DOSSIER SCIENTIFIQUE

RELY+ON™ VIRKON®

Nettoyant désinfectant pour les sols et surfaces

Laboratoire phagogène®
9, Rue Marcel Sembat 44100 NANTES
Tél: +33 (0)2 40 57 56 10 / Fax : +33 (0)2 40 57 56 39
www.phagogene.fr

SOMMAIRE

1. INTRODUCTION

Le **RELY+ONTM VIRKON®** est un nettoyant désinfectant de toutes les surfaces hautes et basses.

Le **RELY+ONTM VIRKON®** est efficace sur plus de 20 familles de virus, 43 genres de bactéries et de nombreuses souches fongiques.

Le **RELY+ONTM VIRKON®** peut être utilisé pour le nettoyage et la désinfection des surfaces pouvant entrer en contact avec les denrées alimentaires si la désinfection est suivie d'un rinçage à l'eau.

Le **RELY+ONTM VIRKON®** peut être utilisé en présence d'animaux dans les animaleries de laboratoire.

2. COMPOSITION

- ✓ Actif désinfectant : Bis(peroxymonosulfate) bis (sulfate) de pentapotassium (N° CAS : 70693-62-8 ; 50,6% m/m)

3. CARACTERISTIQUES ORGANOLEPTIQUES ET PHYSICO-CHIMIQUES

- ✓ Aspect : poudre ou pastille blanche
- ✓ Couleur de la solution reconstituée : rose, devient incolore quand le pourcentage en matière active est insuffisant pour assurer l'efficacité revendiquée
- ✓ pH à 20°C en solution : 2,6 +/- 0,1

4. MODE D'EMPLOI

Utilisation des pastilles

- ✓ Mettre une pastille de 5 g pour 0,5 litre d'eau dans votre contenant (pulvérisateur)
- ✓ Attendre que la (es) pastille(s) soit(ent) complétement dissoute(s) avant utilisation

Utilisation des sachets :

- ✓ Verser un sachet de 50 g pour 5 litres d'eau dans votre contenant (seau, bac de trempage)
- ✓ Mélanger
- ✓ Attendre que la poudre soit complètement dissoute avant utilisation

- ✓ Après dissolution de la poudre ou de la pastille dans l'eau, obtention d'une solution rose (présence d'un marqueur sur le principe actif)
- ✓ Dose à appliquer : 30ml à 40ml/m²
- ✓ Appliquer la solution sur la surface à traiter
- ✓ Laisser agir entre 15 et 30 minutes en fonction de l'action recherchée
- ✓ Rincer à l'eau pour les surfaces pouvant entrer en contact avec les denrées alimentaires

5. ACTIVITE ANTIMICROBIENNE

NORMES	CONCENTRATION	TEMPS (MINUTES)	N° RAPPORT ET DATE	LABORATOIRE D'EXPERTISE
BACTERICIDE				
NF T 72-171 spectre 4 en eau dure (*P.aeruginosa, S.aureus, E.coli et E.hirae*)	0,5%	5 min.	MIC. 95/08-084 ED du 14/09/1995	ADREMI
EN 1276 en cond. saleté (*P.aeruginosa, S.aureus, E.hirae, E.coli et L.monocytogenes, S.typhimurium, Y.enterocolitica*)	1%	5 min.	FH\REP\73540\1 du 07/08/2003	CCFRA Technology Limited
FONGICIDE				
EN 1650 *((C.albicans, A.niger S.cerevisiae)*	2,5% 3% 2,5%	15 min. 15 min. 60 min.	FH\REP\73540\2 du 12/08/2003	CCFRA Technology Limited
VIRUCIDE				
NF T 72-180 (Poliovirus type 1, Adenovirus type 5, orthopoxvirus de la vaccine)	1%	15 min.	29/04/1992	Université Paris Sud
Actif sur Poliovirus type 2	1%	10 min.	9/11/1990	Severn Trent
Actif sur Rotavirus	0,4%	1 min.	-	-
Actif sur Hépatite B	1%	10 min.	01/07/1987	Université de Londres
Actif sur Hépatite C	1%	10 min.	28/06/1999	Micropathology Limited
Actif sur HIV 1	1%	10 min.	1566 du 21/09/1995	ViroMed

Les rapports d'études sont consultables en annexes.

6. CONDUITES D'URGENCE

Conduites d'urgence :

- ✓ Numéro national d'appel d'urgence ORFILA : 01 45 42 59 59

- ✓ La fiche de données de sécurité est à la disposition des utilisateurs sur le site http://www.quickfds.com. Ce site vous permet ensuite d'être informé automatiquement par email de toute mise à jour des fiches de données de sécurité consultées.

7. CONSERVATION

- ✓ Le **RELY+ON™ VIRKON®** a une durée de vie de 36 mois dans les conditions de stockage à température ambiante.
- ✓ La solution reconstituée reste stable pendant 5 jours ou décoloration de la solution

8. CONDITIONNEMENTS

Présentation	Code	Nombre d'unités par carton
Boite de 50 pastilles de 5g	60007	Carton de 12 unités
Sachet de 50g	60008	Carton de 50 unités
Seau de 5kg	60464	Carton de 1 unité

9. ANNEXES

A. D. R. E. M. I. - Tours

LABORATOIRE DE MICROBIOLOGIE - IMMUNOLOGIE
FACULTE DES SCIENCES PHARMACEUTIQUES
31, avenue Monge - 37200 TOURS
Tél. : 47 36 71 91 ou 92 - Fax : 47 36 71 96

VIRKON "S"®

DETERMINATION DE L'ACTIVITE BACTERICIDE EN PRESENCE D'EAU DURE - SPECTRE 4 -

- méthode par filtration sur membranes -

Pr. Jean-Paul CHIRON
Bernard YVONNET
Nicole GUILLET
Virginie MOUSSET

Norme CNEVA - LMV selon
AFNOR NF T 72-171 / eau dure (1988)

A LA DEMANDE DE :

ANTEC International Ltd
Windham Road
Sudbury
SULLFOLK CO10 6XD
England

ETUDE MIC. 95/08-084 ED
TOURS, le 14.09.1995

VIRKON "S"®

DETERMINATION DE L'ACTIVITE BACTERICIDE EN PRESENCE D'EAU DURE - SPECTRE 4 -

- méthode par filtration sur membranes -

SOMMAIRE

ATTESTATION D'EXPERTISE

A la demande des Laboratoires : **ANTEC International Ltd**
Windham Road
Sudbury
SULLFOLK CO10 6XD
England

je soussigné,

Professeur Jean-Paul CHIRON, Expert Analyste (Microbiologie) agréé par le Ministère de la Santé - nomination : B.O. liste n° 82/1, arrêté du 07 Décembre 1981 ; renouvellement : B.O. liste n° 87/1, arrêté du 03 Février 1987 - certifie avoir procédé à l'expertise microbiologique du produit :

VIRKON "S"®
lot du 07 juin 1995

La détermination de l'activité bactéricide, spectre 4, en présence d'eau dure (étude MIC. 95/08-084 ED) a été effectuée selon les exigences minimales émises par le CNEVA - LMV de FOUGERES et la méthodologie de la norme AFNOR NF T 72-171 (1988) - méthode par filtration sur membranes -.

La réalisation technique de cette norme a été assurée par Mesdames Nicole GUILLET et Virginie MOUSSET (techniciennes DELAM) sous la responsabilité de Monsieur Bernard YVONNET, Docteur d'Etat ès-Sciences Pharmaceutiques, Directeur d'Etude.

Le procès verbal est rédigé conformément à la norme. Toutefois, celui-ci est complété par un tableau correspondant à la validation de l'essai proprement dit et quatre annexes (un bulletin de contrôle, une annexe technique et deux annexes rapportant l'ensemble des valeurs expérimentales).

TOURS, le 14 septembre 1995

FACULTÉ DES SCIENCES PHARMACEUTIQUES ADREMI 31, Avenue Monge 37200 TOURS

Professeur Jean-Paul CHIRON
Expert Microbiologie

VIRKON "S"®

DETERMINATION DE L'ACTIVITE BACTERICIDE EN PRESENCE D'EAU DURE - SPECTRE 4 -

- méthode par filtration sur membranes -

1. LABORATOIRE AYANT REALISE L'ESSAI

Laboratoire de Microbiologie - Immunologie
Faculté des Sciences Pharmaceutiques
31, Avenue Monge
37200 TOURS

2. IDENTIFICATION COMPLETE DE L'ECHANTILLON

Nom du produit	:	VIRKON "S"®
Lot n°	:	07 juin 1995
Fabricant	:	ANTEC International Ltd
Date de réception au laboratoire	:	13 juin 1995
Conditions de stockage au laboratoire	:	21 ° ± 1 °C
Période de l'étude	:	08/08/1995 au 07/09/1995
Substance(s) active(s) et concentration(s)	:	Bulletin de contrôle (Annexe 1)

3. CONDITIONS EXPERIMENTALES

3.1. TEMPERATURE DE L'ESSAI : 20 ° ± 1 °C

3.2. TEMPS DE CONTACT : cinq (5) minutes

3.3. DILUANT DU PRODUIT

- recommandé par le fabricant : néant
- utilisé lors des essais : eau déminéralisée stérile

3.4. SUBSTANCE INTERFERENTE

- eau dure : dureté 30 ° Français (§ 7.6.4)

4. MODE OPERATOIRE DETERMINE A LA SUITE DE L'ESSAI PRELIMINAIRE

4.1. MEMBRANES

- Nature : Filtres membrane Durapore hydrophile 0,45 µm
- Référence / lot n° : MILLIPORE HVLP 04700 / lot n° R5DM63370

4.2. LIQUIDE DE RINÇAGE

- Nature : Solution tryptone sel
- Mode de fabrication : Conforme à la norme (§ 7.3)
- Nombre de lavages avec le diluant : trois (3)
- Volume du diluant pour chaque lavage : 50 ml

4.3. NEUTRALISANT(S) AJOUTE(S) AU MILIEU DE DENOMBREMENT (§ 7.5) ET CONCENTRATION(S)

- Neutralisant / Essais préliminaires : D/E (composition dans l'annexe 2)
- Neutralisant / Essais proprement dit : néant

N. B. : L'annexe 2 précise l'ensemble des renseignements et références concernant le matériel, les réactifs et les milieux de culture utilisés pour la réalisation technique de cette norme.

5. RESULTATS DE L'ESSAI PRELIMINAIRE DANS LES CONDITIONS DECRITES CI-DESSUS

Les moyennes des dénombrements (deux essais) et les concentrations étudiées du produit, VIRKON"S"® - lot du 07 juin 1995 -, sont rapportées dans le tableau 1.

Tableau 1 : ESSAIS PRELIMINAIRES (§ 9)
VIRKON "S"® - lot du 07 juin 1995

CONCENTRATIONS ESSAYEES DU PRODUIT (% V/V)	SOUCHES, collection d'origine et numéro dans la collection	N	N'	n	n'
2	*Pseudomonas aeruginosa* CIP A22	***	192	159	153
2	*Escherichia coli* CIP 54.127	182	182	182	193
2	*Staphylococcus aureus* CIP 53.154	129	118	196	156
2	*Enterococcus hirae* CIP 58.55	160	191	246	236

N : Nombre d'unités formant colonies dans 1 ml de la dilution 10^{-6} de la suspension bactérienne en l'absence du mélange produit-substance interférente (dénombrement dans la masse : § 9.2.4)

N' : Nombre d'unités formant colonies dans 1 ml de la dilution 10^{-6} de la suspension bactérienne en l'absence du mélange produit-substance interférente (dénombrement par filtration : § 9.2.6)

n : Nombre d'unités formant colonies dans 1 ml de la dilution 10^{-6} de la suspension bactérienne après contact préalable produit-substance interférente/membrane - *sans neutralisant* - (dénombrement par filtration : § 9.3.3)

n' : Nombre d'unités formant colonies dans 1 ml de la dilution 10^{-6} de la suspension bactérienne après contact préalable produit-substance interférente/membrane - *avec neutralisant* - (dénombrement par filtration : § 9.3.3)

*** : non pratiqué (*P.aeruginosa* : aérobie strict)

Les conditions expérimentales adoptées pour chacune des souches sont applicables lors de l'essai proprement dit pour les concentrations du produit, VIRKON "S"® - lot du 07 juin 1995 -, essayées (§ 9.4) :

- le nombre de bactéries par ml de la suspension bactérienne est compris entre 0,5 et $1,5.10^8$,
- la méthode par filtration n'entraîne pas d'effet antimicrobien ($N' \geq 0,5\ N$),
- le mélange produit-substance interférente aux concentrations étudiées n'entraîne pas, de phénomène de bactériostase ($n \geq 0,5\ N'$) sur la membrane filtrante,
- l'utilisation d'un neutralisant dans le milieu de dénombrement n'est pas nécessaire.

Les valeurs expérimentales des essais préliminaires sont rapportées dans l'annexe 3.

