TEST REPORT SABS

U verw/Your ref: O/No. Ref QF117HealthHyg2006

Ons verw/Our ref: 06-529

Navrae/Enquiries: 428-6269

Datum/Date: 8/12/2006

2423/529/A101797 C

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Health and Hygiene (Pty) Ltd
Attention: Mr. J Temperley
PO Box 347
SUNNINGHILL
2157
ZA

AOAC HARD SURFACE CARRIER TEST ON F10SC VETERINARY DISINFECTANT.

NON POROUS CARRIERS COATED WITH *Pseudomonas aeruginosa* ATCC 15442.

1. DESCRIPTION OF SAMPLES.

The following three batches of F10SC were supplied by the client:

F10SC batch 071107 manufacturing date 11/2005, expiry date 11/2007.
F10SC batch 080310 manufacturing date 3/2006, expiry date 3/2008.
F10SC batch 080511 manufacturing date 5/2006, expiry date 5/2008.

2. TEST REQUESTED.

To determine the efficacy of the product against a standard bacterial strain of *Pseudomonas aeruginosa* at a dilution of 1/250 using the AOAC hard surface carrier test method.

3. METHOD OF TEST.

2000 AOAC International, Official Method 991.49. Testing disinfectants against *Pseudomonas aeruginosa*. Hard surface carrier test method, First action 1991.

Brief summery: The AOAC Use-Dilution test was done with flame polished glass cylinders coated with an exact amount of micro organism. These were dried and exposed to the use-dilution of 1/250 of the disinfectant as indicated by the client for a period of 10 minutes. After neutralization these carriers were cultured to assess the survival of the bacteria.

A single test involved the evaluation of 60 inoculated carriers (one organism) against one batch of the product. Six additional inoculated carriers were used for the determination of the carrier bacterial load. Another set of six additional carriers were prepared at the same time as extras. A total of 72 carriers were required for a single test.

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4. MICRO ORGANISM USED AND PREPARATION OF THE CULTURE:

The test organism used was *Pseudomonas aeruginosa* ATCC No. 15442 supplied by MicroBiologics.

- **Initial preparation of the culture**

The mother culture was opened and prepared according to the AOAC method.
Gram stain reaction after ± 18 hours incubation at 37°C on trypticase soy agar (TSA):
Small gram-negative rods.
Colony morphology on trypticase soy agar: slimy creamy colonies were observed.
Cetrimide agar base: colonies were creamy-green and a yellow-green pigment was present in the agar. Fluorescence was observed under UV light.

- **Additional selective media and biochemical tests used to confirm the genus and species:**

API 20NE from Biomerieux: code obtained 1-3-5-4-5-7-5, 99,9 % certainty, excellent ID as *Pseudomonas aeruginosa.*

- **Preparation of the culture used for the coating of the carriers:**

At least 3 consecutively daily transfers were made in nutrient broth and the last 24 hour old broth culture was used to prepare a lawn on TSA plates. These were incubated for 24 ± 4 hours at 37°C. The growth on each plate was gently removed with a sterile swab and 10 ml sterile nutrient broth. The suspensions from individual plates were pooled in a sterile flask and vortexed for approximately 5 minutes to break up clumps of bacterial cells.

The suspension was left to stand for approximately 10 minutes at room temperature after which an aliquot was diluted with sterile nutrient broth to the required density and bacterial count according to the previously established standard curve and coating test trial records. The suspension so diluted was used within 30 minutes of preparation to coat the required amount of carriers for exactly 15 minutes according to the method.

The coated carriers were removed from the suspension after 15 minutes and dried on sterile filter paper for 40 minutes at 37°C (± 2°C). Immediately thereafter 6 carriers were used for the determination of the carrier load, using the nutrient broth and the required neutralizer for the initial recovery. Counts were done in duplicate on TSA using sterile distilled water for the serial dilutions.

The sponsor requested no additional organic soiling of the carriers.

5. CARRIERS:

- **Description of carriers:**

Disposable fire-polished borosilicate glass carriers were supplied by the sponsor. Size of carriers: length 10mm (± 1 mm); inside diameter 6mm (± 1 mm) and outside diameter 8 mm (± 1 mm). Only cylinders without visible cracks and flaws were used for the tests. The cylinders were washed with ethanol and distilled water according

to the method and autoclaved. They were cooled and stored at room temperature. Carriers were used once only and discarded after use.

- **Exposure period and transfer of carriers:**

Each carrier was exposed to the use-dilution of the product for exactly 10 minutes.
Three sets of 20 tubes were tested individually. Each set was done with 30 second intervals between transfers. The tubes were not swirled after adding the carriers. Transfer of carriers to disinfectant containing tubes was within ± 5 seconds of the actual time of transfer indicated.

