

# Microbiological Activity

## Independent Laboratory Verification



**Samples:** One sample of Citrox BC received from Citrox Limited  
5<sup>th</sup> July 2004  
**Certificate Number:** 04H.246C.  
**Analysis Required:** BS EN 1276 under dirty conditions  
**Samples Tested:** 9<sup>th</sup> July 2004

Product stored at 5<sup>o</sup>C in the dark.  
Active substance: Not declared.  
Batch Number: 033567

### Experimental conditions:

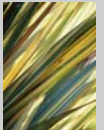
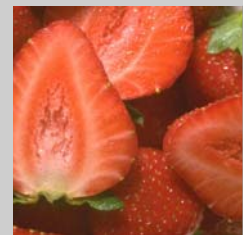
Product diluent used during test	Sterile hard water 300mg/kg CaCO <sub>3</sub>
Product test concentrations	0.2% w/w
Contact time	5 min
Test Temperature	20 <sup>o</sup> C ± 0.5 <sup>o</sup> C
Interfering substance	3.0g/l Bovine albumin
Neutralising solution	3% Tween 80, 3% Saponin, 0.1% Histidine, 0.1% Cysteine
Temperature of incubation	30 <sup>o</sup> C ± 1 <sup>o</sup> C
Identification of bacterial strains used	Campylobacter jejuni NCTC 11322

### Test Results

Sample challenged with 24 hour culture of Campylobacter jejuni with 0.3% bovine serum as organic challenge. After 5 minutes exposure a  $3.26 \times 10^5$  reduction in numbers was observed. This would satisfy the requirements of BS and EN test protocols for efficacy against this organism.

### Conclusion:

Conclusion: According to EN1276 this batch of Citrox BC disinfectant when diluted at 0.2% w/w in hard water possesses satisfactory bactericidal activity in 5 minutes at 20<sup>o</sup>C under dirty conditions (3.0g/l bovine albumin) for the reference strain detailed.



Independent  
Laboratory  
Verification  
by Abbott Analytical



# Microbiological Activity

## Independent Laboratory Verification



**Samples:** One sample of Citrox BC received from Citrox Limited  
5<sup>th</sup> July 2004  
**Certificate Number:** 04H.246C.  
**Analysis Required:** BS EN 1276 under dirty conditions  
**Samples Tested:** 9<sup>th</sup> July 2004

Product stored at 5<sup>o</sup>C in the dark.  
Active substance: Not declared.  
Batch Number: 033567

### Experimental conditions:

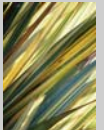
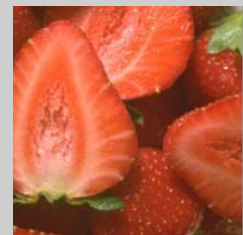
Product diluent used during test	Sterile hard water 300mg/kg CaCO <sub>3</sub>
Product test concentrations	0.2% w/w
Contact time	5 min
Test Temperature	20 <sup>o</sup> C ± 0.5 <sup>o</sup> C
Interfering substance	3.0g/l Bovine albumin
Neutralising solution	3% Tween 80, 3% Saponin, 0.1% Histidine, 0.1% Cysteine
Temperature of incubation	30 <sup>o</sup> C ± 1 <sup>o</sup> C
Identification of bacterial strains used	Campylobacter jejuni NCTC 11322

### Test Results

Sample challenged with 24 hour culture of Campylobacter jejuni with 0.3% bovine serum as organic challenge. After 5 minutes exposure a  $3.26 \times 10^5$  reduction in numbers was observed. This would satisfy the requirements of BS and EN test protocols for efficacy against this organism.

### Conclusion:

Conclusion: According to EN1276 this batch of Citrox BC disinfectant when diluted at 0.2% w/w in hard water possesses satisfactory bactericidal activity in 5 minutes at 20<sup>o</sup>C under dirty conditions (3.0g/l bovine albumin) for the reference strain detailed.



Independent  
Laboratory  
Verification  
by Abbott Analytical



# Microbiological Activity

## Independent Laboratory Verification



**Samples:** One sample of Citrox BC received from Citrox Limited  
20<sup>th</sup> August 2004  
**Certificate Number:** 04H.246cd.CIT  
**Analysis Required:** Activity against Clostridium difficile using EN 13704  
under dirty conditions:  
**Samples Tested:** 8<sup>th</sup> - 9<sup>th</sup> December 2004

Product stored at 5<sup>o</sup>C in the dark.  
Active substance: Not declared.

### Experimental conditions:

Product diluent used during test Sterile Hard Water 300mg/kg CaCO<sub>3</sub>  
Product test concentrations 0.4% v/v  
Contact time 30 min  
Test Temperature 20<sup>o</sup>C ± 1<sup>o</sup>C  
Interfering substance 3.0g/l Bovine albumin  
Neutralising solution 3% Tween 80, 3% Saponin,  
0.1% Histidine, 0.1% Cysteine  
Temperature of incubation 37<sup>o</sup>C ± 1<sup>o</sup>C  
Identification of bacterial strains used Clostridium difficile ATCC 11437

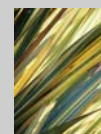
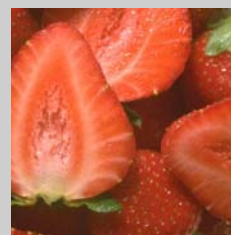
### Test Results

Validation Test	Clostridium difficile
Bacterial Suspension	Vc 254, 206 Nv 2.30 x 10 <sup>3</sup>
Experimental Conditions	Vc 210, 228 A 2.19 x 10 <sup>2</sup>
Neutraliser Control	Vc 198, 224 B 2.11 x 10 <sup>2</sup>
Dilution neutralization Control	Vc 186, 214 C 2.00 x 10 <sup>2</sup>
Bacterial Test Suspension	10 <sup>-6</sup> 228, 200 10 <sup>-7</sup> 25, 23 N 2.27 x 10 <sup>6</sup>
Test results at concentrations	
1 : 33	Vc 724 Na 72400 R 3.93 x 10 <sup>3</sup>

VC = Viable Count  
Nv = Number of cfu/ml in bacterial suspension  
A,B & C = Mean actual count cfu in suspension  
N = Number of cfu in bacterial test suspension  
Na = Number of cfu in test mixture  
R = Reduction on viability

### Conclusion:

According to EN13704 Citrox BC when diluted 1 : 250 in sterile hard water possesses sporicidal activity in 30 minutes at 20<sup>o</sup>C under dirty conditions for the referenced strain detailed.



Independent  
Laboratory  
Verification  
by Abbott Analytical



# Microbiological Activity

## Independent Laboratory Verification



**Samples:** One sample of Citrox BC received from Citrox Limited  
20<sup>th</sup> August 2004  
**Certificate Number:** 04H.246C.  
**Analysis Required:** Activity against Clostridium perfringens using EN 13704  
under dirty conditions  
**Samples Tested:** 7<sup>th</sup> & 8<sup>th</sup> October 2004

Product stored at 5<sup>o</sup>C in the dark.  
Active substance: Not declared.  
Batch Number: 033567

### Experimental conditions:

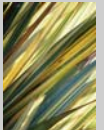
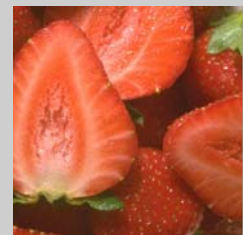
Product diluent used during test	Sterile hard water 300mg/kg CaCO <sub>3</sub>
Product test concentrations	0.4% w/w
Contact time	30 min
Test Temperature	20 <sup>o</sup> C ± 0.5 <sup>o</sup> C
Interfering substance	3.0g/l Bovine albumin
Neutralising solution	3% Tween 80, 3% Saponin, 0.1% Histidine, 0.1% Cysteine
Temperature of incubation	30 <sup>o</sup> C ± 1 <sup>o</sup> C
Identification of bacterial strains used	Clostridium perfringens

### Test Results

Sample challenged at 0.4% w/w. After 30 minutes exposure a  $1.08 \times 10^4$  reduction in numbers was observed. This would satisfy the requirements of BS and EN test protocols for efficacy against this organism.