6. ESSAI PROPREMENT DIT

6.1. RESULTATS DANS LES CONDITIONS DECRITES CI-DESSUS

Les résultats expérimentaux obtenus lors de l'essai proprement dit sont validés pour le produit, VIRKON "S"® - lot du 07 juin 1995 -, à la concentration de 2,0 % (V/V) (tableau 2), en effet :

- le nombre de bactéries (N) par ml de chacune des suspensions bactériennes est compris entre 0,5 et $1,5.10^8$ (§ 10.5.1)
- pour les concentrations maximales d'essai, il est vérifié pour chacune des souches que (§ 11.2) :

$$N' \geq 0,5\ N$$
$$n \geq 0,5\ N'$$

Tableau 2 : *VALIDATION DE L'ESSAI PROPREMENT DIT*
VIRKON "S"® - lot du 07 juin 1995

	N	N'/N	n/N'
Pseudomonas aeruginosa CIP A22	***	***	**0,87**
Escherichia coli CIP 54.127	**159**	**1,10**	**0,95**
Staphylococcus aureus CIP 53.154	**142**	**1,15**	**0,98**
Enterococcus hirae CIP 58.55	**128**	**0,95**	**1,08**

*** : non pratiqué (*P. aeruginosa* : aérobie strict)

Les moyennes des dénombrements (deux essais) et les valeurs de pH minimal et maximal sont rapportées dans le tableau 3. L'annexe 4 précise l'ensemble des données expérimentales de l'essai proprement dit du produit, VIRKON "S"® - lot du 07 juin 1995 -.

Tableau 3 : *ESSAI PROPREMENT DIT*
VIRKON "S"® - lot du 07 juin 1995

SOUCHES, collection d'origine et numéro dans la collection	N	n	N'	X Concentrations en pourcentage (V/V) au contact avec les bactéries					pH	
				2,0	1,0	0,5	0,1	0,05	min	max
Pseudomonas aeruginosa CIP A22	***	108	124	0	1	0	+	+	2,31	4,80
Escherichia coli CIP 54.127	159	166	175	0	0	88	+	+	2,15	4,05
Staphylococcus aureus CIP 53.154	142	160	163	0	6	0	1	1	2,19	4,68
Enterococcus hirae CIP 58.55	128	132	122	0	2	25	+	+	2,29	4,25

*** :non pratiqué (*P. aeruginosa*)

N : Nombre d'unités formant colonies dans 1 ml de la dilution 10^{-6} de la suspension bactérienne en l'absence du mélange produit-substance interférente (dénombrement dans la masse : § 10.5.1)

n : Nombre d'unités formant coloniesdans 1 ml de la dilution 10^{-6} de la suspension bactérienne après contact préalable produit-substance interférente/membrane (dénombrement par filtration : § 10.5.3)

N' : Nombre d'unités formant colonies dans 1 ml de la dilution 10^{-6} de la suspension bactérienne en l'absence du mélange produit-substance interférente (dénombrement par filtration : § 10.5.1)

X : Nombre d'unités formant colonies dans 1 ml de la suspension bactérienne après contact avec le mélange produit-substance interférente (dénombrement par filtration : § 10.5.2)

6.2. CONCENTRATIONS BACTERICIDES DU PRODUIT

Les concentrations bactéricides du produit, VIRKON "S"® - lot du 07 juin 1995 -, sont celles pour lesquelles :

$$X \leq N' \quad si \quad N \approx N' \approx n \ (\S\ 11)$$

soit :

- **0,50 %** (V/V) pour *Pseudomonas aeruginosa* CIP A22
- **0,50 %** (V/V) pour *Escherichia coli* CIP 54.127
- **≤ 0,05 %** (V/V) pour *Staphylococcus aureus* CIP 53.154
- **0,50 %** (V/V) pour *Enterococcus hirae* CIP 58.55

7. CONCLUSION

Le produit, VIRKON "S"® lot du 07 juin 1995, présente une activité bactéricide, spectre 4, en présence d'eau dure (titre hydrotimétrique dans l'essai 30 ° Français) après un temps de contact de cinq (5) minutes à 20° C selon la norme AFNOR NF T 72-171 (1988).

TOURS, le 14 septembre 1995

Nicole GUILLET
Technicienne (DELAM)

Virginie MOUSSET
Technicienne (DELAM)

Bernard YVONNET
Docteur d'Etat ès-Sciences Pharmaceutiques
Directeur d'étude

FACULTE DES SCIENCES PHARMACEUTIQUES ADREMI 31, Avenue Monge 37200 TOURS

Professeur Jean-Paul CHIRON
Expert Microbiologie

ANNEXES

ANNEXE 1 : BULLETIN DE CONTROLE
VIRKON "S"® - lot du 07 juin 1995

ANNEXE 2 : ANNEXE TECHNIQUE

ANNEXE 3 : VALEURS EXPERIMENTALES DE L'ESSAI PRELIMINAIRE
VIRKON "S"® - lot du 07 juin 1995

ANNEXE 4 : VALEURS EXPERIMENTALES DE L'ESSAI PROPREMENT DIT
VIRKON "S"® - lot du 07 juin 1995

ANNEXE 1

BULLETIN DE CONTROLE

VIRKON "S"®

lot du 07 juin 1995

(document fourni par le fabricant)

Antec
International Ltd

Chilton Industrial Estate, Sudbury, Suffolk CO10 6XD,
England. Telephone: Sudbury (01787) 377305 (6 lines)
Telex: 987495 Telefax: (01787) 310846

CERTIFICATE OF ANALYSIS

Consignee: Dr P Chiron
University of Tours
France

ANTEC VIRKON S

Sample Size:	500 G
Batch Dated:	7th June 1995
pH (1%, 2.35-2.65):	2.44
Available Oxygen (Minimum 10 0%):	10.53%

The sample conforms to the Quality Control Specification for Virkon S.

For and on behalf of
ANTEC INTERNATIONAL LIMITED

Mark Squire
Chief Chemist

MS/SI/7.6.95

ANNEXE 2

ANNEXE TECHNIQUE

1. APPAREILLAGE, VERRERIE ET PETIT MATERIEL

- Appareil à osmose inverse MILLI Q WATER SYSTEM MILLI-RO, MILLIPORE
- Four Poupinel THERMOSI® SR 3000
- Autoclave LEQUEUX
- Congélateur à - 80 °C SANYO " ULTRA LOW"
- Hotte à flux laminaire vertical, Microflow Biohazard Classe 2, MDH Ltd
- Etuve bactériologique (37 ° ± 1 °C), MEMMERT type B 40
- Bain d'eau réglé à 20 ° ± 1 °C, n° 3163 BMP 60
- Balance analytique, SARTORIUS 2004 MP
- Agitateur, BIOBLOCK Heidolph Top-Mix 94323, type REAX 2000
- pH-mètre, BIOBLOCK 93302 HI 1211
- Spectrophotomètre, SCIENTIFIC INSTRUMENTS, Cam Spec M302 (longeur d'onde : 620 ± 20 nm)
- Chronomètre, BIOBLOCK Digital timer Cole Parmer
- Appareil de filtration, MILLIPORE de 50 ml de volume utile pour membranes (∅ : 47 à 50 mm)
- Pinces brucelles à extrémités plates, stérilisables par flambage
- Tubes à essai, CORNING Réf. : 400446 (Borosilicate glass)
- Boites de Pétri (∅ 90 mm), CONSORTIUM MATERIELS LABORATOIRES, Réf. 2262S 90 EL
- Flacons à bouchons à visser en verre stérilisable
- Pipettes graduées stériles (1 ml, 5 ml et 10 ml), CORNING, Réf. : 7077-1N, 7077-5N et 7077-10N
- Cuves pour spectrophotométrie, CONSORTIUM MATERIEL LABORATOIRES, SMCU 1
- Membranes filtrantes stériles, MILLIPORE HVLP 04700, lot n° R5DM63370

2. REACTIFS

- Eau déminéralisée obtenue par procédé MILLIPORE à osmose inverse (résistivité = 18 mégohms.cm)
- Chlorure de sodium, PROLABO, lot n° 160YR
- Pastone pancréatique, SANOFI DIAGNOSTICS PASTEUR, lot n° 4L108y
- Thioglycolate de sodium, SERVA, lot n° control D7
- Thiosulfate de sodium, PROLABO, lot n° 89051
- Bisulfite de sodium, PROLABO, lot n° 68191
- Glucose anhydre, LABOSI, lot n° 4595087
- Tween 80, PROLABO, lot n° 129YR
- Lécithine d'oeuf, LABOSI, lot n° 1995124
- Extrait de levure deshydraté pour bactériologie, SANOFI DIAGNOSTICS PASTEUR, lot n° 4A109y
- $CaCl_2$ anhydre, SIGMA, lot n° 53H0617
- $MgCl_2$, SIGMA, lot n° 109F0049
- $NaHCO_3$, PROLABO, lot n° 85 303

3. DILUANTS ET MILIEUX DE CULTURE

- Solution tryptone-sel : préparée conformément à la norme (§ 7.3)
- Gélose trypto caséine soja, SANOFI DIAGNOSTICS PASTEUR, lot n° 4K115y
- Gélose de dénombrement, SANOFI DIAGNOSTICS PASTEUR, lot n° 5G112y

4. SUBSTANCE INTERFERENTE

- Solution A

  $MgCl_2$ anhydre ..... 31,74 g
  $CaCl_2$ anhydre ..... 73,99 g
  dissoudre dans un litre d'eau distillée et stériliser par autoclavage.

- Solution B

  $NaHCO_3$ ..... 56,03 g
  dissoudre dans un litre d'eau distillée et stériliser par filtration sur membrane.

- Préparation

  Eau stérile ..... 50,00 ml
  Solution A ..... 0,75 ml
  Solution B ..... 1,00 ml
  Eau stérile ..... q.s.p. 100,00 ml
  La dureté finale dans l'essai est de 30 ° Français.

5. NEUTRALISANT

- Composition Solution D/E :

Lécithine d'oeuf	7,00 g
Extrait de levure	2,50 g
Thioglycolate de sodium	1,00 g
Thiosulfate de sodium	6,00 g
Bisulfite de sodium	2,50 g
Pastone	5,00 g
Glucose anhydre	10,00 g
Tween 80	5,00 g
Bromocrésol pourpre	0,02 g
Eau distillée	qsp 1000 ml

- Gélose neutralisante :

Gélose trypto caséine soja additionnée de solution neutralisante D/E à 10 % (V/V).

ANNEXE 3 : Valeurs expérimentales de l'essai préliminaire

VIRKON "S"® - lot du 07 juin 1995

Concentrations essayées du produit (% V/V)	SOUCHES, collection d'origine et numéro dans la collection		N (§ 9.2.4)	N' (§ 9.2.6)	n (§ 9.3.3)	n' (§ 9.3.3)	N'/N	n/N
2	***Pseudomonas aeruginosa*** CIP A22	d	***	208/175	152/166	151/155	***	***
		m		192	159	153		
2	***Escherichia coli*** CIP 54.127	d	182/181	186/178	180/184	189/197	1,00	1,00
		m	182	182	182	193		
2	***Staphylococcus aureus*** CIP 53.154	d	128/129	124/112	191/201	159/153	0,91	1,52
		m	129	118	196	156		
2	***Enterococcus hirae*** CIP 58.55	d	155/164	193/189	226/266	234/237	1,19	1,54
		m	160	191	246	236		

d : dénombrements (1er / 2 ème essai)
m : moyenne

*** : non pratiqué (*P. aeruginosa* : aérobie strict)

ANNEXE 4 : Valeurs expérimentales de l'essai proprement dit
VIRKON "S"® - lot du 07 juin 1995

SOUCHES, collection d'origine et numéro dans la collection		X (§ 10.5.2) Concentrations essayées du produit au contact avec les bactéries (% V/V)					N (§ 10.5.1)	n (§ 10.5.3)	N' (§ 10.5.1)
		2,0	1,0	0,5	0,1	0,05			
Pseudomonas aeruginosa	d	0/0	1/1	0/0	+/+	+/+		100/115	128/119
CIP A22	m	0	1	0	+	+	***	108	124
	pH	2,31	2,66	2,93	3,99	4,80			
Escherichia coli	d	0/0	0/0	126/49	+/+	+/+	163/154	161/170	170/180
CIP 54.127	m	0	0	88	+	+	159	166	175
	pH	2,15	2,33	2,58	3,55	4,05			
Staphylococcus aureus	d	0/0	8/3	0/0	0/1	1/0	158/126	157/162	175/150
CIP 53.154	m	0	6	0	1	1	142	160	163
	pH	2,19	2,25	2,55	3,63	4,68			
Enterococcus hirae	d	0/0	3/0	44/5	+/+	+/+	123/133	136/127	118/126
CIP 58.55	m	0	2	25	+	+	128	132	122
	pH	2,29	2,35	2,73	3,41	4,25			

d : dénombrements (1er / 2ème essai) m : moyenne

*** : non pratiqué (*P. aeruginosa* : aérobie strict)

CCFRA Technology Limited
Chipping Campden
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Investigation into the Effectiveness of VIRKON S (FH/73540/1) when Tested in Accordance with BS EN 1276:1997 - 'Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic and institutional areas' (TES-FH-004)

CONFIDENTIAL TO: Mr. Mark Squire,
Chief Chemist,
Antec International Limited,
Windham Road,
Chilton Industrial Estate,
Sudbury,
Suffolk, CO10 2XD

Karen Hall

7th August 2003

Report Approved By : Report Checked By :

Name :

Approved Signatories:	Dr. J.T. Holah	Head of Food Hygiene Department
	Dr. K.L. Brown	Manager, Grade 3

SUMMARY

When tested in accordance with BS EN 1276:1997 the Virkon S tested at 1% v/v possesses bactericidal activity in 5 minutes at 10°C under clean (0.03% bovine albumen) and dirty (0.3% bovine albumen) conditions. Against the four standard organisms - *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli* and *Enterococcus hirae* plus *Listeria monocytogenes, Salmonella* Typhimurium, *Yersinia enterocolitica* and *Escherichia coli* O157:H7. Thus meeting the bacterial requirements only of the M&S protocol TES-FH-013; part 1.

