneal injection in mice (death in 7 days if virus present in cell culture).

* Gibraltar Biological Laboratories, Inc. (unpublished data), presented at ASTM E-25 Committee Meeting: Cleveland, 1977, Philadelphia, 1978.

TABLE C

Lack of Correlation Within Suspension, Cell Culture and In Vivo Systems

Activity Profile on Direct Contact (Suspension in Saline)

Compound	A Col SK	B ECHO 9	C Cox B1	D Infl. A	E Herpes
1. DIQA					
2. Dehydroemetine					
3. Papaverine	▬▬				

Activity Profile in Tissue Culture (Rh M.K)

Compound	Col SK	ECHO 9	Cox B1	Infl. A	Herpes
4. DIQA					
5. Dehydroemetine				▬▬	
6. Papaverine					

Activity Profile In Vivo (Mice)

Compound	Col SK	ECHO 9	Cox B1	Infl. A	Herpes
7. DIQA	▬▬	▬▬		▬▬	▬▬
8. Dehydroemetine			▬▬		
9. Papaverine	▬▬		▬▬		▬▬

↓ NOT PREDICTION ↓

DIQA = Dehydroisoquinoline acetamide

As can be seen, the single contact virucidal activity could not be confirmed in vitro (T.C.),i.e., Papaverine inactivated Col SK virus in saline suspension (A-3) but had no effect against the virus in rhesus monkey kidney cells (A-6). DIQA (C-4 and dehydroemetine (D-5) were effective against Coxsackie B-1 and Influenza A respectively but were inactive in vivo (C-7 and D-8). Furthermore, none of the 8 in vivo activities were predicted from any of the in vitro results.

▬▬ = antiviral effect detected

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TABLE D

ANIMAL ASSAYS AND OFFICIAL TESTS

Virus	Cell Culture In Vitro	Animal	Official Test		
				Europe	USA
Picorna	+	+		Yes	Yes
Myxo	+	+		Yes	Yes
Herpes	+	+		Yes	Yes
Vaccinia	+	+		Yes	Yes
Rhino	+	0		Yes	Yes
Adeno	+	0		Yes	Yes
SV-40	+	+		Yes	No
HBV	0**	+	Chimp MADT*** Culture	0 + 0	+ + 0

* No reliable animal models for rhino or adeno

** No reliable or as yet reproducible method, only endangered chimp in vivo for HBV.

*** Electron microscopy - validated in chimpanzee by Thraenhart Dermietzel, Kuwert and Scheiermann.

TABLE E

Agent		Toxicity in Cell Culture						Intravenous Toxicity In Mice*				
		EPA Test - Virus Dilution										TOLERATED
QUATS FORMULATION	1000 ppm	10^{-1}	T	T	T	T		0	0	0	0	
	10.0 ppm	10^{-2}	T	T	T	T	TOXIC	0	0	0	0	
	1.0 ppm	10^{-3}	0	0	0	0		0	0	0	0	
	0.1 ppm	10^{-4}	0	0	0	0		0	0	0	0	
PHENOLIC FORMULATION	7000 ppm	10^{-1}	T	T	T	T	INVITRO	0	0	0	0	NHANCED
	700 ppm	10^{-2}	T	T	T	T		0	0	0	0	
Polio 10 min +												
Polio 5 min 0	70.0 ppm	10^{-3}	0	0	0	0		0	0	0	0	
Parvo 10 min 0												
Phage 0	7.0 ppm	10^{-4}	0	0	0	0		0	0	0	0	
GLUTERALDEHYDE FORMULATION	2000 ppm	10^{-1}	T	T	T	T	TOXICITY	T	0	0	0	INVIVO
	200 ppm	10^{-2}	T	T	T	T		0	0	0	0	
Polio 10 min +												
Polio 5 min 0	20 ppm	10^{-3}	0	0	0	0		0	0	0	0	
Parvo 10 min 0												
Phage	2.0 ppm	10^{-4}	0	0	0	0		0	0	0	0	
GLUTERALDEHYDE AND PHENOL		10^{-1}	T	T	T	T	UNALTERED	T	T	T	T	TOXICITY
		10^{-2}	T	T	T	T		T	T	0	T	
Polio 10 min +												
Polio 5 min 0		10^{-3}	0	0	0	0		0	0	0	0	
Parvo 10 min 0												
Phage 0		10^{-4}	0	0	0	0		0	0	0	0	