### Conclusion:

Conclusion: According to EN13704 this batch of Citrox BC disinfectant when diluted at 0.4% w/w in hard water possesses satisfactory bactericidal activity in 30 minutes at 20<sup>o</sup>C under dirty conditions (3.0g/l bovine albumin) for the reference strain detailed.



Independent  
Laboratory  
Verification  
by Abbott Analytical



# Microbiological Activity

## Independent Laboratory Verification



**Samples:** One sample of Citrox BC received from Citrox Limited 23rd June 2005  
**Certificate Number:** 05F.214.CIT  
**Analysis Required:** BS EN 1276 under dirty conditions  
**Samples Tested:** 24th June 2005

Product stored at 5°C in the dark.  
Active substance: Not declared.  
Batch Number: 363016 / 363017

### Experimental conditions:

Product diluent used during test Sterile hard water 300mg/kg  
CaCO<sub>3</sub>  
Product test concentrations 0.2% w/w  
Contact time 5 min  
Test Temperature 20°C ± 0.5°C  
Interfering substance 3.0g/l Bovine albumin  
Neutralising solution 3% Tween 80, 3% Saponin,  
0.1% Histidine, 0.1% Cysteine  
Temperature of incubation 30°C ± 1°C

Identification of bacterial strains used *Pseudomonas aeruginosa* ATCC 15442  
*Escherichia coli* NCTC 10418  
*Staphylococcus aureus* NCTC 6571  
*Enterococcus hirae* ATCC 8043

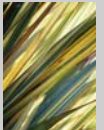
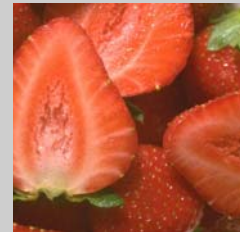
### Test Results

Validation test	<i>Pseudomonas aeruginosa</i>	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	<i>Enterococcus hirae</i>
Bacterial suspension	Vc 184, 166 Nv 1.75 x 10 <sup>3</sup>	Vc 154, 138 Nv 1.46 x 10 <sup>3</sup>	Vc 136, 147 Nv 1.41 x 10 <sup>3</sup>	Vc 212, 185 Nv 1.98 x 10 <sup>3</sup>
Experimental conditions	Vc 210, 180 A 1.95 x 10 <sup>2</sup>	Vc 166, 192 A 1.79 x 10 <sup>2</sup>	Vc 151, 142 A 1.46 x 10 <sup>2</sup>	Vc 210, 232 A 2.21 x 10 <sup>2</sup>
Neutraliser control	Vc 188, 196 B 1.92 x 10 <sup>2</sup>	Vc 158, 142 B 1.50 x 10 <sup>2</sup>	Vc 160, 146 B 1.53 x 10 <sup>2</sup>	Vc 208, 182 B 1.95 x 10 <sup>2</sup>
Dilution-neutralisation control	Vc 220, 186 C 2.03 x 10 <sup>2</sup>	Vc 182, 164 C 1.73 x 10 <sup>2</sup>	Vc 155, 136 C 1.45 x 10 <sup>2</sup>	Vc 230, 216 C 2.23 x 10 <sup>2</sup>
Bacterial Test Suspension	10 <sup>-6</sup> 354, 416 10 <sup>-7</sup> 38, 37 N 3.80 x 10 <sup>8</sup>	10 <sup>-6</sup> 408, 372 10 <sup>-7</sup> 44, 40 N 4.05 x 10 <sup>8</sup>	10 <sup>-6</sup> 127, 139 10 <sup>-7</sup> 12, 15 N 1.34 x 10 <sup>8</sup>	10 <sup>-6</sup> 224, 272 10 <sup>-7</sup> 22, 20 N 2.29 x 10 <sup>8</sup>
Test results				
0.2% w/w Vc	2	0	0	0
Na	200	<100	<100	<100
R	1.9 x 10 <sup>6</sup>	>4.05 x 10 <sup>6</sup>	>1.34 x 10 <sup>6</sup>	>2.29 x 10 <sup>6</sup>

Vc = Viable Count.  
N = Number of cfu/ml of the bacterial test suspension.  
Nv = Number of cfu in bacterial suspension.  
R = Reduction in viability.  
Na = Number of cfu/ml in the test mixture

### Conclusion:

According to BS EN1276 this batch of Citrox BC when diluted at 1:250 (v/v) in hard water possess satisfactory bactericidal activity in 5 minutes at 20°C under dirty conditions (3.0g/l bovine albumin) for the organisms detailed.



Independent  
Laboratory  
Verification  
by Abbott Analytical



# Microbiological Activity

## Independent Laboratory Verification



**Samples:** One sample of Citrox BC received from Citrox Limited  
31st May 2006

**Certificate Number:** 06E.184.CIT

**Analysis Required:** Activity against Enterococcus Faecalis under dirty conditions

**Samples Tested:** 1st June 2006

Product stored at 5°C in the dark.  
Active substance: Not declared.  
Batch number :366362

### Experimental conditions:

Product diluent used during test Artificial Hard Water 300mg CaCO<sub>3</sub>  
Product test concentrations 0.6% v/v  
Contact time 5 min  
Test Temperature 20°C ± 0.5°C  
Interfering substance  
a) 3.0g/l Bovine Albumin  
b) 3.0g/l Bovine Albumin/  
3.0ml/l sheep erythrocytes  
c) 10.0g/l Bovine Albumin/  
10.0g/l Yeast Extract

Temperature of incubation 30°C ± 1°C

Identification of bacterial strains used Enterococcus Faecalis NCTC 8213

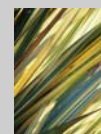
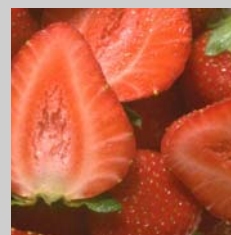
### Test Results

Validation Test	Enterococcus Faecalis
Bacterial Suspension	Vc 156, 172 Nv 1.64 x 10 <sup>3</sup>
Experimental Conditions	Vc 138, 166 A 1.52 x 10 <sup>2</sup>
Neutraliser Control	Vc 128, 154 B 1.41 x 10 <sup>2</sup>
Dilution neutralization Control	Vc 133, 115 C 1.24 x 10 <sup>2</sup>
Bacterial Test Suspension	10 <sup>-6</sup> 124, 144 10 <sup>-7</sup> 11, 15 N 1.32 x 10 <sup>8</sup>
Test results	
A.	Vc 0 Na <100 R >1.32 x 10 <sup>6</sup>
B.	Vc 8 Na 800 R 1.65 x 10 <sup>5</sup>
C.	Vc 11 Na 1100 R 1.20 x 10 <sup>5</sup>

VC = Viable Count  
Nv = Number of cfu/ml in bacterial suspension  
A,B & C = Mean actual count cfu in suspension  
N = Number of cfu in bacterial test suspension  
Na = Number of cfu in test mixture  
R = Reduction on viability

### Conclusion:

According to EN1276 Citrox BC when diluted 1 : 166 (v/v) in hard water possesses satisfactory bactericidal activity in 5 minutes at 20°C under dirty conditions (10g/l bovine serum, 10g/l yeast extract) for Enterococcus Faecalis, under the conditions of Norwich Hospital protocol there is a possibility of visible growth of organisms in broth culture after 7 days.