INTRODUCTION

Antec International requested the Food Hygiene Department of CCFRA Technology Limited (CCFRA) to assess the efficacy of Virkon S, for bactericidal activity in accordance with BS EN 1276:1997 - 'Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic and institutional areas' (TES-FH-004). With the addition of *Listeria monocytogenes, Salmonella* Typhimurium, *Yersinia enterocolitica* and *Escherichia coli* O157:H7.

METHOD

For the bactericidal tests, BS EN 1276:1997 - 'Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic and institutional areas' (TES-FH-004) was followed.

Bactericidal activity in general use conditions

a)	IDENTIFICATION OF THE TEST LABORATORY	**Food Hygiene Department, CCFRA**
b)	IDENTIFICATION OF THE SAMPLE	
	Name of the product	**VIRKON S**
	Batch number	**PJW 1/87/B 500g**
	Manufacturer	**Antec International**
	Condition on receipt	**OK**
	Date of delivery	**13th June, 2003**
	Storage conditions	**Food Hygiene Sample Store, Dark, Ambient**
	Product diluent recommended by the manufacturer for use	**Water of Standard Hardness (WSH)**
	Active substance(s) and its (their) concentrations(optional)	**N/A**
c)	EXPERIMENTAL CONDITIONS	
	Period of analysis	**16th June, 2003 - 15th July, 2003**
	Product diluent used during the test	**Water of Standard Hardness (WSH)**
	Product test concentrations	**0.5%, 1.0% and 2.0%**
	Appearance product dilutions	**Clear**
	Contact time	**Bactericidal Tests – 5 mins ±10 secs**
	Test temperature	**Bactericidal Tests - 10°C ±1°C**
	Interfering substance	**Bovine Serum Albumin @ 0.03% ("CLEAN") and 0.30% ("DIRTY") in Sterile Distilled Water (SDW)**
	Stability of the mixture (interfering substance and product diluted in hard water)	**Some precipitation formation at 2% in Dirty conditions. Breaks down on agitation**
	Temperature of incubation	**Bactericidal Tests - 37°C ± 1°C**

c) EXPERIMENTAL CONDITIONS (CONTINUED)

Identification of bacterial strains used	**Standard bactericidal test strains -**
	Escherichia coli **Ec FH 64/a**
	Enterococcus hirae **Eh FH 65/a**
	Pseudomonas aeruginosa **Pa FH 72/a**
	Staphylococcus aureus **Sa FH 73/a**
	plus
	Listeria monocytogenes **Lm FH 66/a**
	***Salmonella* Typhimurium** **St FH 68/a**
	Yersinia enterocolitica **Ye FH 67/a**

d) OPERATING PROCEDURE

Methods used	**Bactericidal Test – BS EN 1276:1997**
Deviations from methods	**NONE**
Neutraliser method	**Dilution Neutralisation**
Neutraliser/ rinse media	**BS Inactivator**

e) TEST RESULTS **PASS @ 1.0%**

f) CONCLUSION **According to BS EN 1276:1997 the supplied 'Virkon S' (FH/73540/1), at the in use concentration (1%) possess bactericidal activity in 5 minutes at 10°C under clean and dirty conditions for the referenced strains…**

Escherichia coli
Enterococcus hirae
Pseudomonas aeruginosa
Staphylococcus aureus
plus
Listeria monocytogenes
***Salmonella* Typhimurium**
Yersinia enterocolitica

N.B. KEY TO CODES ON RESULT SHEETS...

Vc:	viable count
R:	reduction in viability
Na:	the number of cfu/ml in the test mixture (dilution factor of 10^{-1})
C:	the number of cfu/ml in the dilution neutralisation test control, or of the membrane filtration test control
Nv:	the number of cfu/ml of the bacterial test suspension (dilution factor of 10^{-1})
A:	the number of cfu/ml of the experimental conditions control
B:	the number of cfu/ml of the neutraliser toxicity control or of the filtration control
N:	the number of cfu/ml in the bacterial or fungal suspension (dilution factor of 10^{-6} for Bacterial Tests, 10^{-5} For Fungicidal Tests

Test criteria	Validation Test				Test suspension	Test procedure at concentration % (v/v / w/w)				Result
						cfu/ml in test pot	0.5%	1.0%	2.0%	Pass/Fail/ Invalid
Escherichia coli FH 64/a Clean	Vc: 148, 166 –2 13, 21 Nv: 1.6 x 10^3	Vc: 135, 154 A: 1.4 x 10^2	Vc: 172, 167 B: 1.7 x 10^2	Vc: 269, 218 C: 2.4 x 10^2	Vc: 171, 182 –7 22, 21 N: 1.8 x 10^8	1.8 x 10^7	Vc: 0, 0 Na: <1.5 x 10^2 R: >10^5	Vc: 0, 0 Na: <1.5 x 10^2 R: >10^5	Vc: 0, 0 Na: <1.5 x 10^2 R: >10^5	**Pass @ 0.5%**
Escherichia coli FH 64/a Dirty	Vc: 148, 166 –2 13, 21 Nv: 1.6 x 10^3	Vc: 165, 167 A: 1.6 x 10^2	Vc: 172,167 B: 1.7 x 10^2	Vc: 187, 218 C: 2.0 x 10^2	Vc: 171, 182 –7 22, 21 N: 1.8 x 10^8	1.8 x 10^7	Vc: 0, 0 Na: <1.5 x 10^2 R: >10^5	Vc: 2, 3 Na: <1.5 x 10^2 R: >10^5	Vc: 0, 0 Na: <1.5 x 10^2 R: >10^5	**Pass @ 0.5%**
Staphylococcus aureus FH 73/b Clean	Vc: 208, 252 –2 22, 23 Nv: 2.3 x 10^3	Vc: 195, 220 A: 2.1 x 10^2	Vc: 259, 255 B: 2.6 x 10^2	Vc: 229,276 C: 2.5 x 10^2	Vc: 266, 271 –7 28, 26 N: 2.7 x 10^8	2.7 x 10^7	Vc: 0, 0 Na: <1.5 x 10^2 R: >10^5	Vc: 0, 0 Na: <1.5 x 10^2 R: >10^5	Vc: 0, 0 Na: <1.5 x 10^2 R: >10^5	**Pass @ 0.5%**
Staphylococcus aureus FH 73/b Dirty	Vc: 208, 252 –2 22, 23 Nv: 2.3 x 10^3	Vc: 250, 229 A: 2.4 x 10^2	Vc: 259, 255 B: 2.6 x 10^2	Vc: 252, 273 C:2.6 x 10^2	Vc: 266, 271 –7 28, 26 N: 2.7 x 10^8	2.7 x 10^7	Vc: >300, >300 Na: >1.5 x 10^2 R: <10^5	Vc: 2, 1 Na: <1.5 x 10^2 R: >10^5	Vc: 0, 0 Na: <1.5 x 10^2 R: >10^5	**Pass @ 1.0%**
Enterococcus hirae FH 65/a Clean	Vc: 217,216 –2 13, 21 Nv: 2.1 x 10^3	Vc: 209, 219 A:2.1 x 10^2	Vc: 224, 222 B: 2.2 x 10^2	Vc: 234, 200 C: 2.2 x 10^2	Vc: 230, 241 –7 21, 29 N: 2.4 x 10^8	2.4 x 10^7	Vc: 10, 10 Na: <1.5 x 10^2 R: >10^5	Vc: 0, 0 Na: <1.5 x 10^2 R: >10^5	Vc: 0, 2 Na: <1.5 x 10^2 R: >10^5	**Pass @ 0.5%**
Enterococcus hirae FH 65/a Dirty	Vc: 217, 216 –2 13, 21 Nv: 2.1 x 10^3	Vc: 178, 250 A: 2.1 x 10^2	Vc: 224, 222 B: 2.2 x 10^2	Vc: 240, 236 C: 2.4 x 10^2	Vc: 230, 241 –7 21, 29 N: 2.4 x 10^8	2.4 x 10^7	Vc: >300, >300 Na: >1.5 x 10^2 R: <10^5	Vc: 20, 24 Na: 2.2 x 10^2 R: >10^5	Vc: 0, 0 Na: <1.5 x 10^2 R: >10^5	**Pass @ 1.0%**
Pseudomonas aeruginosa FH 72/b Clean	Vc: 247, 209 –2 33, 33 Nv: 2.4 x 10^3	Vc: 272, 276 A: 2.7 x 10^2	Vc: 190, 208 B: 1.9 x 10^2	Vc: 212, 234 C: 2.2 x 10^2	Vc: 268, 324 –7 23, 29 N: 2.9 x 10^8	2.9 x 10^7	Vc: >300, >300 Na: >1.5 x 10^2 R: <10^5	Vc: 0, 0 Na: <1.5 x 10^2 R: >10^5	Vc: 2, 0 Na: <1.5 x 10^2 R: >10^5	**Pass @ 1.0%**
Pseudomonas aeruginosa FH 72/b Dirty	Vc: 247, 209 –2 33, 33 Nv: 2.4 x 10^3	Vc: 282, 263 A: 2.7 x 10^2	Vc: 190, 208 B: 1.9 x 10^2	Vc: 296, 275 C: 2.8 x 10^2	Vc: 268, 324 –7 23, 29 N: 2.9 x 10^8	2.9 x 10^7	Vc: 0, 0 Na: <1.5 x 10^2 R: >10^5	Vc: 1, 0 Na: <1.5 x 10^2 R: >10^5	Vc: 1, 0 Na: <1.5 x 10^2 R: >10^5	**Pass @ 0.5%**

Test criteria	Validation Test				Test suspension	Test procedure at concentration % (v/v / w/w)				Result
						cfu/ml in test pot	0.5%	1.0%	2.0%	Pass/Fail/ Invalid
Listeria monocytogenes FH66/*a* Clean	Vc: 261, 288 –2 21, 24 Nv: 2.7 x 10^3	Vc: 292, 260 A: 2.8 x 10^2	Vc: 271, 256 B: 2.6 x 10^2	Vc: 261, 314 C: 2.9 x 10^2	Vc: 261, 288 –7 21, 24 N: 2.7 x 10^8	2.7 x 10^7	Vc: 0, 0 Na: <1.5 x 10^2 R: >10^5	Vc: 0, 0 Na: <1.5 x 10^2 R: >10^5	Vc: 0, 0 Na: <1.5 x 10^2 R: >10^5	**Pass @ 0.5%**
Listeria monocytogenes FH66/*a* Dirty	Vc: 261, 288 –2 21, 24 Nv: 2.7 x 10^3	Vc: 294, 256 A: 2.7 x 10^2	Vc: 271, 256 B: 2.6 x 10^2	Vc: 241, 313 C: 2.8 x 10^2	Vc: 261, 288 –7 21, 24 N: 2.7 x 10^8	2.7 x 10^7	Vc: >300, >300 Na: >1.5 x 10^2 R: <10^5	Vc: 30, 24 Na: 2.7 x 10^2 R: 10^5	Vc: 2, 0 Na: <1.5 x 10^2 R: >10^5	**Pass @ 1.0%**
Salmonella Typhimurium *FH 68/a* Clean	Vc: 300, 274 –2 33, 27 Nv: 2.9 x 10^3	Vc: 341 346 A: 3.4 x 10^2	Vc: 285, 302 B: 2.9 x 10^2	Vc: 248, 251 C: 2.5 x 10^2	Vc: 177, 179 –7 18, 27 N: 1.8 x 10^8	1.8 x 10^7	Vc: 0, 0 Na: <1.5 x 10^2 R: >10^5	Vc: 0, 0 Na: <1.5 x 10^2 R: >10^5	Vc: 0, 0 Na: <1.5 x 10^2 R: >10^5	**Pass @ 0.5%**
Salmonella Typhimurium *FH 68/a* Dirty	Vc: 300, 274 –2 33, 27 Nv: 2.9 x 10^3	Vc: 256, 294 A: 2.7 x 10^2	Vc: 285, 302 B: 2.9 x 10^2	Vc: 258, 246 C: 2.5 x 10^2	Vc: 177, 179 –7 18, 27 N: 1.8 x 10^8	1.8 x 10^7	Vc: 0, 0 Na: <1.5 x 10^2 R: >10^5	Vc: 0, 0 Na: <1.5 x 10^2 R: >10^5	Vc: 0, 0 Na: <1.5 x 10^2 R: >10^5	**Pass @ 0.5%**
Yersinia enterocolitica FH 67/a Clean	Vc: 178, 171 –2 14, 9 Nv: 1.7 x 10^3	Vc: 149, 122 A: 1.3 x 10^2	Vc: 154, 148 B: 1.5 x 10^2	Vc: 207, 185 C: 1.9 x 10^2	Vc: 176, 173 –7 15, 16 N: 1.7 x 10^8	1.7 x 10^7	Vc: 0, 0 Na: <1.5 x 10^2 R: >10^5	Vc: 0, 0 Na: <1.5 x 10^2 R: >10^5	Vc: 0, 0 Na: <1.5 x 10^2 R: >10^5	**Pass @ 0.5%**
Yersinia enterocolitica FH 67/a Dirty	Vc: 178, 171 –2 14, 9 Nv: 1.7 x 10^3	Vc: 164, 145 A: 1.5 x 10^2	Vc: 154, 148 B: 1.5 x 10^2	Vc: 195, 163 C: 1.8 x 10^2	Vc: 176, 173 –7 15, 16 N: 1.7 x 10^8	1.7 x 10^7	Vc: 0, 0 Na: <1.5 x 10^2 R: >10^5	Vc: 0, 0 Na: <1.5 x 10^2 R: >10^5	Vc: 0, 0 Na: <1.5 x 10^2 R: >10^5	**Pass @ 0.5%**
Escherichia coli O157:H7 FH Clean	Vc: 161, 157 –2 26, 12 Nv: 1.6 x 10^3	Vc: 110, 120 A: 1.1 x 10^2	Vc: 142, 163 B: 1.5 x 10^2	Vc: 142, 161 C: 1.5 x 10^2	Vc: 154, 165 –7 21, 16 N: 1.6 x 10^8	1.6 x 10^7	Vc: 0, 0 Na: <1.5 x 10^2 R: >10^5	Vc: 0, 0 Na: <1.5 x 10^2 R: >10^5	Vc: 0, 0 Na: <1.5 x 10^2 R: >10^5	**Pass @ 0.5%**
Escherichia coli O157:H7 FH 72/a Clean	Vc: 161, 157 –2 26, 12 Nv: 1.6 x 10^3	Vc: 90, 112 A: 1.0 x 10^2	Vc: 142, 163 B: 1.5 x 10^2	Vc: 124, 116 C: 1.2 x 10^2	Vc: 154, 165 –7 21, 16 N: 1.6 x 10^8	1.6 x 10^7	Vc: 0, 0 Na: <1.5 x 10^2 R: >10^5	Vc: 0, 0 Na: <1.5 x 10^2 R: >10^5	Vc: 0, 0 Na: <1.5 x 10^2 R: >10^5	**Pass @ 0.5%**