Antemortem Signs

*Tremors, convulsions, straub reaction, ataxia, increase respiration (one or all) or death (similar results in the chick embryo)

Combination	Yes	No
	Increased spectrum	
B + C ⟶ D	[ ]	[X]
	Increased Cytotoxicity (in vitro)	
B + C ⟶ D	[ ]	[X]
	Increased CNS and Respiratory Effects	
B + C ⟶ D	[X]	[ ]

ZIG ZAG VECTORING

(Patient→Instrument→Patient)

INVASIVE TRANSMISSION

Bronchoscopy
Angioscopy
Tracheoscopy
Colonoscopy
(Other endoscopic procedures)

Lungs:	Myxoviruses, VZV, Adeno, CMV, EB
Trachea:	Myxoviruses, VZV, Adeno, CMV, EB
Sigmoid:	Rota, Norwalk, Coxsackie, HBA, HIV, ECHO
Intestine:	Rota, Norwalk, Coxsackie, HBA, HIV, ECHO
Kidneys:	CMV, Vaccinia
Blood:	EMC, Coxsacke, HSV, Measles, HBV, HIV, EB
Brain:	Prions, Measles, Coxsackie, Encephalitides
Catheters:	

TOPICAL TRANSMISSION

Hands:	Picorna viruses, Rota, HSV, HIV, HBV
Eyes:	Picorna viruses, Rota, HSV, HIV, HBV

HBV

Woodchuck Hepatitis - low titers, availability

Duck Hepatitis - phylogenetically too far removed

Chimpanzee -

(a) Validated and accepted (Bond, Favero and Thraenhart)

(b) Note: Wasteful of the species

Morphological -

(a) Validated

(b) Unmanipulated human virions (Plasma)

(c) Time and Dilution Variables

Transfected Cultures - No marker - Research only

Newer Cell Cultures - (HEP-2G)
(Human hepatocytes etc.) (non-hepatic)
Continued research should be pursued, but:

(a) Not shown to be reproducible as of now

(b) Receptor apparatus "manipulated"

(c) Sensitivity to low titers of either normal or partially "injured" DANE particles not established .

(d) Transfected lines not necessarily infected by inact virus

(e) Viral parts can give false positive by IF, DNAase etc.

(f) Difficult to infect consistently → high concentration of intact virions

HEPATITIS VIRUSES
GERMICIDES

	Type	Official Test Europe	USA
HAV	Picorna		+
HBV	Hepadna (I) Chimp	0	+
	(II) MADT	+	+
	(III) Culture*	0	0
HCV (Non-A, None-B)	Toga (E) Flavi- (RNA)	0	0
HDV (Delta)	Viroid (RNA)	0	0
HEV (enteric Non-A, Non-B)	Calici- (RNA)	0	0

* Research in progress world-wide

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TABLE 1 A

INCUBATION TIME - VIRUCIDE ACTIVITY

RHINO			ADENO			PARVO		
7 Days	10 Days	14 Days	7 Days	10 Days	14 Days	7 Days	10 Days	14 Days
0	0	+	0	0	+	0	+	+

0 = No replication (Passes Test)

+ = Replication (Fails Test)