Independent  
Laboratory  
Verification  
by Abbott Analytical



# Microbiological Activity

## Independent Laboratory Verification



**Samples:** One sample of Citrox BC received from Citrox Limited  
1<sup>st</sup> July 2004  
**Certificate Number:** 04G.0031.CIT  
**Analysis Required:** Activity against *Lactobacillus acidophilus* using modified  
EN 1276 under dirty conditions.  
**Samples Tested:** 7<sup>th</sup> & 8<sup>th</sup> July 2004

Product stored at 5°C in the dark.  
Active substance: Not declared.

### Experimental conditions:

Product diluent used during test Sterile hard water 300mg/kg CaCO<sub>3</sub>  
Product test concentrations 0.2% v/v  
Contact time 5 min  
Test Temperature 20°C ± 1°C  
Interfering substance 3.0g/l Bovine albumin  
Neutralising solution 3% Tween 80, 3% Saponin,  
0.1% Histidine, 0.1% Cysteine  
Temperature of incubation 37°C ± 1°C

Identification of bacterial strains used *Lactobacillus acidophilus* ATCC 4356

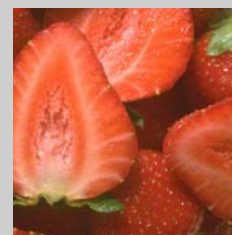
### Test Results

Validation Test	Lactobacillus Acidophilus
Bacterial Suspension	Vc 166, 132 Nv 1.49 x 10 <sup>3</sup>
Experimental Conditions	Vc 155, 111 A 1.33 x 10 <sup>2</sup>
Neutraliser Control	Vc 128, 102 B 1.15 x 10 <sup>2</sup>
Dilution neutralization Control	Vc 152, 169 C 1.60 x 10 <sup>2</sup>
Bacterial Test Suspension	10 <sup>-6</sup> 252 206 10 <sup>-7</sup> 26 27 N 2.47 x 10 <sup>8</sup>
Test results at concentrations	
1 : 500	Vc 21 Na 2100 R 1.17 x 10 <sup>5</sup>

Vc = Viable Count  
Nv = Number of cfu/ml in bacterial suspension  
A,B & C = Mean actual count cfu in suspension  
N = Number of cfu in bacterial test suspension  
Na = Number of cfu in test mixture  
R = Reduction on viability

### Conclusion:

According to EN 1276 Citrox BC when diluted 1 : 500 in sterile hard water possesses bacterial activity in 5 minutes at 20°C under dirty conditions for the referenced strains detailed.



Independent  
Laboratory  
Verification  
by Abbott Analytical



# Microbiological Activity

## Independent Laboratory Verification



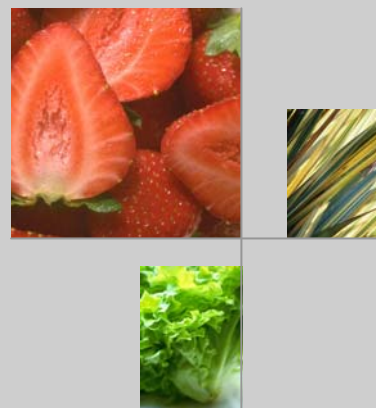
**Samples:** One sample of Citrox BC disinfectant received from Citrox Limited 23<sup>rd</sup> April 2003  
**Certificate Number:** 03D.3141.GWP  
**Analysis Required:** Activity against Legionella pneumophila at 0.6% v/v under simulated EN 1276 test conditions  
**Samples Tested:** 14<sup>th</sup> May 2003

### Test Results

Legionella pneumophila NCTC 11192

Reduction in numbers after 15 minutes  $1.90 \times 10^4$

Reduction in numbers after 30 minutes  $1.20 \times 10^6$



Independent  
Laboratory  
Verification  
by Abbott Analytical



# Microbiological Activity

## Independent Laboratory Verification

**Samples:** One sample of Citrox BC disinfectant received from Citrox Limited 5<sup>th</sup> March 2004

**Certificate Number:** 04C.100.CIT

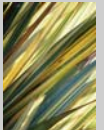
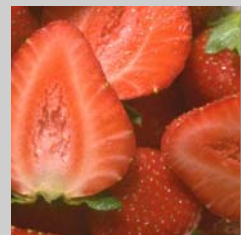
**Analysis Required:** Activity against *Listeria* on surfaces using an Electrostatic sprayer giving 20 micron droplet size.

**Samples Tested:** 5<sup>th</sup> March 2004

An overnight broth culture of *Listeria monocytogenes* was sprayed on to a food grade plastic clad wall using an atomizer and allowed to dry for approximately 2 hours. The wall was then divided in squares for approximately 500mm x 500mm with masking tape. A swab was taken from one of the squares before any treatment, placed in 10ml of recovery medium shaken vigorously to disperse the organisms and plated on to *Listeria* selective agar to establish the base level of contamination. Subsequent squares were treated with 2.5%v/v, 10%v/v and 25%v/v respectively of Citrox BC in sterile tap water by spraying from the electrostatic sprayer for a measured time of 2 minutes. After each square had been treated it was allowed to be in contact with the contaminated area for 5 minutes before the area was swabbed with a fresh sterile swab which was then placed in 10ml of a sterile neutralizing solution before examining for surviving organisms as with the control. Only after the swabbing was done was then next square sprayed. The results obtained for survival of *Listeria* after treatment are recorded below.

### Test Results

Control	2.5%	10%	25%
1920 cfu per sq.	465 (16.8% kill)	0 (100% kill)	0 (100% kill)



Independent  
Laboratory  
Verification  
by Abbott Analytical



# Microbiological Activity

## Independent Laboratory Verification

**Samples:** One sample of Citrox BC received from Citrox Limited  
13<sup>th</sup> February 2002

**Certificate Number:** 02b.136.GWP. Interim report

**Analysis Required:** Challenge tested against Moulds as indicated using  
adaptation of EN 1650

**Samples Tested:** 13<sup>th</sup> February 2002

Product stored at 5°C in the dark.  
Active substance: Not declared.

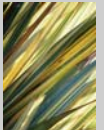
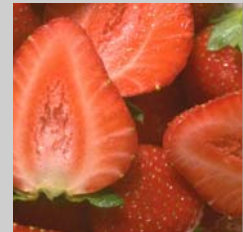
### Experimental conditions:

Product diluent used during test	Sterile hard water 300mg/kg CaCO <sub>3</sub>
Product test concentrations	1.5% w/w
Contact time	30 min
Test Temperature	20°C ± 0.5°C
Interfering substance	5% w/v yeast suspension
Neutralising solution	3% Tween 80, 3% Saponin, 0.1% Histidine, 0.1% Cysteine
Temperature of incubation	30°C ± 1°C
Identification of mould strains used	penicillium digitatum

### Test Results

Sample challenged with spore suspension of the mould under test. After 30 minutes exposure the following reduction in numbers was observed.