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Investigation into the Effectiveness of VIRKON S (FH/73540/1) when Tested in Accordance with BS EN 1650:1998 - 'Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of fungicidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic, and institutional areas'

CONFIDENTIAL TO: Mr. Mark Squire,
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Report Approved By	:	Report Checked By	:
Name	:		

Approved Signatories:	Dr. J.T. Holah	Head of Food Hygiene Department
	Dr. K.L. Brown	Manager, Grade 3

SUMMARY

When tested in accordance with BS EN 1650:1998 the Virkon S tested at 2.5% v/v possesses yeasticidal activity against *Candida albicans* only in 15 minutes at 20°C under clean (0.03% bovine albumen) and dirty (0.3% bovine albumen) conditions.

When tested in accordance with BS EN 1650:1998 the Virkon S tested at 2.5% v/v did not possess yeasticidal activity against the additional test organism *Saccharomyces cerevisiae* in 15 minutes at 20°C under clean (0.03% bovine albumen) and dirty (0.3% bovine albumen) conditions.

When tested in accordance with BS EN 1650:1998 the Virkon S tested at 2.5% v/v possesses yeasticidal activity against the additional test organism *Saccharomyces cerevisiae* in 60 minutes at 20°C under clean (0.03% bovine albumen) and dirty (0.3% bovine albumen) conditions.

When tested in accordance with BS EN 1650:1998 the Virkon S tested at 3% v/v did not possess fungicidal activity against *Aspergillus niger* only in 60 minutes at 20°C under clean (0.03% bovine albumen) and dirty (0.3% bovine albumen) conditions.

When tested in accordance with BS EN 1650:1998 the Virkon S tested at 3% v/v possesses fungicidal activity against *Aspergillus niger* only in 15 minutes at 40°C under clean (0.03% bovine albumen) and dirty (0.3% bovine albumen) conditions.

INTRODUCTION

Antec International requested the Food Hygiene Department of CCFRA Technology Limited (CCFRA) to assess the efficacy of Virkon S for fungicidal activity in accordance with BS EN 1650:1998 - 'Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of fungicidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic, and institutional areas' against the two standard microorganisms plus *Saccharomyces cerevisiae.* After discussion with Antec International the test programme was revised to test at higher temperatures to assess the effectiveness against *A. niger.*

METHOD

For the bactericidal tests, BS EN 1650:1998 - 'Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of fungicidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic, and institutional areas' was followed. Additionally the higher temperature was used against *A. niger.*

There are two potential outcomes to EN 1650. If the product is effective against *C. albicans* only under the standard conditions of the test (15 min, 20°C, clean and dirty conditions), a 'yeasticidal' claim can be made for the product. If the product is effective against *both C. albicans* and *A. niger* under the standard conditions of the test (15 min, 20°C, clean and dirty conditions), a 'fungicidal' claim can be made.

NOTES

Higher temperature used in the assessment of efficacy against *A. niger* only. Longer contact time used (60 minutes) in the assessment of efficacy against *S. cerevisiae*

Bactericidal / fungicidal activity in general use conditions

a)	IDENTIFICATION OF THE TEST LABORATORY	**Food Hygiene Department, CCFRA**
b)	IDENTIFICATION OF THE SAMPLE	**FH/73540/2**
	Name of the product	**Virkon S**
	Batch number	**PJW2/4**
	Manufacturer	**Antec International**
	Condition on receipt	**OK**
	Date of delivery	**8/12/03**
	Storage conditions	**Food Hygiene Sample Store, Dark, Ambient**
	Product diluent recommended by the manufacturer for use	**Water of standard hardness (WSH)**
	Active substance(s) and its (their) concentrations(optional)	**N/A**
c)	EXPERIMENTAL CONDITIONS	
	Period of analysis	**8/12/03-3/2/03**
	Product diluent used during the test	**WSH**
	Product test concentrations	**2.5%, 3.0% and 4.0%**
	Appearance product dilutions	**Clear**
	Contact time	**Fungicidal Tests - 15 min ± 10 sec (also 30 min ± 10 sec/60 min ± 10 sec for *Aspergillus niger* and *S. cerevisiae*)**
	Test temperature	**Fungicidal Tests - 20°C ± 1°C**
	Interfering substance	**Bovine Serum Albumin @ 0.03% ("CLEAN") and 0.30% ("DIRTY") in Sterile Distilled Water (SDW)**
	Stability of the mixture (interfering substance and product diluted in hard water)	**At 4% ppt forms in contact with 3.0% Bovine Albumen**
	Temperature of incubation	**Fungicidal Tests - 30°C ± 1°C** **(Either Food Hygiene Incubator 1 or Microbiology walk-in 30°C)**

c) EXPERIMENTAL CONDITIONS (CONTINUED)

Identification of bacterial strains used	***Candida albicans***	**CaFH69/a**
	Aspergillus niger	**AnFH74/a**
	Saccharomyces cerevisiae	**ScFH68/a**

d) OPERATING PROCEDURE

Methods used	**Fungicidal Test - EN 1650 (1998)**
Deviations from methods	**Higher temperature used for assessment of *A. niger* Extended contact time used for assessment of *S. cerevisiae***
Neutraliser method	**Membrane filtration**
Neutraliser/ rinse media	**BS Diluent**

e) TEST RESULTS **See Tables**

f) CONCLUSION ***C. albicans* pass @ 2.5% 15 min contact 20°C**
***S. cerevisiae* pass @ 2.5% 60 min contact 20°C**
***A. niger* pass @ 3.0% 15 min contact 40°C**

N.B. KEY TO CODES ON RESULT SHEETS...

Vc:	viable count
R:	reduction in viability
Na:	the number of cfu/ml in the test mixture (dilution factor of 10^{-1})
C:	the number of cfu/ml in the dilution neutralisation test control, or of the membrane filtration test control
Nv:	the number of cfu/ml of the bacterial test suspension (dilution factor of 10^{-1})
A:	the number of cfu/ml of the experimental conditions control
B:	the number of cfu/ml of the neutralizertoxicity control or of the filtration control
N:	the number of cfu/ml in the bacterial or fungal suspension (dilution factor of 10^{-6} for Bacterial Tests, 10^{-5} For Fungicidal Tests)

Table 1: Pass results

Test Organism	Validation Test				Test Suspension	Test procedure at concentration % (v/v / w/w)				Result
						cfu/ml in test pot	2.5	3.0	4.0	Pass/Fail/ Invalid
Candida albicans Ca FH69/a Clean	Vc: 190, 180 –2 25, 23 Nv: 1.9 x 10^3	Vc: 219, 205 A: 2.1 x 10^2	Vc: 220, 213 B: 2.2 x 10^2	Vc: 265, 308 C: 2.9 x 10^2	Vc: 201, 176 –6 23, 21 N: 1.9 x 10^7	1.9 x 10^6	Vc: 0, 0 Na: <1.5 x 10^2 R: >10^4	Vc: 0, 0 Na: <1.5 x 10^2 R: >10^4		PASS @ 2.5% VALID
Candida albicans Ca FH69/a Dirty	Vc: 190, 180 –2 25, 23 Nv: 1.9 x 10^3	Vc: 166, 237 A: 2.0 x 10^2	Vc: 220, 213 B: 2.2 x 10^2	Vc: 299, 291 C: 2.9 x 10^2	Vc: 201, 176 –6 23, 21 N: 1.9 x 10^7	1.9 x 10^6	Vc: 6, 6 Na: <1.5 x 10^2 R: >10^4	Vc: 0, 0 Na: <1.5 x 10^2 R: >10^4		PASS @ 2.5% VALID
Saccharomyces cerevisiae Sc FH68/a 60 min Clean	Vc: 165, 169 –2 20, 19 Nv: 1.7 x 10^3	Vc: 176, 125 A: 1.5 x 10^2	Vc: 188, 178 B: 1.8 x 10^2	Vc: 179, 182 C: 1.8 x 10^2	Vc: 212, 188 –6 14, 16 N: 1.9 x 10^7	1.9 x 10^6	Vc: 0, 0 Na: <1.5 x 10^2 R: >10^4	Vc: 0, 0 Na: <1.5 x 10^2 R: >10^4		PASS @ 2.5% VALID 60 minute contact
Saccharomyces cerevisiae Sc FH68/a 60 min Dirty	Vc: 165, 169 –2 20, 19 Nv: 1.7 x 10^3	Vc: 164, 179 A: 1.7 x 10^2	Vc: 188, 178 B: 1.8 x 10^2	Vc: 168, 196 C: 1.8 x 10^2	Vc: 212, 188 –6 14, 16 N: 1.9 x 10^7	1.9 x 10^6	Vc: 0, 0 Na: <1.5 x 10^2 R: >10^4	Vc: 0, 0 Na: <1.5 x 10^2 R: >10^4		PASS @ 2.5% VALID 60 minute contact
Aspergillus niger An FH74/a Clean	Vc: 112, 106 –2 28, 25 Nv: 1.2 x 10^3	Vc: 126, 119 A: 1.2 x 10^2	Vc: 104, 115 B: 1.1 x 10^2	Vc: 122, 124 C: 1.2 x 10^2	Vc: 144, 143 –6 19, 25 N: 1.5 x 10^7	1.5 x 10^6		Vc: 0, 0 Na: <1.5 x 10^2 R: >10^4	Vc: 0, 0 Na: <1.5 x 10^2 R: >10^4	PASS @ 3.0% VALID 40°C
Aspergillus niger An FH74/a Dirty	Vc: 112, 106 –2 28, 25 Nv: 1.2 x 10^3	Vc: 143, 117 A: 1.3 x 10^2	Vc: 104, 115 B: 1.1 x 10^2	Vc: 131, 120 C: 1.2 x 10^2	Vc: 144, 143 –6 19, 25 N: 1.5 x 10^7	1.5 x 10^6		Vc: 0, 0 Na: <1.5 x 10^2 R: >10^4	Vc: 0, 0 Na: <1.5 x 10^2 R: >10^4	PASS @ 3.0% VALID 40°C