TABLE 2 A

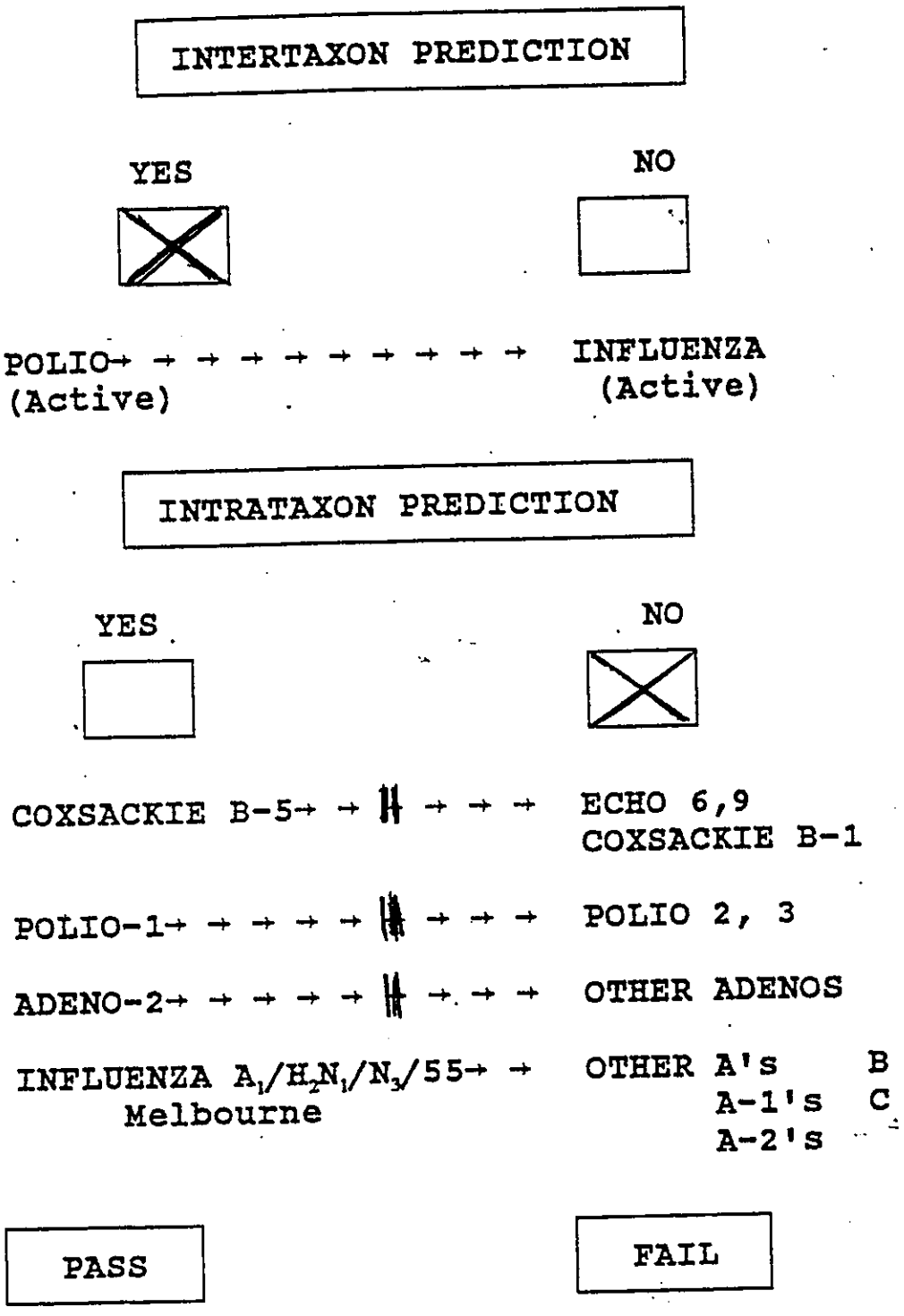
INTERTAXON PREDICTION
YES
NO
POLIO (Active)
INFLUENZA (Active)
INTRATAXON PREDICTION
YES
NO
COXSACKIE B-5
ECHO 6,9
COXSACKIE B-1
POLIO-1
POLIO 2, 3
ADENO-2
OTHER ADENOS
INFLUENZA $A_1/H_2N_1/N_3/55$ Melbourne
OTHER A's B
A-1's C
A-2's
PASS
FAIL

TABLE 3A

Human Pathogenicity of Viruses

Most Lethel

I. Marburg
Ebola
Lassa

II. Rabies

III. HIV

IV Prions

Morbidity

I Myxo virus
II Herpes viruses
III Rhino viruses
IV Adeno viruses
V Entero viruses
VI (Smallpox) (lab workers)

Least Lethal

I. Norwalk Agent
Parvo

II. Rhino virus

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TABLE 4 A

EPA/AOAC	FDA	EUROPE	ASTM
Suspension Tests	**Suspension Tests**	**Suspension Tests**	**Suspension and Carrier Tests**
• TB (phenol coefficient) • E. coli (detergent-sanitizers) • Staph (detergent-sanitizers) • Pathogenic fungi • Algae	• Spores	• Bacteria • Fungi • Viruses • (Disinfectants antiseptics-hands)	
Carrier Tests	**Carrier Tests**		
• TB • Spores • Bacteria • Fungi • Viruses	• AOAC • Spores		
	• Organic Soil • Refeeding • Cell Lines		

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