Penicillium digitatum 2.16 x 10<sup>6</sup>



Independent  
Laboratory  
Verification  
by Abbott Analytical

# Microbiological Activity

## Independent Laboratory Verification



**Samples:** One sample of Citrox BC disinfectant received from Citrox Limited 23<sup>rd</sup> April 2003  
**Certificate Number:** 0.3D.314m.GWP.  
**Analysis Required:** Activity against Mycobacterium fortuitum at 0.6% v/v under simulated EN 1276 test conditions  
**Samples Tested:** 16<sup>th</sup> May 2003

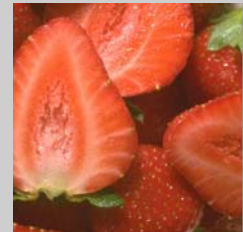
### Test Results

Mycobacterium fortuitum is considered a suitable organism disinfectant materials for tuberculocidal activity. It forms the organism of choice for testing for tuberculocidal activity of a product for registration under MAFF Bovine TB Orders (BS 6734)

Mycobacterium fortuitum NCTC 8573

Reduction in numbers after 15 minutes  $9.15 \times 10^3$

Reduction in numbers after 30 minutes  $7.25 \times 10^5$



Independent  
Laboratory  
Verification  
by Abbott Analytical



# Microbiological Activity

## Independent Laboratory Verification



**Samples:** One sample of Citrox BC received from Citrox Limited  
10<sup>th</sup> December 2003  
**Certificate Number:** 3m.155.CIT  
**Analysis Required:** Challenge test against *Phytophthora* Spp as indicated  
using adaptation of EN 1650  
**Samples Tested:** 15<sup>th</sup> January 2004

Product stored at 5<sup>o</sup>C in the dark.  
Active substance: Not declared.

### Experimental conditions:

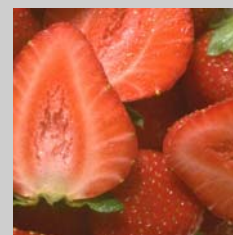
Product diluent used during test	Sterile hard water 300mg/kg CaCO <sub>3</sub>
Product test concentrations	0.12%, 0.25% & 0.5% v/v
Contact time	30 min
Test Temperature	20 <sup>o</sup> C ± 0.5 <sup>o</sup> C
Interfering substance	5% w/v yeast suspension
Neutralising solution	3% Tween 80, 3% Saponin, 0.1% Histidine, 0.1% Cysteine
Temperature of incubation	30 <sup>o</sup> C ± 1 <sup>o</sup> C
Identification of bacterial strains used	<i>Phytophthora ramorum</i>

### Test Results

Sample challenged with spore suspension of the mold under test. After 30 minutes exposure the following reduction in numbers was observed:

0.12%	0.25%	0.5%
3.72 x 10 <sup>3</sup>	8.46 x 10 <sup>3</sup>	2.76 x 10 <sup>4</sup>

At 0.5% v/v Citrox BC shows significant reduction in numbers of *Phytophthora* under the test conditions described.



Independent  
Laboratory  
Verification  
by Abbott Analytical



# Microbiological Activity

## Independent Laboratory Verification



**Samples:** One sample of Citrox BC received from Citrox Limited  
13<sup>th</sup> January 2003  
**Certificate Number:** 03A.102.GWP  
**Analysis Required:** BSEN 1276 under dirty conditions  
**Samples Tested:** 17<sup>th</sup> January 2003

Product stored at 5<sup>o</sup>C in the dark.  
Active substance: Not declared.  
Batch number: BC Aus / 14W

### Experimental conditions:

Product diluent used during test Sterile Hard Water 300mg/kg CaCO<sub>3</sub>  
Product test concentrations 3% v/v  
Contact time 5 min  
Test Temperature 20<sup>o</sup>C ± 0.5<sup>o</sup>C  
Interfering substance 3.0g/l Bovine albumin  
Neutralising solution 3% Tween 80, 3% Saponin,  
0.1% Histidine, 0.1% Cysteine  
Temperature of incubation 30<sup>o</sup>C ± 1<sup>o</sup>C

Identification of bacterial strains used Pseudomonas aeruginosa ATCC 15442

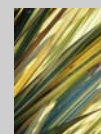
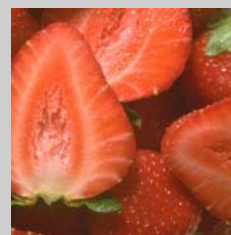
### Test Results

Validation Test	Pseudomonas aeruginosa
Bacterial Suspension	Vc 184, 166 Nv 1.75 x 10 <sup>3</sup>
Experimental Conditions	Vc 210, 180 A 1.95 x 10 <sup>2</sup>
Neutraliser Control	Vc 188, 196 B 1.92 x 10 <sup>2</sup>
Dilution neutralization Control	Vc 220, 186 C 2.03 x 10 <sup>2</sup>
Bacterial Test Suspension	10 <sup>-6</sup> 54, 76 10 <sup>-7</sup> 5, 5 N 5.57 x 10 <sup>8</sup>
Test results at concentrations	
1 : 33	Vc 37 Na 3700 R 1.55 x 10 <sup>5</sup>

VC = Viable Count  
Nv = Number of cfu/ml in bacterial suspension  
A,B & C = Mean actual count cfu in suspension  
N = Number of cfu in bacterial test suspension  
Na = Number of cfu in test mixture  
R = Reduction on viability

### Conclusion:

According to EN 1276 this batch of Citrox BC when diluted at 1 : 33 (v/v) in hard water possesses satisfactory bactericidal activity in 5 minutes at 20<sup>o</sup>C under dirty conditions (3.0g/l bovine albumin) for Pseudomonas.



Independent  
Laboratory  
Verification  
by Abbott Analytical





# Microbiological Activity

## Independent Laboratory Verification

**Samples:** One sample of Citrox Hand Gel received from Citrox Bioscience Ltd, 4th August 2005  
**Certificate Number:** 5H.099a.KAE  
**Analysis Required:** Activity against EN 1500  
**Samples Tested:** 22nd August 2005

### Principle of test:

The number of test organisms released from the fingertips of artificially contaminated hands is assessed before and after the hygienic handrub. The ratio of the two resulting values is called the reduction factor. It represents a measure of antimicrobial activity of the hygienic handwash product tested. In order to achieve the necessary precision a large number of subjects has to be used because of the possible variation in bacterial flora found on human skin. In this case a total of **twelve (12)** healthy adults were chosen each one carrying out the test procedure in precisely the same way as the others. To compensate for extraneous influences the test sample reduction factor (P) is compared with the reduction factor obtained with a parallel reference handwash procedure (R) which is performed with the same subjects, on the same day and under comparable environmental conditions.

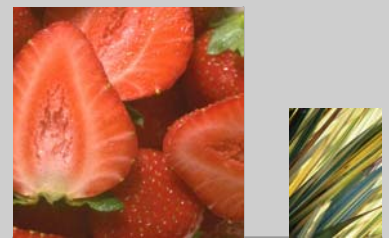
Experimental procedure:

#### 1) Application of the contamination fluid.

Each of the 12 subjects was asked to wash their hands for 1 minute in soft soap to remove natural commensal organisms and dried thoroughly on a paper towel. The hands were then contaminated with very large numbers of bacteria well in excess of that experienced in normal everyday occurrence. The hands were immersed in the contamination fluid (containing an overnight culture of the test organism in this instance E. coli at a concentration of approximately  $10^8$  cfu per ml) in a suitable sized container for 5 seconds. The hands were removed from the contamination fluid and surplus liquid allowed to drain back into the container. This time the hands were allowed to air dry for approximately 3 minutes holding them horizontally with fingers spread out and rotating them to and fro to avoid the formation of droplets.