Table 2 RAW DATA

Test Organism	Validation Test				Test Suspension	Test procedure at concentration % (v/v / w/w)				Result
						cfu/ml in test pot	0.5%	1.0%	2.0%	Pass/Fail/ Invalid
Candida albicans Ca FH69/a Clean	Vc: 211, 189 –2 28, 20 Nv: 2.0 x 10^3	Vc: 93, 162 A: 1.3 x 10^2	Vc: 5, 7 B: 6 x 10^0	Vc: 114, 182 C: 1.5 x 10^2	Vc: 206, 213 –6 33, 25 N: 2.2 x 10^7	2.2 x 10^6	Vc: 1, 2 Na: <1.5 x 10^2 R: >10^4	Vc: 0, 0 Na: <1.5 x 10^2 R: >10^4	Vc: 0, 0 Na: <1.5 x 10^2 R: >10^4	PASS @ 1.0% INVALID
Candida albicans Ca FH69/a Dirty	Vc: 211, 189 –2 28, 20 Nv: 2.0 x 10^3	Vc: 115, 72 A: 9.3 x 10^1	Vc: 5, 7 B: 6 x 10^0	Vc: 83, 57 C: 7.0 x 10^1	Vc: 206, 213 –6 33, 25 N: 2.2 x 10^7	2.2 x 10^6	Vc: >300, >300 Na: >1.5 x 10^2 R: <10^4	Vc: >300, >300 Na: >1.5 x 10^2 R: <10^4	Vc: >300, >300 Na: >1.5 x 10^2 R: <10^4	FAIL@ 2.0% INVALID
Candida albicans Ca FH69/a Clean	Vc: 289, 254 –2 29, 31 Nv: 2.7 x 10^3	Vc: 135, 127 A: 1.3 x 10^2	Vc: 0, 0 B: 0	Vc: 0, 0 C: 0	Vc: 283, 257 –6 29, 28 N: 2.7 x 10^7	2.7 x 10^6	Vc: >300, >300 Na: >1.5 x 10^2 R: <10^4	Vc: 0, 0 Na: <1.5 x 10^2 R: >10^4	Vc: 0, 0 Na: <1.5 x 10^2 R: >10^4	PASS @ 1.0% INVALID
Candida albicans Ca FH69/a Dirty	Vc: 289, 254 –2 29, 31 Nv: 2.7 x 10^3	Vc: 242, 248 A: 2.4 x 10^2	Vc: 0, 0 B: 0	Vc: 0, 0 C: 0	Vc: 283, 257 –6 29, 28 N: 2.7 x 10^7	2.7 x 10^6	Vc: >300, >300 Na: >1.5 x 10^2 R: <10^4	Vc: >300, >300 Na: >1.5 x 10^2 R: <10^4	Vc: >300, >300 Na: >1.5 x 10^2 R: <10^4	FAIL @ 2.0% INVALID
Saccharomyces cerevisiae Sc FH68/a 15 min Clean	Vc: 165, 169 –2 20, 19 Nv: 1.7 x 10^3	Vc: 176, 125 A: 1.5 x 10^2	Vc: 188, 178 B: 1.8 x 10^2	Vc: 183, 184 C: 1.8 x 10^2	Vc: 212, 188 –6 14, 16 N: 1.9 x 10^7	1.9 x 10^6	Vc: 0, 0 Na: <1.5 x 10^2 R: >10^4	Vc: 0, 0 Na: <1.5 x 10^2 R: >10^4		PASS @ 2.5% VALID 60 minute contact
Saccharomyces cerevisiae Sc FH68/a 15 min Dirty	Vc: 165, 169 –2 20, 19 Nv: 1.7 x 10^3	Vc: 164, 179 A: 1.7 x 10^2	Vc: 188, 178 B: 1.8 x 10^2	Vc: 212, 198 C: 2.0 x 10^2	Vc: 212, 188 –6 14, 16 N: 1.9 x 10^7	1.9 x 10^6	Vc: >300, >300 Na: >1.5 x 10^2 R: <10^4	Vc: >300, >300 Na: >1.5 x 10^2 R: <10^4		FAIL @ 2.5% VALID 15 minute contact
Aspergillus niger An FH74/a Clean 15 min 20°c	Vc: 91, 106 –2 25, 14 Nv: 1.0x 10^3	Vc: 63, 63 A: 6.3 x 10^1	Vc: 85, 79 B: 8.2 x 10^1	Vc: 100, 78 C: 8.9 x 10^1	Vc: 133, 110 –6 16, 17 N: 1.2 x 10^7	1.2 x 10^6		Vc: >300, >300 Na: >1.5 x 10^2 R: <10^4	Vc: >300, >300 Na: >1.5 x 10^2 R: <10^4	FAIL @ 2.0% VALID

Table 2 RAW DATA

Test Organism	Validation Test				Test Suspension	Test procedure at concentration % (v/v / w/w)				Result
						cfu/ml in test pot		3.0%	4.0%	Pass/Fail/ Invalid
Aspergillus niger An FH74/a Dirty 15 min 20°c	Vc: 91, 106 –2 25, 14 Nv: 1.0×10^3	Vc: 76, 72 A: 7.4×10^1	Vc: 85, 79 B: 8.2×10^1	Vc: 96, 82 C: 8.9×10^2	Vc: 133, 110 –6 16, 17 N: 1.2×10^7	1.2×10^6		Vc: >300, >300 Na: $>1.5 \times 10^2$ R: $<10^4$	Vc: >300, >300 Na: $>1.5 \times 10^2$ R: $<10^4$	FAIL @ 4% VALID
Aspergillus niger An FH74/a Clean 30 min 20°c	Vc: 91, 106 –2 25, 14 Nv: 1.0×10^3	Vc: 63, 63 A: 6.3×10^1	Vc: 85, 79 B: 8.2×10^1	Vc: 111, 106 C: 1.0×10^2	Vc: 133, 110 –6 16, 17 N: 1.2×10^7	1.2×10^6		Vc: >300, >300 Na: $>1.5 \times 10^2$ R: $<10^4$	Vc: >300, >300 Na: $>1.5 \times 10^2$ R: $<10^4$	FAIL @ 4% VALID
Aspergillus niger An FH74/a Dirty 30 min 20°c	Vc: 91, 106 –2 25, 14 Nv: 1.0×10^3	Vc: 76, 72 A: 7.4×10^1	Vc: 85, 79 B: 8.2×10^1	Vc: 77, 66 C: 7.1×10^1	Vc: 133, 110 –6 16, 17 N: 1.2×10^7	1.2×10^6		Vc: >300, >300 Na: $>1.5 \times 10^2$ R: $<10^4$	Vc: >300, >300 Na: $>1.5 \times 10^2$ R: $<10^4$	FAIL @ 4% VALID
Aspergillus niger An FH74/a Clean 60 min 20°c	Vc: 91, 106 –2 25, 14 Nv: 1.0×10^3	Vc: 63, 63 A: 6.3×10^1	Vc: 85, 79 B: 8.2×10^1	Vc: 99, 96 C: 9.7×10^1	Vc: 133, 110 –6 16, 17 N: 1.2×10^7	1.2×10^6		Vc: >300, >300 Na: $>1.5 \times 10^2$ R: $<10^4$	Vc: 103, 104 Na: 1.0×10^3 R: $<10^4$	FAIL @ 4% VALID
Aspergillus niger An FH74/a Dirty 60 min 20°c	Vc: 91, 106 –2 25, 14 Nv: 1.0×10^3	Vc: 76, 72 A: 7.4×10^1	Vc: 85, 79 B: 8.2×10^1	Vc: 85, 73 C: 7.9×10^1	Vc: 133, 110 –6 16, 17 N: 1.2×10^7	1.2×10^6		Vc: >300, >300 Na: $>1.5 \times 10^2$ R: $<10^4$	Vc: >300, >300 Na: $>1.5 \times 10^2$ R: $<10^4$	FAIL @ 4% VALID
Aspergillus niger An FH74/a Clean 15 min 20°C	Vc: 199, 154 –2 15, 16 Nv: 1.7×10^3	Vc: 138, 126 A:1.3×10^2	Vc: 131, 120 B:1.2×10^2	Vc: 126, 113 C: 1.2×10^2	Vc: 199, 154 –6 15, 16 N: 1.7×10^7	1.7×10^6		Vc: >300, >300 Na: $>1.5 \times 10^2$ R: $<10^4$	Vc: >300, >300 Na: $>1.5 \times 10^2$ R: $<10^4$	FAIL @ 4% VALID
Aspergillus niger An FH74/a Dirty 15 min 20°C	Vc: 199, 154 –2 15, 16 Nv: 1.7×10^3	Vc: 135, 132 A:1.3×10^2	Vc: 131, 120 B:1.2×10^2	Vc: 126, 113 C: 1.2×10^2	Vc: 199, 154 –6 15, 16 N: 1.7×10^7	1.7×10^6		Vc: >300, >300 Na: $>1.5 \times 10^2$ R: $<10^4$	Vc: >300, >300 Na: $>1.5 \times 10^2$ R: $<10^4$	FAIL @ 4% VALID
Aspergillus niger An FH74/a Clean 30 min 20°C	Vc: 199, 154 –2 15, 16 Nv: 1.7×10^3	Vc: 138, 126 A:1.3×10^2	Vc: 131, 120 B:1.2×10^2	Vc: 126, 113 C: 1.2×10^2	Vc: 199, 154 –6 15, 16 N: 1.7×10^7	1.7×10^6		Vc: >300, >300 Na: $>1.5 \times 10^2$ R: $<10^4$	Vc: >300, >300 Na: $>1.5 \times 10^2$ R: $<10^4$	FAIL @ 4% VALID

Table 2 RAW DATA

Test Organism	Validation Test				Test Suspension	Test procedure at concentration % (v/v / w/w)				Result
						cfu/ml in test pot		3.0%	4.0%	Pass/Fail/ Invalid
Aspergillus niger An FH74/a Dirty 30 min 20°C	Vc: 199, 154 –2 15, 16 Nv: 1.7×10^3	Vc: 135, 132 A:1.3×10^2	Vc: 131, 120 B:1.2×10^2	Vc: 126, 113 C: 1.2×10^2	Vc: 199, 154 –6 15, 16 N: 1.7×10^7	1.7×10^6		Vc: >300, >300 Na: $>1.5 \times 10^2$ R: $<10^4$	Vc: >300, >300 Na: $>1.5 \times 10^2$ R: $<10^4$	FAIL @ 4% VALID
Aspergillus niger An FH74/a Clean 60 min 20°C	Vc: 199, 154 –2 15, 16 Nv: 1.7×10^3	Vc: 138, 126 A:1.3×10^2	Vc: 131, 120 B:1.2×10^2	Vc: 126, 113 C: 1.2×10^2	Vc: 199, 154 –6 15, 16 N: 1.7×10^7	1.7×10^6		Vc: >300, >300 Na: $>1.5 \times 10^2$ R: $<10^4$	Vc: 140, 156 Na: 1.4×10^3 R: $<10^4$	FAIL @ 4% VALID
Aspergillus niger An FH74/a Dirty 60 min 20°C	Vc: 199, 154 –2 15, 16 Nv: 1.7×10^3	Vc: 135, 132 A:1.3×10^2	Vc: 131, 120 B:1.2×10^2	Vc: 126, 113 C: 1.2×10^2	Vc: 199, 154 –6 15, 16 N: 1.7×10^7	1.7×10^6		Vc: >300, >300 Na: $>1.5 \times 10^2$ R: $<10^4$	Vc: >300, >300 Na: $>1.5 \times 10^2$ R: $<10^4$	FAIL @ 4% VALID
Aspergillus niger An FH74/a Clean 30 min 40°C	Vc: 112, 106 –2 28, 25 Nv: 1.2×10^3	Vc: 126, 119 A: 1.2×10^2	Vc: 104, 115 B: 1.1×10^2	Vc: 122, 124 C: 1.2×10^2	Vc: 144, 143 –6 19, 25 N: 1.5×10^7	1.5×10^6		Vc: 0, 0 Na: $<1.5 \times 10^2$ R: $>10^4$	Vc: 0, 0 Na: $<1.5 \times 10^2$ R: $>10^4$	PASS @ 3% VALID
Aspergillus niger An FH74/a Dirty 30 min 40°C	Vc: 112, 106 –2 28, 25 Nv: 1.2×10^3	Vc: 143, 117 A: 1.3×10^2	Vc: 104, 115 B: 1.1×10^2	Vc: 131, 120 C: 1.2×10^2	Vc: 144, 143 –6 19, 25 N: 1.5×10^7	1.5×10^6		Vc: 0, 0 Na: $<1.5 \times 10^2$ R: $>10^4$	Vc: 0, 0 Na: $<1.5 \times 10^2$ R: $>10^4$	PASS @ 3% VALID
Aspergillus niger An FH74/a Clean 60 min 40°C	Vc: 112, 106 –2 28, 25 Nv: 1.2×10^3	Vc: 126, 119 A: 1.2×10^2	Vc: 104, 115 B: 1.1×10^2	Vc: 122, 124 C: 1.2×10^2	Vc: 144, 143 –6 19, 25 N: 1.5×10^7	1.5×10^6		Vc: 0, 0 Na: $<1.5 \times 10^2$ R: $>10^4$	Vc: 0, 0 Na: $<1.5 \times 10^2$ R: $>10^4$	PASS @ 3% VALID
Aspergillus niger An FH74/a Dirty 60 min 40°C	Vc: 112, 106 –2 28, 25 Nv: 1.2×10^3	Vc: 143, 117 A: 1.3×10^2	Vc: 104, 115 B: 1.1×10^2	Vc: 131, 120 C: 1.2×10^2	Vc: 144, 143 –6 19, 25 N: 1.5×10^7	1.5×10^6		Vc: 0, 0 Na: $<1.5 \times 10^2$ R: $>10^4$	Vc: 0, 0 Na: $<1.5 \times 10^2$ R: $>10^4$	PASS @ 3% VALID

UNIVERSITÉ DE PARIS-SUD

FACULTÉ DE PHARMACIE

Département de

MICROBIOLOGIE ET IMMUNOLOGIE

Châtenay le, 29 Avril 1992

A.M. QUERO, Professeur

RAPPORT

A la demande de la Société ANTEC INTERNATIONAL Chilton Industrial Estate, Sudbury, Suffolk Co 10 6 X D, ENGLAND, transmise par les Laboratoires PHAGOGENE, ZI de Carros, 1ère Avenue, 06510 CARROS, nous avons étudié les propriétés virucides du produit dénommé :

VIRKON lot n° 5572

dont la composition comprend des composés péroxygénés, surfactant, acides organiques et un système de tampon inorganique.

La méthode utilisée a été une de celle préconisée par la norme expérimentale publiée par l'AFNOR (T 72-180 Décembre 1989).

"Antiseptiques et désinfectants
Utilisés à l'état liquide, miscible à l'eau
Détermination de l'activité virucide
Virus des vertébrés".

à savoir la méthode par dilution.