- **Primary subculture and neutralizer:**

The same sequence of transfer was followed for subculturing. Three sets of 20 tubes were tested individually. Each set was done with 30 second intervals between transfer from disinfectant to primary subculture medium. Transfer of carriers to primary subculture tubes was also within ± 5 seconds of the actual time of transfer indicated.

- **Sequence of transfer:**

Each set of 20 tubes was treated as follows:
A calibrated electronic clock-timer was used in 'count up" mode.

Tube/carrier reference	Carrier: Drop time	Carrier: transfer time	Tube/carrier reference	Carrier: Drop time	Carrier: transfer time
1	0 sec	10 min	**11**	5 min	15 min
2	30 sec	10 min 30 sec	**12**	5 min 30 sec	15 min 30 sec
3	1 min	11 min	**13**	6 min	16 min
4	1 min 30 sec	11 min 30 sec	**14**	6 min 30 sec	16 min 30 sec
5	2 min	12 min	**15**	7 min	17 min
6	2 min 30 sec	12 min 30 sec	**16**	7 min 30 sec	17 min 30 sec
7	3 min	13 min	**17**	8 min	18 min
8	3 min 30 sec	13 min 30 sec	**18**	8 min 30 sec	18 min 30 sec
9	4 min	14 min	**19**	9 min	19 min
10	4 min 30 sec	14 min 30 sec	**20**	9 min 30 sec	19 min 30 sec

6. PREPARATION OF THE PRODUCT:

F10SC veterinary disinfectant contains quaternary ammonium and biguanidine compounds (5,8%), non-toxic ampholytic surfactants and sequesterants.
Storage conditions indicated by the manufacturer: below 30°C in dry conditions.

- **Use-dilution of product indicated and preparation thereof:**

The recommended dilution of 1/250 was used for the test. A total of 4 ml of the super concentrate was diluted with sterile distilled water at room temperature to a total volume of 1 L using "A" grade volumetric glassware. This preparation was dispensed in 10 ml portions into 60 (plus a few extra) tubes and placed into a water bath at 20°C (± 0,5°C) approximately 30 minutes before the test started. Testing started once the contents of the tubes had reached water bath temperature. The disinfectant at use-dilution was tested within less than 3 hours of preparation.

- **Neutralizer used and preparation thereof:**

Letheen broth according to the AOAC method was not used as the primary subculture medium. The most suitable neutralizer for F10SC is the following and had been established during previous disinfectant trials:

TSB + 3% Asolectin + 20 % Tween 80 neutralizer.

Preparation:
To each 100 ml of Tryptone soy broth prepared according to the manufacturer's instructions, add 3 gram of Asolectin and 20 gram of Tween 80. Boil with constant stirring until all asolectin granules have dissolved. Allow the mixture to cool before adjusting the pH to between 7,0 and 7,4. Dispense in the volumes required and autoclave for 20 minutes at 121°C (± 2°C).

Nutrient broth was used as the primary subculture medium with 0,1 ml of the above neutralizer added to each tube containing 10 ml of broth.

The following ingredients were used for the preparation of the neutralizer:

Biolab Tryptone soy broth (TSB), supplier Merck, batch 1027483.
ACE Tween 80 CP (polyoxyethylene sorbitan mono-oleate) supplier Associated Chemical Enterprises, Batch 20430/6851.
Fluka Asolectin from soybean (soy lecithin) supplier Sigma Aldrich, Batch 1096966 21005050.

- **Confirmation of adequate neutralization of the disinfectant residues carried over into the subculture broth:**

Four sets of four negative tubes each were randomly chosen from the sixty tubes of Batch 080511 tested.

A 24 hour culture grown on TSA plates was diluted in nutrient broth to a transmittance of 84 % T at 580 nm and the organism load was estimated at approximately 1×10^8 cfu/ml. This suspension was serially diluted and the respective dilutions ranging from 10^{-5} to 10^{-8} were used to inoculate each negative tube selected in a specific set, as well as for the determination of the organism count of each dilution on TSA pour plates. (The tubes were to be inoculated with the bacterial suspension to contain between 5 – 100 cells per tube.)

Tubes and plates were incubated at 37°C (± 2°C) for 48 hours. Colonies on the plates were counted to confirm the organism load.

Tubes were examined for turbidity. Growth in the tubes indicated effective neutralization of the disinfectant.

The organisms in the tubes were transferred to the selective growth media indicated and also Gram stained after 24 hours growth on TSA to confirm the identity of the cultures.