#### 2) Pre-values.

Immediately after drying, each of the 12 subjects was asked to rub their fingertips, including the thumbs for 1 minute on the base of a petri dish, using a separate petri dish for each hand, containing 10ml of maximum recovery diluent (MRD) without neutraliser, in order to assess the release of test organisms before treatment of the hands. Dilutions of these sample fluids were prepared to  $10^{-3}$  and  $10^{-4}$ . A 1ml aliquot of each dilution for each hand was placed in a separate petri dish 10 - 15ml of Tryptone Soy Agar sterilised and cooled to  $45^{\circ}\text{C}$  added and mixed thoroughly. Plates were allowed to set and incubated at  $37^{\circ}\text{C}$  for 24 hours. Each plate was then examined for growth of the test organism..



Independent  
Laboratory  
Verification  
by Abbott Analytical



# Microbiological Activity

## Independent Laboratory Verification

Certificate number: 05h.099h.KAE

Page 2 of 4

### 3) Hygienic Handrub procedure.

Each of the 12 subjects was asked to pour 3ml of propan-2-ol into the cupped hands and rub vigorously for 30s onto the skin up to the wrists in accordance with the standard handrub procedure. This comprises five strokes backwards and forwards palm to palm, right palm over left dorsum and left palm over right dorsum, palm to palm with fingers interlaced, back of fingers to opposing palms with fingers interlocked, rotational rubbing of right thumb clasped in left palm and left thumb clasped in right palm, rotational rubbing with clasped fingers of right hand in palm of left hand and clasped fingers of left hand in right palm. Repeat with a further 3ml propan-2-ol to give a total rubbing time of 60s. After 60 seconds the hands are rinsed under running tap water for 5 seconds, excess water is shaken off.

### 4) Handrub procedure with test product (P).

The above procedure was repeated exactly using the product Citrox Hand Gel in place of propan-2-ol.

### 5) Post-values.

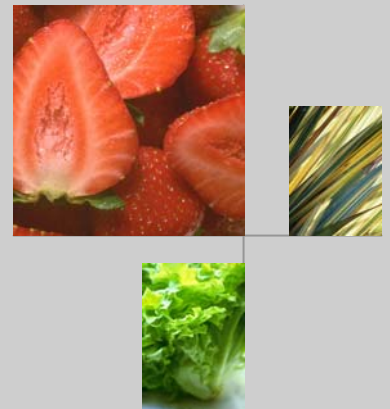
Immediately after rinsing the 12 subjects were asked to rub the fingertips on the base of a petri dish containing 10ml of MRD with neutraliser for 1 minute using a separate petri dish for each hand. Then 1ml of each of the undiluted sample fluids was placed in a petri dish and covered with 15ml of TSA mixed thoroughly and allowed to set. Plates were then incubated overnight at 37°C and examined for growth of the test organism.

### 6) Calculation.

The number of colony forming units (CFU) per plate for each dilution was recorded and the number of cfu's per ml of sample fluid calculated. For both reference and test procedure the log counts from right and left hands of each subject were averaged separately for pre-values and post-values.

From the difference between this individual combined log pre-value and the log post-value a log reduction factor is established for each subject. Then the two arithmetic means of all individual log reduction factors are calculated for both the reference and the test procedure. For **Citrox Hand Gel** to pass the criteria of EN 1500 the mean log reduction factor obtained must not be significantly smaller than that obtained for the alcohol rub. Test of significance of log reduction factors of P against R is carried out using the Wilcoxon matched pairs signed ranks test.

D C Watson



Independent  
Laboratory  
Verification  
by Abbott Analytical



# Microbiological Activity

## Independent Laboratory Verification

Certificate number: 05h.099h.KAE

Page 3 of 4

### Results:

Reference Handwash Procedure (R) Handwash with Citrox Hand Gel (P)

Subject	Log x	Log y	Log z	Log x	Log y	Log z
1	6.41	0	6.41	6.56	1.70	4.86
2	6.49	0	6.49	6.46	1.90	4.56
3	6.29	1.48	4.81	6.59	1.60	4.99
4	6.42	1.30	5.12	6.63	1.48	5.15
5	6.50	0	6.50	6.38	0	6.38
6	6.27	0	6.27	6.44	1.60	4.84
7	6.22	0	6.22	6.51	1.84	4.67
8	6.38	0	6.38	6.55	2.04	4.51
9	6.44	1.70	4.74	6.29	1.30	4.99
10	6.50	0	6.50	6.39	0	6.39
11	6.40	1.48	4.92	6.44	0	6.44
12	6.51	0	6.51	6.48	1.70	4.78
X	6.40	0.50	5.90	6.48	1.26	5.22
N	12	12	12	12	12	12

Where:

Log x = log pre-value.

Log y = log post-value

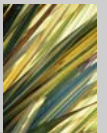
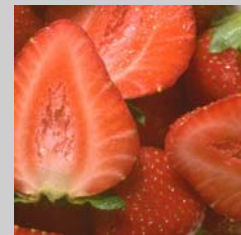
Log z = log reduction factor

X = overall mean value of log x, log y and log z.

N = number of subjects in each column.

The above data table shows that all criteria of the Test Validation (ref EN 1500:1997 clause 5.8) were satisfied.

The mean log reduction factor for the test material, Citrox Hand Gel, was 5.22 compared to 5.90 for the reference material, propan-2-ol. Therefore, the Wilcoxon matched-pairs signed-ranks test was used to assess whether the mean log reduction factor for Citrox Hand Gel is significantly smaller than that obtained with propan-2-ol.



Independent  
Laboratory  
Verification  
by Abbott Analytical



# Microbiological Activity

## Independent Laboratory Verification

Certificate number: 05h.099h.KAE

Page: 4 of 4

### Wilcoxon matched-pairs signed-ranks test:

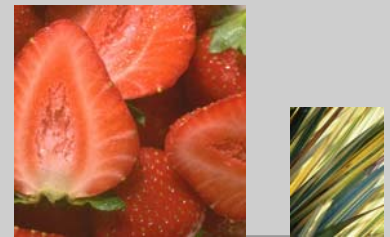
Log RF derived from    Difference    Rank of difference

Subject	R	P	R - P	Without sign	With sign
1	6.41	4.86	1.55	8.5	8.5
2	6.49	4.56	1.93	12	12
3	4.81	4.99	-0.18	4	- 4
4	5.12	5.15	-0.03	1	- 1
5	6.50	6.38	0.12	3	3
6	6.27	4.84	1.43	6	6
7	6.22	4.67	1.55	8.5	8.5
8	6.38	4.51	1.87	11	11
9	4.74	4.99	-0.25	5	- 5
10	6.50	6.39	0.11	2	2
11	4.92	6.44	-1.52	7	- 7
12	6.51	4.78	1.73	10	10
Sum of ranks (+)					61 (+)
Sum of ranks (-)					17 (-)

The results of the Wilcoxon matched-pairs signed-ranks test shows that there is no significant difference in the log reduction values at the required level of significance ( $p = 0.1$ ) as the smaller sum of ranks for the test material is 17 compared to minimum value of 12 (ref EN 1500:1997 Table C.5) for 12 subjects.

Therefore, the conclusion is that Citrox Hand Gel, when used as received, conforms to the requirements of EN 1500 and can be considered a suitable hygienic handrub when used under the procedures described above.

D C Watson



Independent  
Laboratory  
Verification  
by Abbott Analytical



# Microbiological Activity

## Independent Laboratory Verification

**Samples:** Five samples of Handwash received from Citrox Limited  
29<sup>th</sup> September 2003

**Certificate Number:** 03J.375.GWP

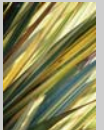
**Analysis Required:** Persistence on hands over working day using a contact  
technique by Ciba products.