MATERIEL ET METHODES

1 - Principe Général

La méthode consiste à mettre en contact la solution du désinfectant et la suspension virale pendant des durées variables. Le mélange est ensuite dilué brutalement à froid pour arrêter l'action virucide et cytotoxique du désinfectant.

Le titre de la dilution est comparé à celui de la suspension virale de départ traitée dans les mêmes conditions.

2 - Cellules

Cellules VERO : cellules rénales d'origine simienne en lignée dont le milieu de croissance est le milieu essentiel de Eagle (MEM) additionné de 10 % de sérum de veau foetal et d'antibiotiques (Pénicilline 200 U/ml, Streptomycine 0,04 mg/ml) et le milieu d'entretien MEM additionné de 2 % de sérum de veau foetal et d'antibiotiques.

Cellules KB : Lignée d'origine humaine cultivée en utilisant les mêmes milieux que pour les cellules VERO.

3 - Virus

3 virus préconisés par la norme AFNOR

- Enterovirus Polio 1, souche SABIN, cultivé sur cellules VERO
- Adenovirus h 5, cultivé sur cellules KB
- Orthopoxvirus de la vaccine, cultivé sur cellules VERO

Le titrage des virus est réalisé en microplaques par la mesure de l'effet cytopathique sur les cellules sensibles, le titre est évalué par la méthode de Fisher avec utilisation des tables de Wyshak et Detré qui permet de déterminer le nombre le plus probable d'unités infectieuses par ml (UI/ml).

4 - Désinfectant

Le VIRKON se présente sous la forme d'une poudre rose. Une solution à 1% dans de l'eau distillée est préparée extemporanément.

5 - Protocole opératoire

5.1. Essais préliminaires

5.1.1. Recherche de la dilution subcytotoxique de VIRKON à 1%

Une série de dilution du désinfectant dans le milieu de culture est mise en contact avec les cellules pendant 5 jours. La dilution subcytotoxique est appréciée par examen de la couche cellulaire au microscope inversé.

Elle correspond à la plus forte concentration pour laquelle on observe une nappe cellulaire intacte.

5.1.2. Témoin de l'élimination de l'effet virucide :

La suspension virale est mise en contact pendant 1h à + 21°C avec la dilution subcytotoxique du désinfectant puis elle est titrée parallèlement à une suspension virale témoin.

5.2. Essais proprement dit

Deux tubes sont préparés :

- un tube réaction R qui contient 0,5ml de la suspension virale et 4,5ml du désinfectant à la concentration étudiée.

- Un tube témoin T qui contient 0,5 ml de la suspension virale et 4,5ml de la solution saline tamponnée (PBS).

Les tubes sont placés à 21°C. Après 15mn, 30mn, 60mn, un échantillon de 1ml est prélevé dans le tube R dilué à dilution subcytotoxique dans une solution saline tamponnée placé dans la glace fondante. Après 60 mn, un échantillon de 1 ml est également prélevé dans le tube T et dilué dans les mêmes conditions. Le titrage

simultané des dilutions obtenues permet d'apprécier l'action virucide du désinfectant par comparaison des titres en virus des échantillons R et T.

5.3. Dosage de Protéines

La teneur en protéine des souches virales et du milieu réactionnel est évaluée par la méthode de Lowry.

6 - Résultats

Les résultats obtenus lors de l'étude des propriétés virucides de VIRKON à 1% par la méthode par dilution sont les suivants :

6.1. Essais préliminaires

6.1.1. Elimination de l'effet cytotoxique (Tableau I)

L'examen des couches cellulaires traitées par la dilution au 1/20 de VIRKON à 1% indique que cette dilution n'est plus cytotoxique pour les cellules Véro ni pour les cellules KB.

6.1.2. Elimination de l'effet virucide (Tableau II)

La dilution au 1/20 de VIRKON à 1% n'est pas virucide pour les virus Poliomyélitique, Adenovirus h5 et vaccine.

6.1.3. Dosage des protéines (Tableau III)

Les teneurs en protéines des souches virales et des milieux réactionnels sont rassemblées dans le Tableau III.

6.2. Essais proprement dits

Les résultats obtenus avec VIRKON à 1% sur les 3 suspensions virales sont rassemblés dans le Tableau IV. Ils indiquent que VIRKON à 1% entraîne un

abaissement du titre viral supérieur à 4log pour les trois virus essayés : Enterovirus polio 1, Adenovirus h5, Orthopoxvirus de la vaccine.

CONCLUSION

Le produit VIRKON à 1 % est virucide selon la norme T72180 puisqu'il entraîne un abaissement du titre viral supérieur à 4 logarithmes pour les trois virus étudiés.

Pr. A.M. QUERO

TABLEAU I

Elimination de l'effet cytotoxique de VIRKON à 1%

Dilution infracytotoxique

Cellules VERO	1/20
Cellules KB	1/20

TABLEAU II

Elimination de l'effet virucide de VIRKON à 1%

(Méthode par dilution)

Dilution d'arrêt 1/20

Titres des suspensions virales. UI/ml (log)

	Témoin virus	Dilution DSF 1/20 + virus (1h à 20°C)
Enterovirus Polio 1	8,01	7,82
Adenovirus h 5	8,38	8,31
Vaccine	8,01	7,82

TABLEAU III

TENEUR EN PROTEINES DES SOUCHES VIRALES

ET DES MILIEUX REACTIONNELS.

	SOUCHES VIRALES	MILIEUX
Enterovirus polio 1	1,2mg/ml	0,12mg/ml
Adenovirus h 5	1,3mg/ml	0,13mg/ml
Orthopoxvirus de la vaccine	1,3mg/ml	0,13mg/ml

TABLEAU IV

ACTIVITE VIRUCIDE DE VIRKON à 1%

Titres des suspensions virales (log)

	TV	R15	R30	R60	Abaissement du titre viral
Enterovirus Polio 1	7,71	<3,64	<3,64	<3,64	>4,07
Adenovirus h 5	8,01	<3,64	<3,64	<3,64	>4,39
Orthopoxvirus de la vaccine	7,80	<3,64	<3,64	<3,64	>4,16

INACTIVATION OF POLIOVIRUS TYPE 2

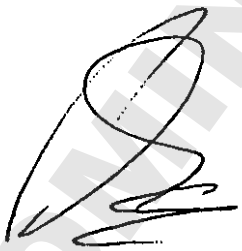
EFFICACY OF VIRKON S DISINFECTANT

Investigation 1.
Inactivation of poliovirus type 2
by Virkon S disinfectant in the absence of organics.

Carried out for
Antec International Ltd
Chilton Industrial Estate
Sudbury, Suffolk CO10 6XD

by

Severn Trent Laboratories
St Martin's Road
Coventry CV3 6PR



Dr R Morris
Virologist
9th November 1990

OBJECTIVES

To determine the efficacy of Virkon S disinfectant in inactivating poliovirus type 2 under laboratory conditions in the absence of organic material.

VIRUS

Poliovirus type 2 (MEF-1 strain), originally obtained from the American Type Culture Collection via Wellcome Research Laboratories (ref ATCC VR-61). Propagated in Vero cells (two passages) and BGM cells (two passages). Virus preparation filtered through 50nm porosity filter to remove viral aggregates (monodispersion).

CELL CULTURES

African green monkey kidney continuous cell line (BGM) obtained from Flow Laboratories propagated in Eagles minimal essential medium supplemented with antibiotics and foetal calf serum.

ASSAY PROCEDURE

Poliovirus was detected and enumerated using the suspended plaque assay system with BGM cells described by Morris & Waite (1980).

PREPARATION OF DISINFECTANT

Virkon S powder was provided by Antec International (for batch details see appendix). The powder was dispersed in sterile deionised water at concentrations double the working level. The disinfectant stock concentrations were prepared fresh for each experiment.

CONDUCT OF THE TEST

2.5ml of monodispersed poliovirus type 2 was mixed with an equal volume of double strength disinfectant and allowed to interact at the test temperature for the required time. After the required time had elapsed an equal volume (5ml) of universal neutraliser (appendix) was added and the mixture assayed immediately. Each inactivation was carried out in triplicate.

RESULTS

Experiment 1: Virkon S was used at final concentrations ranging from 0 to 4% w/v with contact times of 30 and 120 minutes. All inactivations were carried out at 37°C. Table 1 details the virus levels detected in each of the triplicate samples. At no time was there evidence of cytotoxicity attributable to the disinfectant.

Table 1. Inactivation of poliovirus type 2 by Virkon S at 37°C at two contact times (experiment 1).

Concn Virkon S %	Contact time mins	plaque forming units per test sample
0	30	495,000; 540,000; 570,000
1	30	<100; <100; <100
2	30	<100; <100; <100
3	30	<100; <100; <100
4	30	<100; <100; <100
0	120	790,000; 550,000; 565,000
1	120	<100; <100; <100
2	120	<100; <100; <100
3	120	<100; <100; <100
4	120	<100; <100; <100

Under these conditions Virkon S was capable of inactivating at least 99.999% of poliovirus type 2 at a concentration of 1% w/v.

Experiment 2: experiment 1 was repeated using a contact time of 10 minutes with the reaction being carried out at room temperature (22°C). Table 2 details the levels of viruses detected after the contact time. At no time was there ecidence of cytotoxic effect.

Table 2. Inactivation of poliovirus type 2 by Virkon S disinfectant at room temperature with two contact times.

Concn Virkon S %	Contact time mins	plaque forming units per test sample
0	10	2,000,000
1	10	<10; ,10; <10
2	10	<10; <10; <10
3	10	<10; <10; <10
0	30	4,000,000
1	30	<10; <10; <10
2	30	<10; <10; <10
3	30	<10; <10; <10

Under these conditions Virkon S was capable in reducing the levels of poliovirus type 2 by at least 99.999%.

CONCLUSIONS

Virkon S disinfectant is effective in reducing poliovirus type 2 levels by at least 99.999% when used under conditions of minimal organic contamination at a concentration of 1% w/v at both room temperature and at elevated temperatures (37°C).

REFERENCE

Morris, R & Waite, WM (1980). Evaluation of procedures for recovery of viruses from water. 2. Detection systems. *Water Research, 14,* 795-798.

APPENDIX

Universal neutraliser

Deionised water	960ml
Sodium thiosulphate	5g
0.25N phosphate buffer	10ml
(34g potassium dihydrogen orthophosphate/litre)	
Tween 80	30ml
L-histidine	1g
Lecithin	3g

Virkon S - details of batch used in the evaluations

Provided at 500g powder shaker
Batch number 2348
Manufacture date January 1990
Expiry date 1992
pH 2.48
Available oxygen 10.12%

Antec reference MS/EAC/MS07/DISC2/28.2.90

VIRUCIDAL ACTIVITY OF VIRKON AGAINST ROTAVIRUSES.

Panteleeva L.G., Kirianova E.V., Blokhina T.A., Avakov A.A.

As it follows from it's name, Virkon is a virucidal disinfectant. Independent studies performed by scientists of well-known laboratories have proved Virkon's activity against 17 families of human and animal viruses. Virkon inactivates viruses having weak resistance to chemical disinfectants (influenza viruses, parainfluenza virus, herpes simplex virus) and highly resistant viruses (Hepatitis B, Picornaviridae, Rotaviridae).

Investigations of Rotaviruses' resistance to disinfectants have been launched by our group. A part of the work devoted to a comparative study of human Rotavirus and monkey Rotavirus resistance to Virkon is presented in this paper.

Why does this type of virus interest us? The first reason is that Rotaviruses are the causal microorganisms of rotaviral gastroenteritis among children and adults. The infection causes epidemiologic outbreaks and is most active in winter season. Secondly, according to data of foreign investigators Rotaviruses are highly resistant to disinfectant activity and not every disinfectant can be applied in a nidus of rotaviral gastroenteritis.

Difficulties in isolating and cultivating human Rotaviruses restrict investigations with the virus, since such investigations are not available for every laboratory.

More often a laboratory strain of monkey Rotavirus SA-II is used for investigations. It is similar in antigen structure and physical-chemical properties to human Rotavirus. However, data of different researchers have some contradictions concerning comparative resistance of human Rotavirus and monkey Rotavirus, strain SA-II.

In this connection, we have used the possibility to evaluate comparative resistance of both viruses to Virkon. After selecting the most resistant viruses we could proceed to work out regimens for disinfection in cases of viral gastroenteritis.

METHODS AND MATERIALS.

Monkey Rotavirus SA-II was given by L.A. Shekoyan from the Institute of Poliomyelitis and Viral Encephalitis of the Academy of Medical Sciences of the USSR. The virus was cultivated in a roll-tube culture of cells RAMT (kidney cells of monkeys Cercopithecus ephyop); the titre of the virus was 10^6 TCD_{50} /ml.

Human Rotavirus (strain N° 134) was isolated from a patient with rotaviral gastroenteritis /I/ in a primary culture of monkey kidney cells (the authors of the strain are V.I. Vasilieva, N.N. Gracheva, N.V. Karazhas, T.A. Blokhina, A.A. Avakov). The strain was deposited in the State Collection of Viruses in the D.I. Ivanovsky Institute of Virusology in 1986, N° 1997. The virus was identified by the method of electron microscopy in reactions of agglutination, neutralization, binding compliment, latex agglutination.