**Samples Tested:** 7<sup>th</sup> October 2003

**Protocol:** Each product was tested by a different volunteer under carefully supervised conditions. Hands were washed twice with 3ml of product. Activity on the hands was assessed by pressing a finger tip for 30 seconds on agar plates seeded with the test bacteria which in this instance was *Staphylococcus aureus*. This was repeated at 2 hourly intervals through the working day. Volunteers were advised to go about their normal business during this time. The presence of the product on the fingers was indicated by a reduction in growth of the test organism on the agar plate. The results obtained were scored on a subjective basis with 5+ being total kill of the organism to 1+ being substantial growth of the test organism in the area tested. A count of 0 shows that there was no activity registered.

## Test Results

	Time 0	2 hours	4 hours	6 hours
Citrox	+++++	++++	++++	++
Sterisol	+++++	++++	++	0
Purell	+++++	+++	++	0
Pfizer Alcohol based Sanitising gel	+++++	++	+	0



Independent  
Laboratory  
Verification  
by Abbott Analytical



# Microbiological Activity

## Independent Laboratory Verification



**Samples:** One sample of Citrox Surface Cleaner received from Citrox Limited 11th April 2005  
**Certificate Number:** 05D.121.GWP  
**Analysis Required:** BS EN 1276 under dirty conditions  
**Samples Tested:** 13th April 2005

Product stored at 5°C in the dark.  
 Active substance: Not declared.  
 Batch Number: 70

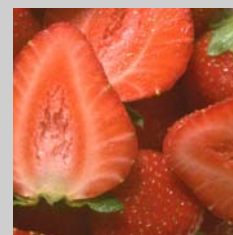
### Experimental conditions:

Product diluent used during test Sterile hard water 300mg/kg  
 CaCO<sub>3</sub>  
 Product test concentrations 2% v/v  
 Contact time 5 min  
 Test Temperature 20°C ± 0.5°C  
 Interfering substance 3.0g/l Bovine albumin  
 Neutralising solution 3% Tween 80, 3% Saponin,  
 0.1% Histidine, 0.1% Cysteine  
 Temperature of incubation 30°C ± 1°C

Identification of bacterial strains used Pseudomonas aeruginosa ATCC 15442  
 Escherichia coli NCTC 10418  
 Staphylococcus aureus NCTC 6571  
 Enterococcus hirae ATCC 8043

### Conclusion:

According to EN1276 this batch of Citrox Surface Cleaner when diluted at 1:50 (v/v) in hard water possesses satisfactory bactericidal activity in 5 minutes at 20°C under dirty conditions (3.0g/1 bovine albumin) for reference organisms indicated.



## Test Results

Validation test	Pseudomonas aeruginosa	Escherichia coli	Staphylococcus aureus	Enterococcus Hirae
Bacterial Suspension	Vc 184, 166 Nv 1.75 x 10 <sup>3</sup>	Vc 154, 138 Nv 1.46 x 10 <sup>3</sup>	Vc 136, 147 Nv 1.41 x 10 <sup>3</sup>	Vc 212, 185 Nv 1.98 x 10 <sup>3</sup>
Experimental Conditions	Vc 210, 180 A 1.95 x 10 <sup>2</sup>	Vc 166, 192 A 1.79 x 10 <sup>2</sup>	Vc 151, 142 A 1.46 x 10 <sup>2</sup>	Vc 210, 232 A 2.21 x 10 <sup>2</sup>
Neutraliser Control	Vc 188, 196 B 1.92 x 10 <sup>2</sup>	Vc 158, 142 B 1.50 x 10 <sup>2</sup>	Vc 160, 146 B 1.53 x 10 <sup>2</sup>	Vc 208, 182 B 1.95 x 10 <sup>2</sup>
Dilution neutralization Control	Vc 220, 186 C 2.03 x 10 <sup>2</sup>	Vc 182, 164 B 1.73 x 10 <sup>2</sup>	Vc 155, 136 C 1.45 x 10 <sup>2</sup>	Vc 230, 216 C 2.23 x 10 <sup>2</sup>
Bacterial Test Suspension	10 <sup>-6</sup> 856, 820 10 <sup>-7</sup> 87, 86 N 8.52 x 10 <sup>8</sup>	10 <sup>-6</sup> 344, 306 10 <sup>-7</sup> 34, 36 N 3.37 x 10 <sup>8</sup>	10 <sup>-6</sup> 416, 388 10 <sup>-7</sup> 44, 42 N 4.16 x 10 <sup>8</sup>	10 <sup>-6</sup> 472, 434 10 <sup>-7</sup> 48, 50 N 4.71 x 10 <sup>8</sup>
Test results				
1 : 50 Vc Na R	34 3400 2.50 x 10 <sup>5</sup>	0 <100 >3.37 x 10 <sup>6</sup>	3 300 1.38 x 10 <sup>6</sup>	14 1400 3.36 x 10 <sup>6</sup>

Vc = Viable Count  
 N = Number of cfu/ml of the bacterial test suspension  
 Nv = Number of cfu in bacterial suspension  
 R = Reduction in viability  
 Na = Number of cfu/ml in the test mixture

Independent  
 Laboratory  
 Verification  
 by Abbott Analytical



# Microbiological Activity

## Independent Laboratory Verification



**Samples:** One sample of Citrox Surface Spray received from Citrox Limited  
**Certificate Number:** 03A.102  
**Analysis Required:** BS EN 1276 under dirty conditions  
**Samples Tested:** 13<sup>th</sup> July 2003

Product stored at 5°C in the dark.  
 Active substance: Not declared.

### Experimental conditions:

Product diluent used during test: Sterile hard water 300mg/kg CaCO<sub>3</sub>  
 Product test concentrations: Neat  
 Contact time: 5 min  
 Test Temperature: 20°C ± 0.5°C  
 Interfering substance: 3.0g/l Bovine albumin  
 Neutralising solution: 3% Tween 80, 3% Saponin, 0.1% Histidine, 0.1% Cysteine  
 Temperature of incubation: 30°C ± 1°C

Identification of bacterial strains used: Pseudomonas aeruginosa ATCC 15442  
 Escherichia coli NCTC 10418  
 Staphylococcus aureus ACTA 6571  
 Enterococcus hirae ATCC 8043

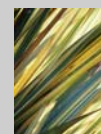
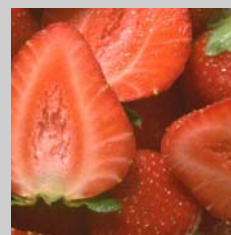
### Test Results

Validation test	Pseudomonas aeruginosa	Escherichia coli	Staphylococcus aureus	Enterococcus hirae
Bacterial suspension	Vc 184, 166 Nv 1.75 x 10 <sup>3</sup>	Vc 154, 138 Nv 1.46 x 10 <sup>3</sup>	Vc 136, 147 Nv 1.41 x 10 <sup>3</sup>	Vc 212, 185 Nv 1.98 x 10 <sup>3</sup>
Experimental Conditions	Vc 210, 180 A 1.95 x 10 <sup>2</sup>	Vc 166, 192 A 1.79 x 10 <sup>2</sup>	Vc 151, 142 A 1.46 x 10 <sup>2</sup>	Vc 210, 232 A 2.21 x 10 <sup>2</sup>
Neutraliser control	Vc 188, 196 B 1.92 x 10 <sup>2</sup>	Vc 158, 142 B 1.50 x 10 <sup>2</sup>	Vc 160, 146 B 1.53 x 10 <sup>2</sup>	Vc 208, 182 B 1.95 x 10 <sup>2</sup>
Dilution-neutralisation Control	Vc 220, 186 C 2.03 x 10 <sup>2</sup>	Vc 182, 164 C 1.73 x 10 <sup>2</sup>	Vc 155, 136 C 1.45 x 10 <sup>2</sup>	Vc 230, 216 C 2.23 x 10 <sup>2</sup>
Bacterial Test Suspension	10-6 83, 94 10-7 8, 8 N 8.45 x 10 <sup>8</sup>	10-7 78, 92 10-7 6, 8 N 7.75 x 10 <sup>8</sup>	10-7 52, 66 10-7 6, 6 N 5.95 x 10 <sup>8</sup>	10-7 34, 18 10-7 4, 5 N 3.55 x 10 <sup>8</sup>
Test result				
1 : 50 Vc	102	58	47	29
Na	10200	5800	4700	2900
R	1.25 x 10 <sup>5</sup>	1.34 x 10 <sup>5</sup>	1.26 x 10 <sup>5</sup>	1.22 x 10 <sup>5</sup>