Reinoculated kidney cells of monkeys RAMT and 4647 were cultivated in IGL - MEM medium with 10% bovine inactivated serum on flat-bottomed polysterol 96 - spot plates (of local manufacture) in the air atmosphere with 5% of carbon dioxide. The dose was 8.10^4 cells per 1 spot. After 24 hour incubation at 37°C the culture of cells was used in tests. Before contamination of a cellular monolayer the samples of Rotaviruses were activated for 0.5 hour at 37°C in IGL - MEM medium containing 10 mkg/ml of trypsin. There were prepared tenfold dilutions of viral samlpes and each dilution was used for contaminating of no less than 3 parallel spots. Before contamination the cellular monolayer was thoroughly washed with the medium without serum. Adsobtion of the virus was carried out at 37°C during 1.5 hours in the air atmosphere with 5% carbon dioxide. Then the virus containing liquid was removed and the cellular monolayer was poured with the medium IGL - MEM containing 1 mkg/ml of trypsin and was incubated for 5 days till the development of cytopathogenic effect. The cytopathogenic effect was evaluated by chracteristic application of the monolayer. The virus titre was expressed in TCD_{50} (tissue cytopathogenic dose was 50%).

Virkon solutions were prepared by dissolving the powder in distilled water. Solutions' concentrations were calculated according to the agent. 1% solution of sodium thiosulphonate was used as neutralizer for 0.1 - 0.7% Virkon solutions.

Tests on inactivating Rotaviruses were carried out with the help of suspension method.

One volume of the viral suspension in IGL - MEM medium was mixed with 9 volumes of Virkon solution in corresponding concentration and was incubated at the room temperature for a definite period of time. A blank sample was mixed with distilled water. After the incubation the viral sample was mixed (1 : 9) with 1% solution of sodium thiosulphate diluted with IGL - MEM medium. The virus titre was determined by the above-mentioned method. For evaluation of the virucidal activity solutions in concentrations 0.1, 0.15, 0.2, 0.3, 0.4% were used.

RESULTS OF THE STUDY.

Monkey Rotavirus SA-II was exposed to 0.1, 0.15, 0.2, 0.3% Virkon's activity (see the table). The reduction of the virus titre by 3 log was achieved after 60 minutes contact with 0.1% solution; 0.15% solution produced the same effect in 10 minutes. 0.2% solution reduced monkey Rotavirus titre by 3 log in 5 minutes; 0.3% solution reduced the titre in less than 1 minute.

The human Rotavirus titre was reduced by 3 log after 60 minutes contact with 0.1% Virkon solution. 0.2% Virkon showed this effect in 30 minutes and 0.3% solution - in 20 minutes.

0.4% Virkon solution inactivated human Rotavirus by reducing it's titre by 3 log in 10 minutes.

Comparative evaluation of Virkon virucidal activity on human Rotavirus and monkey Rotavirus shows that human Rotavirus is more resistant to Virkon than monkey virus SA-II. Thus, 0.2% Virkon solution reduced human Rotavirus

titre by 3 log in 30 minutes, whereas monkey Rotavirus titre was redused in 5 minutes; contact time with 0.3% Virkon solution was 20 min. and one minute respectively.

To reduce a quantity of human Rotavirus in suspension by 1000 times after 10 minutes contact, it was necessary to use 0.4% Virkon solution, whereas for monkey virus SA-II 0.15% concentration was sufficient.

To achieve a similar effect in hydrogen peroxide tests on monkey Rotavirus SA-II it was necessary to use 3% solution (according to active substance) or 9.9% according to the agent.

Thus, hydrogen peroxide is 10 times less active than Virkon.

The obtained data allow us to make the following conclusions:

1. Virkon shows virucidal activity against human Rotaviruses and monkey Rotavirus in 0.1% - 0.4% solutions, reducing virus titre by 3 log after 1 - 60 min. contact time.
2. Human Rotavirus shows higher resistance to Virkon than monkey Rotavirus SA-II.
3. Virkon's virucidal activity against monkey Rotavirus SA-II is higher than that of hydrogen peroxide.

Table.

Virucidal activity of Virkon and hydrogen peroxide against human and monkey Rotaviruses.

Agent	Virkon		Hydrogen peroxide		
	Agent concentration, %	Inactivation time, min. by 3 log	Virus	Concentration, % according to agent	Inactivation time, min. by 3 log
Type					
Monkey Rotavirus SA-II	0.1	60	Monkey Rotavirus SA-II	3.3	55
	0.15	10		6.6	40
	0.2	5		9.9	12
	0.3	1			
Human Rotavirus	0.1	60	Human Rotavirus	-	-
	0.2	30			
	0.3	20			
	0.4	10			

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DEPARTMENT OF
MEDICAL MICROBIOLOGY

1 July 1987

INACTIVATION OF HEPATITIS B VIRUSES:

EVALUATION OF EFFICACY OF DISINFECTANTS

Human hepatitis B virus belongs to a group called hepadnaviruses. A characteristic of these viruses is that they contain a circular DNA that is partially double-stranded. The virions contain an endogenous DNA polymerase that carries out repair synthesis to fill in the single-stranded gap. The ability of the enzyme to utilize exogenous nucleotides to complete the gap in in vitro reactions is an indirect means of detecting infectious virions. Hence, loss of DNA polymerase activity is a primary indicator of inactivated virus particles. This assay was therefore used throughout the evaluation.

TEST PRODUCT:	VIRKON S
WORKING SOLUTION USED:	2% w/v aqueous solution
MINIMUM EFFECTIVE CONCENTRATION FOUND:	1% final solution
MINIMUM EFFECTIVE CONTACT TIME FOUND:	10 minutes

Dr K N Tsiquaye, PhD, MRCPath
Senior Lecturer in Virology

INHIBITION OF DNA POLYMERASE IN VITRO

Various concentrations of disinfectants/antiseptics were exposed to known virus preparations for varying periods of time in order to determine the lowest concentration which completely inhibits DNA polymerase activity in the shortest time.

VIRUS PREPARATIONS

Positive Plasma: a) HBsAg positive plasma spiked with excess concentrated HBV.

b) DHBsAg positive plasma.

Control Plasma: a) HBsAg negative plasma.

b) DHBsAg negative plasma.

BRIEF DESCRIPTIONS OF METHOD

Aliquots of positive and negative plasma samples were each exposed to an equal volume of test product for defined periods of time. The test product was controlled with phosphate buffered saline (PBS). Each sample was then treated with a mixture of NP-40 and 2-mercaptoethanol. A volume of reaction mixture containing exogenous nucleotides including 3H-thymidine triphosphate was added to each mixture. Samples were incubated for 3 hours to allow for the incorporation of nucleoside monophosphate in the chain elongation of viral DNA by the viral polymerase. The extent of incorporation was monitored by the detection of radioactivity (3H-thymidine monophosphate) in counts per minute (cpm). Each sample was tested in duplicate and the mean cpm is given.

RESULTS

The final results of the series of experiments are given in tables 1 and 2. Values are presented in the form of a ratio of Positive:Negative. Ratios $\geq$ 2.1 for a test sample is considered to contain hepatitis B virus particles with active DNA polymerase.

CONCLUSION

Under the conditions in which the tests were carried out VIRKON S is effective in destroying totally the activity of the enzyme, DNA polymerase, of hepatitis B viruses at:

2% working solution for a minimum exposure time of 10 minutes.

Dr K N Tsiquaye, PhD, MRCPath
Senior Lecturer in Virology

RESULTS

TABLE 1: VIRKON S and Human Hepatitis B Virus.

EXPOSURE TIME (mins)	HBV-DNA POLYMERASE ACTIVITY (cpm)	
	Positive Plasma* + VIRKON S / Negative Plasma* + VIRKON S	Positive Plasma* + PBS / Negative Plasma* + PBS
5	37/25	218/38
10	40/32	205/33
20	33/34	213/31

* Normal Virus-Positive and Negative plasma and VIRKON S were allowed to interact, diluted with PBS and centrifuged at 35,000 rpm for 4 hours. Pellets were resuspended to give 20 x concentrated of HBV in original plasma and assayed for DNA polymerase activity.

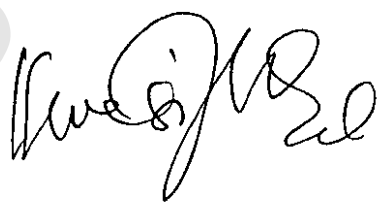
Dr K N Tsiquaye, PhD, MRCPath
Senior Lecturer in Virology

RESULTS

TABLE 2: VIRKON S and Human Hepatitis B Virus.

EXPOSURE TIME (mins)	HBV-DNA POLYMERASE ACTIVITY (cpm)	
	Positive Plasma* + VIRKON S / Negative Plasma + VIRKON S	Positive Plasma* + PBS / Negative Plasma + PBS
5	40/20	396/28
10	25/26	380/26
20	45/36	367/38

* HBsAg positive plasma to which excess concentrated HBV was added.



Dr K N Tsiquaye, PhD, MRCPath
Senior Lecturer in virology

RESULTS

TABLE 3: VIRKON S and Duck Hepatitis B Virus.

EXPOSURE TIME (mins)	DHBV-DNA POLYMERASE ACTIVITY (cpm)	
	Positive Plasma + VIRKON S / Negative Plasma + VIRKON S	Positive Plasma* + PBS / Negative Plasma + PBS
5	75/69	905/110
10	56/44	826/98
20	52/35	897/107

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Director A J Stevens MA BVSc MRCVS DipBact

Your reference

Our reference

Date

POULTRY DEPARTMENT

CENTRAL VETERINARY LABORATORY

Laboratory Evaluation of ANTEC 'VIRCON S' Disinfectant as a Viricidal Agent against Avian Influenza Virus

Client	Antec International Ltd., Sudbury, Suffolk
Test Product	Antec Vircon S Powder. A stock solution was prepared by dissolving in hard water.
Test Organism	Avian influenza, strain A/Chicken/Germany/34 (H7N1)
Test Procedure	v/v dilutions of the disinfectant were tested for viricidal activity using the standard protocol used for approval of disinfectants against fowl pest but substituting the avian influenza virus.

Micropathology Ltd

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University of Warwick Science Park, Barclays Venture Centre,
Sir William Lyons Road, Coventry CV4 7EZ

Activity of Virkon against hepatitis C virus measured using destruction of viral specific molecules as a marker

Report Prepared 28/6/99 for Antec International Ltd.

Infection with hepatitis C virus (HCV) occurs to varying degrees around the world. Its prevalence in western Europe is estimated by the World Health Organisation at being 1-2.4% of the general population. In certain population groups however, such as those with a current or past history of intra-venous drug use, the incidence of infection is much higher.

HCV can cause chronic disease for which there is currently no effective treatment and vaccination has yet to be developed. The virus may persist at a high concentration for many years in the bloodstream of infected individuals and has also been detected in other types of body fluids. Therefore its control is of importance in any situation where people are exposed to spillages of blood and possibly other body fluids.

Difficulties exist in measuring the effectiveness of disinfectants against the virus. HCV cannot be grown in the laboratory and the only animal model is the chimpanzee. Therefore the only practicable source of virus is the blood of those infected. This protocol uses an assay for measuring the concentration of a virus specific molecule in an infected blood sample following exposure of the sample to the disinfectant (and to distilled water as control). The loss of detectable virus specific molecules is used as a marker of virus 'killing'. The assay is likely to underestimate the effectiveness of disinfectants against HCV because the molecule detected is relatively resistant to chemical degradation; it is, however, essential for infectivity and so its disappearance following treatment is a good indication of virus inactivation.

Method

Virkon solution was prepared by adding one complete sachet to 5 litres of water pre-warmed to 35°C. The solution was stirred to completely dissolve the powder.

10μl aliquots of an infectious HCV serum sample were exposed to either

a. 990μl of prepared Virkon solution, or
b. 990μl of distilled water.

These treatments were performed at room temperature (~20°C) for a contact time of 10 minutes.

Immediately after the treatment an extraction procedure was used to isolate virus specific molecules from the mixture. Residual disinfectant was removed at this stage and a control molecule was introduced to check for interference by any remaining disinfectant during the subsequent detection step.

The concentration of remaining detectable virus specific molecules was measured using a very sensitive quantitative assay, the results of which are expressed as copies of molecule detected per ml of blood.

Results

No interference of the detection assay was observed.

Virkon treatment

NO detectable virus specific molecules remaining.

Distilled water treatment

11,623 virus specific molecules per ml detected.

Comment

Virkon reduced the detectable virus specific molecule in the blood sample after 10 minutes contact time to below the limit of detection of the assay and it was therefore successful in this indirect estimation of its activity against HCV. Virkon was tested with a light organic challenge such as might be encountered in a protocol with a pre-wash step.

As Micropathology Ltd has no control over the usage of this material, this testing does not constitute an endorsement of this product by Micropathology Ltd in any application. Whilst Micropathology Ltd has tested this material in the manner indicated, the Company specifically excludes any reference to its name in any literature as promotional material related to any product so cited.

Steven Read BSc Hons
Molecular Scientist

VIRUCIDAL EFFICACY ASSAY

REPORT TITLE

10 Minute Inactivation of Human Immunodeficiency Virus Type 1 by Virkon S

DATA REQUIREMENTS

U.S. EPA 40 CFR Part 158
"Data Requirements for Registration"

PRODUCT IDENTITY

Virkon S,
Lot 1698 and Lot 2050

PROJECT NUMBER

1566

AUTHOR

C. Sue Brady, M.T.
Study Director

FINAL REPORT

September 21, 1995

PERFORMING LABORATORY

ViroMed Laboratories, Inc.
6101 Blue Circle Drive
Minneapolis, MN 55343

SPONSOR

Antec International LTD.
Chilton Industrial Estate
Sudbury, Suffolk C010 6XD
England

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA Section 10 (d) (1) (A), (B), or (C).

Company:_____

Company Agent:_____ Date:_____

GOOD LABORATORY PRACTICE STATEMENT

The study referenced in this report was conducted in compliance with U.S. Environmental Protection Agency Good Laboratory Practice (GLP) regulations set forth in 40 CFR Part 160. The studies not performed by or under the direction of ViroMed Laboratories, Inc. are exempt from this Good Laboratory Practice statement and include: characterization and stability of the compound(s).

Study Director: C. Sue Brady _____ 9/21/95
C. Sue Brady, M.T. (ASCP/NCA) — Date

Submitter: _____ _____
Date

Sponsor: _____ _____
Date

QUALITY ASSURANCE UNIT SUMMARY

Study: Virucidal Efficacy

The objective of the Quality Assurance Unit is to monitor the conduct and reporting of nonclinical laboratory studies. These studies have been performed under Good Laboratory Practice regulations (40 CFR Part 160) and in accordance to standard operating procedures and standard protocols. The Quality Assurance Unit maintains copies of study protocols and standard operating procedures and has inspected this study on the date(s) listed below. Studies are inspected at time intervals to assure the integrity of the study. The findings of these inspections have been reported to management and the Study Director.

Phase Inspected:	Project Set-up	Date: September 5, 1995
	In Process	Date: September 11, 1995
	Final Reading	Date: September 12, 1995
	Final Report	Date: September 21, 1995
	Study Director Review	Date: September 21, 1995
	Management Review	Date: September 21, 1995

Professional personnel involved:

Bonita L. Baskin, Ph.D.	- Laboratory Director
C. Sue Brady, M.T.	- Study Director
Karen M. Ramm, B.A.	- Research Assistant II
Katherine A. Paulson, C.L.A.	- Research Assistant I
Joyce A. Nelson	- Quality Assurance Director

Documentation of the above Quality Assurance audits have been reviewed.

Quality Assurance Director: Joyce A. Nelson Date: Sept. 21, 1995

Study Director: C. Sue Brady Date: 9/21/95

REPORT

EVALUATION OF VIRUCIDAL EFFICACY OF VIRKON S IN AN INANIMATE SURFACE ASSAY AGAINST HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 (HIV-1).

TEST OBJECTIVE

The objective of this study is to determine the virucidal efficacy of this product on an inanimate environmental surface when in contact with HIV-1 for a 10 minute exposure time.

SPONSOR: Antec International LTD.
Chilton Industrial Estate
Sudbury, Suffolk C010 6XD
England

SAMPLE NAME OR CODE: Virkon S, Lot 1698 and Lot 2050

DATE SAMPLE RECEIVED: Lot 1698 - January 13, 1995
Lot 2050 - February 15, 1995

TEST ARTICLE CHARACTERIZATION

The identity, strength, purity, stability, and chemical composition was not provided by Sponsor to ViroMed Laboratories, Inc.

TEST FACILITY: ViroMed Laboratories, Inc.
6101 Blue Circle Drive
Minneapolis, MN 55343

DATA RETENTION

A certified copy of this report, materials and data pertinent to this study will be stored at ViroMed Laboratories, Inc., 6101 Blue Circle Drive, Minneapolis, MN 55343. The test substances will be returned to Sponsor.

INITIATION DATE: July 12, 1995
COMPLETION DATE: September 21, 1995

SUMMARY OF RESULTS:

Disinfectant:	Virkon S
Dilution:	1:100 in 400 ppm AOAC synthetic hard water
Virus:	HIV-1, Strain HTLV III_B
Exposure Time:	10 minutes
Exposure Temperature:	Room temperature (25°C)
Organic Soil Load:	5% fetal bovine serum (fbs)
Efficacy Result:	**VIRUCIDAL** under these test conditions

MATERIALS

1. Virus
The HTLV-III_B strain of HIV-1 was used in this study. The virus was obtained from Dr. Neal T. Wetherall, Vanderbilt University, Nashville, TN and demonstrated cytopathic effects (CPE) typical of HIV-1 on MT-2 cells.

2. Test Cell Cultures
MT-2 cells were originally obtained from Dr. Robert Shoemaker of the National Cancer Institute, Frederick, MD. Cultures were grown and propagated in house and used in suspension in disposable tissue culture labware.

3. Test Media:
The test media used in this study was RPMI 1640 supplemented with 15% (v/v) fetal bovine serum (fbs) heat-inactivated at 56°C for 30 minutes. The medium was also supplemented with 2 mM L-glutamine, 50 µg/ml gentamicin and 25 mM Hepes.

The following table lists the test and control groups, the dilutions assayed, and the numbers of cultures used. See text for a more detailed explanation.

Number of Dilutions and Cultures for Virucidal Efficacy Study			
Test or Control Group	Dilutions to be Assayed (log_{10})	Cultures per dilution	Total Cultures
Cell Control		4	4/group
Virus Control (Group A)	-2,-3,-4,-5,-6,-7	4	28
Sample batch #1+virus (Group B)	-2,-3,-4,-5,-6,-7	4	28
Sample batch #2+virus (Group B)	-2,-3,-4,-5,-6,-7	4	28
Toxicity of batch #1 (Group C)	-2,-3,-4,-5,-6,-7	4	28
Toxicity of batch #2 (Group C)	-2,-3,-4,-5,-6,-7	4	28
Non-Virucidal level - batch #1 (Group D)	-2,-3,-4,-5,-6,-7	4	28
Non-Virucidal level - batch #2 (Group D)	-2,-3,-4,-5,-6,-7	4	28

METHODS

1. Preparation of Disinfectant

Virkon S was tested at a 1:100 dilution in 400 ppm AOAC synthetic hard water as requested by the Sponsor (50 grams test substance + 5 liters 400 ppm hard water). The product was soluble upon use for testing. Disinfectant dilution was prepared on day of use.

The 400ppm AOAC Hard Water used for Lot 1698 was made using 24ml of Solution 1 + 3600ml sterile deionized water, 24ml of Solution 2, and another 2352ml of sterile deionized water. The 400ppm hard water was prepared, titrated (actual titration result = 404ppm), and used for testing on the day of test set-up.

The 400ppm AOAC Hard Water used for Lot 2050 was made using 24ml of Solution 1 + 3600ml sterile deionized water, 24ml of Solution 2, and another 2352ml of sterile deionized water. The 400ppm hard water was prepared, titrated (actual titration result = 400ppm), and used for testing on the day of test set-up.

2. Preparation of Virus Films

Films of the virus were prepared by spreading 0.2ml amounts of undiluted virus suspension on the bottoms of sterile glass petri dishes. Films were kept at room temperature (25°C) and ambient humidity until dry (35 minutes). The virus was further dried for 30 minutes at 37°C.

3. Treatment of Virus Films with Disinfectant

Dried virus films were exposed to 2.0 mls of the disinfectant for a 10 minute exposure time at 25°C. Following the exposure time, the plate was scraped with a plastic cell scraper to resuspend the contents of the plate and the virus disinfectant mixture was immediately added to 18ml of LBS broth. The mixture (10^{-2} dilution) was immediately titered by serial dilution and assayed for infectivity.

4. Treatment of Virus Control Films

A virus film was prepared as previously described. The control was run in parallel to the test virus, but 2 mls of test media was added in lieu of the disinfectant. The control virus was scraped as previously described and the mixture was immediately added to the neutralizer, titered by serial dilution and assayed for infectivity. (The control was exposed to the media for the same amount of time as the test film was exposed to the disinfectant).

5. Cytotoxicity Controls

An aliquot of disinfectant plus the neutralizer was diluted serially in media and inoculated into cell cultures. Cytotoxicity of cell cultures were scored at the same time as virus/disinfectant and virus control cultures.

6. Assay of Non-Virucidal Level of Disinfectant

Each dilution of the disinfectant (cytotoxicity control) was mixed with an aliquot of the stock virus, and the resulting mixtures of dilutions were assayed for infectivity in order to determine the dilution(s) of disinfectant at which virucidal activity, if any, was retained. Dilutions that showed virucidal activity were not considered in determining reduction in infectivity by the disinfectant.

7. Neutralization

As described previously, following the exposure period of the disinfectant to the dried virus film, the virus disinfectant mixture was immediately added to the neutralizer (LBS broth) to decrease the virucidal action of the disinfectant. Following the addition of the neutralizer, the virus-disinfectant-neutralizer mixture was immediately titered by serial dilution to further decrease the virucidal properties of the disinfectant. Controls were run in parallel following the same method.

8. Infectivity Assays

The MT-2 cell line, which exhibits lytic Cytopathic Effect (CPE) in the presence of HIV, was used as the indicator cell line in the infectivity assays. Dilutions of virus-disinfectant mixtures and the controls were inoculated into the MT-2 cell cultures in quadruplicate. The cultures were inoculated with 200µl of each dilution and 50ul of MT-2 cells (5×10^4/well) were added. The cultures were incubated at 36-38°C in 5-7% CO_2. The cells were observed for 7 days and Cytopathic Effect (CPE) and cytotoxicity were recorded. The CPE demonstrated was typical of HIV-1 on MT-2 cells (multinucleated giant cells).

9. Calculations

The method of Karber was used to calculate 50 percent end points.

$$-1 - \left[\frac{\text{(Sum of \% mortality at each dilution)}}{100} - 0.5 \times \text{(logarithm of dilution)}\right]$$

ANALYSIS AND CONCLUSION

Results of tests with two lots of disinfectant against HIV-1 are shown in Table 1. The titer of the virus control was 6.75 $\log_{10}$. Infectivity was not detected in the virus-disinfectant mixture for either lot at any dilution tested ($\leq 2.5 \log_{10}$). Toxicity was observed for both lots at 2.5 $\log_{10}$. The neutralization control (non-virucidal level of the disinfectant) indicates that the disinfectant was neutralized at 2.5 $\log_{10}$. Taking the toxicity and neutralization control results into consideration the reduction in virus titer was greater than or equal to 4.25 $\log_{10}$ for both batches of disinfectant. Tests on two lots of Virkon S demonstrated complete inactivation of HIV-1 indicating **VIRUCIDAL** activity under these test conditions.

REFERENCES

1. ASTM Standards on Materials and Environmental Microbiology, 1987, E1053-85.
2. U.S. Environmental Protection Agency Pesticide assessment Guidelines, Subdivision G: Product Performance, November, 1982. U.S. Environmental Protection Agency, Registration Division, Office of Pesticide Programs, DIS/TSS-7, November 12, 1981.
3. Blackwell, J.H., and J.H.S. Chen. 1970. Effects of various germicidal chemicals on HEP-2 cell culture and Herpes simplex virus. J. AOAC 53:1229-1236.
4. Diagnostic Procedures for Viral, Rickettsial, and Chlamydial Infections. Lennette, E.H. and Schmidt, N.J. editors. Fifth edition, 1979. p. 32-35.

TABLE 1

Effects of Virkon S, Lot 1698 and Lot 2050 on HIV-1 Dried on an Inanimate Surface when Exposed for 10 Minutes.

DILUTION	DRIED VIRUS CONTROL (GROUP A)	Virkon S + HIV-1 Lot 1698 (GROUP B)	Virkon S + HIV-1 Lot 2050 (GROUP B)
Cell Control	0 0 0 0	0 0 0 0	0 0 0 0
10^{-2}	+ + + +	T T T T	T T T T
10^{-3}	+ + + +	0 0 0 0	0 0 0 0
10^{-4}	+ + + +	0 0 0 0	0 0 0 0
10^{-5}	+ + + +	0 0 0 0	0 0 0 0
10^{-6}	+ + + +	0 0 0 0	0 0 0 0
10^{-7}	0 + 0 0	0 0 0 0	0 0 0 0
$TCID_{50}$/0.2ml	$10^{6.75}$	$\leq 10^{2.5}$	$\leq 10^{2.5}$

DILUTION	CYTOTOXICITY Lot 1698 (GROUP C)	CYTOTOXICITY Lot 2050 (GROUP C)
Cell Control	0 0 0 0	0 0 0 0
10^{-2}	T T T T	T T T T
10^{-3}	0 0 0 0	0 0 0 0
10^{-4}	0 0 0 0	0 0 0 0
10^{-5}	0 0 0 0	0 0 0 0
10^{-6}	0 0 0 0	0 0 0 0
10^{-7}	0 0 0 0	0 0 0 0
TCD_{50}/0.2ml	$\leq 10^{2.5}$	$\leq 10^{2.5}$

(+) = positive for the presence of test virus
(0) = no test virus recovered and/or no cytotoxicity present
(T) = Cytotoxicity present

Non-Virucidal Level (control)

DILUTION	Virus Control + Cyto. Control Lot 1698 (GROUP D)	Virus Control + Cyto. Control Lot 2050 (GROUP D)
Cell Control	0 0 0 0	0 0 0 0
10^{-2}	T T T T	T T T T
10^{-3}	+ + + +	+ + + +
10^{-4}	+ + + +	+ + + +
10^{-5}	+ + + +	+ + + +
10^{-6}	+ + + +	+ + + +
10^{-7}	+ + + +	+ + + +

Results of the Non-virucidal level control indicate that the test substance was neutralized at 2.5 $\log_{10}$ ($TCID_{50}$).

(+) = positive for the presence of test virus
(0) = no test virus recovered and/or no cytotoxicity present
(T) = Cytotoxicity present