Vc = Viable Count  
 N = Number of cfu/ml of the bacterial test suspension  
 Nv = Number of cfu in bacterial suspension  
 R = Reduction in viability  
 Na = Number of cfu/ml in the test mixture

### Conclusion:

According to EN1276 this batch of Citrox Surface Spray when tested neat possesses satisfactory bactericidal activity in 2 minutes at 20°C under dirty conditions (3.0g/l bovine albumin) for the organisms listed.



Independent  
 Laboratory  
 Verification  
 by Abbott Analytical





# Microbiological Activity

## Independent Laboratory Verification



**Samples:** One sample of Hand Foam received from Citrox Limited, Unit 1 River Court, Brighthouse Road, Riverside Park, Middlesbrough TS2 1RT 26<sup>th</sup> February 2007

**Certificate Number:** 07B.115.CIT

**Analysis Required:** BSEN 12054

**Samples Tested:** 28<sup>th</sup> February 2007

Product stored at room temperature in the dark.  
Active substance: Not declared.

### Experimental conditions:

Product test concentrations Neat as received  
Contact time 1 min  
Test Temperature 20°C ± 0.5°C  
Interfering substance 3.0g/l Bovine albumin  
Neutralising solution 3% Tween 80, 3% Saponin, 0.1% Histidine, 0.1% Cysteine  
Temperature of incubation 30°C ± 1°C

Identification of bacterial strains used Pseudomonas aeruginosa ATCC 15442  
Escherichia coli NCTC 10418  
MRSA NCIMB 50143  
Enterococcus hirae ATCC 8043

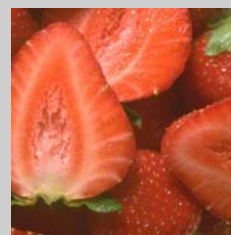
### Test Results

Validation test	Pseudomonas aeruginosa	Escherichia coli	MRSA NCIMB 50143	Enterococcus hirae
Bacterial suspension	Vc 536, 447 Nv 4.91 x 10 <sup>3</sup>	Vc 372, 338 Nv 3.55 x 10 <sup>3</sup>	Vc 392, 438 Nv 4.15 x 10 <sup>3</sup>	Vc 432, 455 Nv 4.43 x 10 <sup>3</sup>
Experimental conditions	Vc 416, 442 A 4.29 x 10 <sup>2</sup>	Vc 300, 354 A 3.27 x 10 <sup>2</sup>	Vc 372, 414 A 3.93 x 10 <sup>2</sup>	Vc 440, 394 A 4.17 x 10 <sup>2</sup>
Neutraliser control	Vc 488, 496 B 4.92 x 10 <sup>2</sup>	Vc 344, 282 B 3.13 x 10 <sup>2</sup>	Vc 358, 446 B 4.02 x 10 <sup>2</sup>	Vc 456, 424 B 4.40 x 10 <sup>2</sup>
Dilution-neutralisation control	Vc 520, 486 C 5.03 x 10 <sup>2</sup>	Vc 290, 336 C 3.13 x 10 <sup>2</sup>	Vc 375, 436 C 4.05 x 10 <sup>2</sup>	Vc 422, 356 C 3.89 x 10 <sup>2</sup>
Bacterial Test Suspension	10 <sup>-6</sup> 944, 638 10 <sup>-7</sup> 43, 59 N 6.50 x 10 <sup>8</sup>	10 <sup>-6</sup> 330, 312 10 <sup>-7</sup> 20, 28 N 2.80 x 10 <sup>8</sup>	10 <sup>-6</sup> 334, 416 10 <sup>-7</sup> 48, 52 N 4.37 x 10 <sup>8</sup>	10 <sup>-6</sup> 448, 512 10 <sup>-7</sup> 64, 47 N 5.17 x 10 <sup>8</sup>
Test results				
Neat	Vc 0 Na <100 R >6.50 x 10 <sup>6</sup>	0 <100 >2.80 x 10 <sup>6</sup>	0 <100 >4.37 x 10 <sup>8</sup>	0 <100 >5.17 x 10 <sup>6</sup>

Vc = Viable Count  
N = Number of cfu/ml of the bacterial test suspension  
Nv = Number of cfu in bacterial suspension  
R = Reduction in viability  
Na = Number of cfu/ml in the test mixture

### Conclusion:

According to EN12054 this batch of Hand Foam when used neat as received possesses satisfactory bactericidal activity in 1 minute at 20°C



Independent  
Laboratory  
Verification  
by Abbott Analytical



# MATERIAL SAFETY DATA SHEET

## 1. Product & Company Identification

**Chemical Name:** Citrox Antibacterial Hand Foam  
**Chemical Formula:**  
**Synonyms:** None  
**Manufacturer:** Citrox Limited  
Unit 9 River Court  
Brighthouse Road  
Riverside Park  
Middlesbrough  
TS2 1RT  
Tel: 01642 241777  
Fax: 01642 219217  
E-mail: [info@citrox-products.co.uk](mailto:info@citrox-products.co.uk)

## 2. Composition & Information on Ingredients

**Components:** Organic Acids                      Citrox Natural Biocide  
**Conc:** Above 10%                              Below 10%  
**EINECS No:**  
**CAS No:**  
**Hazard:**  
**Risk Phrases:**

## 3. Hazardous Identification

Not regarded as a health hazard under current legislation.

## 4. First Aid Measures

**Eyes:** Immediately flush eye with eye-wash solution or clean water for at least 10 minutes.  
**Skin:** N/A  
**Ingestion:** N/A  
**Inhalation:** Non-volatile liquid product.

## 5. Fire Fighting Measures

**Extinguishing Media:** Product is non-combustible  
**Special Hazards:** None

## 6. Accidental Release Measures

**Personal Precautions:** N/A  
**Environmental Precautions:** No particular/specific measures required.  
**Spillage Treatment:** Small Spillage - N/A  
Large Spillage - N/A

## 7. Handling and Storage

**Handling:** Avoid contact with eyes  
**Storage:** Store at ambient temperature  
**Packaging:** 100ml spray bottles

## 8. Exposure Control / Personal Protection

**General:** No specific measures are required provided the product is handled in accordance with the general rules of occupational hygiene and safety. Avoid contact with eyes.

**Personal Protection:** The following personal protective equipment should be provided.

**Skin Protection:** N/A

**Hand Protection:** N/A

**Eye Protection:** N/A

## 9. Physical and Chemical Properties

**Appearance:** Light yellow liquid

**Odour:** N/A

**Density:** N/A

**pH:** N/A

**Solubility:** N/A

## 10. Stability and Reactivity

**Stability:** Stable under normal storage conditions

**Conditions to Avoid:** Protect from frost

**Materials to Avoid:** None

**Decomposition Products:** N/A

## 11. Toxicological Information

**Acute Toxicity:** Non-hazardous substance.

**Eyes:** Mild irritation on contact. Unlikely to cause eye damage.

**Skin:** No known effects

**Ingestion:** No known adverse effects.

**Inhalation:** No known effects.

## 12. Ecological Information

**Mobility:** Product dissolves completely in water

**Degradability:** Product is above 98% biodegradable

**Accumulation:** None known

**Ecotoxicity:** No adverse effects

## 13. Disposal Considerations

Flush to drain with copious quantities of water. Disposal should be in accordance with waste disposal or water authority regulations.

#### 14. Transport Information

**UN No:** Not classified  
**Hazard Class:** Not classified  
**Packaging Group:** Not classified

Only transport in original containers in upright position. Never remove containers from cardboard containers before transporting. Unless containers are palletised never transport stacked more than 3 high.

#### 15. Regulatory Information

**Chemicals (hazard information and packaging for supply) Regulations 1996**  
**Classification: Non-hazardous.**

NEAT PRODUCT

Risk Phrase Safety Phrases	S26	In case of contact with eyes, rinse immediately with plenty of water and seek medical advice if irritation persists.
----------------------------------	-----	--

Use of this product may be governed by the following regulations: Chemical Hazard Information and Packaging Regulations 1996, Carriage of Dangerous Goods by Road and Rail (C.P.L) Regulations 1996.

Control of Substances Hazardous to Health Regulation 1996.

#### 16. Additional Information

This product is a non-toxic, Hand Sanitiser for use in the medical and associated industries.

This product is registered with the National Poison Information Service. Medical staff can obtain additional health and safety information from this service by telephoning 0171 635 9191. New Cross Hospital, Avonley Road, London SE14 5ER.

This information given is based on our knowledge of the product at the time of publication. The attention of the user is drawn to the possible risks incurred by using the product for any other purpose other than that for which it was intended.

# FINAL REPORT

## CONFIRMATORY VIRUCIDAL EFFECTIVENESS TEST Using Feline Calicivirus (Surrogate for Norwalk virus (Norovirus))

TEST AGENT:  
Decosan (also known as Citrox AV20)

Data Requirements  
EPA Guidelines 810.2100 (g)

Author  
Peggy R. Cherwoo

Study Completion Date  
May 15, 2006

Performing Laboratory  
MICROBIOTEST  
105 Carpenter Drive  
Sterling, Virginia 20164

Laboratory Project Identification Number  
557-106

Submitted to: BIOLOGICAL SOLUTIONS LLC  
900 East Liberty Street  
Sumter, SC 29153  
(Citrox Limited exclusive agent in USA)

## TEST SUMMARY

- TITLE:** Confirmatory Virucidal Effectiveness Test Using Feline calicivirus (Surrogate for Norwalk virus)
- STUDY DESIGN:** This study was performed according to the signed protocol and project sheets issued by the Study Director.
- See Project Sheets (Appendix I)  
See signed protocol (Appendix 2)
- TEST MATERIALS:** Decosan, Lot No. 33106D, received at MICROBIOTEST, 04/04/06 and assigned DS No. 8194.
- SPONSOR:** BIOLOGICAL SOLUTIONS, LLC  
900 East Liberty Street  
Sumter, SC 29153

## TEST CODITIONS

Challenge Virus:

Deline calicivirus, University of Ottawa (CREM)

Host:

CrFK cells, American BioResearch Laboratories

Active ingredient in test product:

Citric acid / Bioflavonoid complex

Neutraliser used:

Fluid thioglycollate medium +20% Newborn calf serum  
+1% Polysorbate 80

Dilution:

Ready to use

Spray distance:

6 inches

Contact time:

1 minute

Contact temperature:

22°C

Organic load:

Viral stock contained at least 5% organic load

Media and reagents:

Newborn calf serum (NCS)

RPMI 1640 containing 10% NCS

Fluid thioglycollate medium

Polysorbate 80

## **STUDY DATES AND FACILITIES**

The laboratory phase of this test was performed at MICROBIOTEST, 105 Carpenter Drive, Sterling, VA 20164, from 04/26/06 to 05/02/06. The study director signed the protocol 04/25/06. The study completion date is the date the study director signed the final report.

All changes or revisions of the protocol we documented, signed by the study director, dated and maintained with the protocol.

## **RECORDS TO BE MAINTAINED**

All testing data, protocol, protocol modification, test material records, the final report, and correspondence between MICROBIOTEST and the sponsor will be stored in the archives at MICROBIOTEST, 105, Carpenter Drive, Sterling, VA 20164, or at a controlled facility off site.

## **RESULTS**

Results are presented in Tables 1 – 4. A titration was performed to determine the titer of the viral stock. The  $\log_{10}$  reduction (LR) of infectious virus observed as cytopathic effects of Feline calicivirus was determined using the Most Probable Number (MPN) method as described in EPA-Statistic Primer (EPA-SP). The cell viability control demonstrated CrFK cell viability and media sterility. Virus was not recovered in the cell viability control. The  $\log_{10}$  reduction was calculated in the following manner:

$\text{Log}_{10}$  reduction = Infectious virus titer from plate recovery control – Infectious virus titer recovered from test.

## RESULTS (Continued)

Table 1  
Test Results

Dilution	Decosan	
	Lot No. 33106D	
	Replicate 1	Replicate 2
$10^{-2}$	CCCC	CCCC
$10^{-3}$	CCCC	CCCC
$10^{-4}$	0000	0000
$10^{-5}$	0000	0000
$10^{-6}$	0000	0000
$10^{-7}$	0000	0000
Log <sub>10</sub> MPN/mL	3.37985	3.37985
Mean (Log <sub>10</sub> MPN/mL)	3.37985	

Table 2  
Neutraliser Effectiveness and Cytotoxicity Related Controls  
Lot No. 33106D

Dilution	Neutraliser Effectiveness Control	Cytotoxicity Control	Cytotoxicity-related Viral Interference Control
$10^{-2}$	CCCC	CCCC	CCCC
$10^{-3}$	CCCC	CCCC	CCCC
$10^{-4}$	++++	0000	++++

Key: + = Feline calicivirus infected cells were detected, cytopathic effects observed  
 0 = Feline calicivirus infected cells were not detected, no cytopathic effects observed,  
 no cytotoxicity observed.  
 C = Cytotoxicity observed

## RESULTS (Continued)

Table 3  
Control Results

Dilution	Feline calicivirus Plate Recovery Control	
	Replicate 1	Replicate 2
$10^{-2}$	++++	++++
$10^{-3}$	++++	++++
$10^{-4}$	++++	++++
$10^{-5}$	++++	++++
$10^{-6}$	++++	++++
$10^{-7}$	++++	++++
$\text{Log}_{10}\text{MPN/mL}$	$\geq 7.37983$	$\geq 7.37983$
Mean ( $\text{log}_{10}\text{MPN/mL}$ )	$\geq 7.37983$	

Key: + = Feline calicivirus infected cells were detected, cytopathic effects observed.  
0 = Feline calicivirus infected cells were not detected, no cytopathic effects observed,  
no cytotoxicity observed.  
C = Cytotoxicity.

Table 4  
 $\text{Log}_{10}$  Reduction

Decosan
Lot No. 33106D
3.99998

## CONCLUSION

According to the regulatory agencies, the test agent passes the test if there is complete inactivation of the virus at all dilutions. When cytotoxicity is evident, at least a three-log reduction in titer must be demonstrated beyond the cytotoxic level. When tested as described, Decosan (also known as Citrox AV20) passed the Virucidal Effectiveness Test when Feline calicivirus was exposed to the test agent for 1 minute at 22°C. All of the controls met the criteria for a valid test. These conclusions are based on observed data.