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First set:

Tube selected:	Initial result: (in nutrient broth with inactivator)	Spiked with the 24 hour culture of the test organism (10^{-5} dilution was added to each tube.) Result after 48 hours:
1	Negative	Positive
13	Negative	Positive
25	Negative	Positive
37	Negative	Positive

Plate count results (TSA) done in triplicate: too numerous to count (equal to 1 ml).
Result not used, counts not within limits. Too high.

Second set:

Tube selected:	Initial result: (in nutrient broth with inactivator)	Spiked with the 24 hour culture of the test organism (10^{-6} dilution was added to each tube.) Result after 48 hours:
4	Negative	Positive
9	Negative	Positive
12	Negative	Positive
18	Negative	Positive

Plate count results (TSA) done in triplicate: 71, 85 and 92 cfu/plate (equal to 1 ml).
(Average of 83 within the stated limits of 5-100 cells per tube)

Third set:

Tube selected:	Initial result: (in nutrient broth with inactivator)	Spiked with the 24 hour culture of the test organism (10^{-7} dilution was added to each tube.) Result after 48 hours:
15	Negative	Positive
17	Negative	Positive
21	Negative	Positive
23	Negative	Positive

Plate count results (TSA) done in triplicate: 8, 9 and 6 cfu/plate (equal to 1 ml).
(Average of 7,7 within the stated limits of 5-100 cells per tube)

Fourth set:

Tube selected:	Initial result: (in nutrient broth with inactivator)	Spiked with the 24 hour culture of the test organism (10^{-8} dilution was added to each tube.) Result after 48 hours:
27	Negative	Positive
29	Negative	Positive
32	Negative	Negative
34	Negative	Positive

Plate count results (TSA) done in triplicate: 1, 0 and 0 cfu/plate (equal to 1 ml).
Result not used, counts not within limits. Too low.

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Confirmation of the results:

All the positive tubes were cultured on trypticase soy agar for ± 24 hours at 37°C for Gram staining.
All were small Gram negative rods resembling the mother culture.
Colony morphology on trypticase soy agar: slimy creamy colonies were observed.

All the positive tubes were also cultured on cetrimide agar base to confirm the presence *Pseudomonas aeruginosa.*
Colonies were creamy-green and a yellow-green pigment was present in the agar.
Fluorescence was observed under UV light.

The results confirmed effective germicide neutralization.

7. MEDIA AND API 20 NE SYSTEM USED:

Description of media	Supplier	Batch No:
Nutrient Broth	Biolab	1027183
Cetrimide agar base	Biolab	1022440
Trypticase soy agar (TSA)	Biolab	1026753
API 20NE	Biomerieux	804699201

Quality control of media:

The media was prepared and/or autoclaved according to the manufacturers' instructions.

Negative controls of all media used were incubated for approximately 24 hours at 37°C (± 2°C) to verify sterility.

Positive controls were done using the mother culture as the reference to verify growth promotion as well as colony characteristics, pigment production and fluorescence, Gram stain reactions and biochemical reactions on the different selective media.

8. INCUBATION PERIOD:

Tubes were incubated for 48 – 54 hours at 37°C (± 2°C).

9. INTERPRETATION OF RESULTS:

Each tube was shaken prior to recording of the results to determine the absence or presence of turbidity.

A positive culture was one in which the primary subculture broth appeared turbid. Positive/turbid tubes were subcultured on TSA and cetrimide agar base. Confirmation was done using Gram stain reactions, cell morphology and reactions on cetrimide agar base (yellow-green to blue-green pigment production and fluorescence under UV light). Additional confirmation was done with the API 20NE identification system.

A negative result was one in which the primary subculture broth appeared clear. A specific number of negative tubes (10% of the total) were also used for spiking to prove the efficacy of the neutralizer used.

Performance standard: Not more than 2 positive carriers out of every sixty tested.

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10. RESULTS:

10.1 Efficacy of F10SC veterinary disinfectant **Batch 071107** against *Pseudomonas aeruginosa* ATCC 15442 on non porous hard surface carriers.

Description of sample:	**F10SC Veterinary disinfectant**
Batch tested and expiry date:	**# 071107**
Recommended dilution:	1/250
Recommended exposure time:	10 minutes
Media used for primary subculture:	10 ml Nutrient broth + inactivator
Inactivator used:	Tryptone Soy Broth containing 3% asolectin and 20 % Tween 80
Volume of inactivator added to 10 ml media	0,1 ml
Preparation of carriers: a) % T of suspension at 580 nm b) drying time	 a) 96,9 % T b) 40 minutes
Temperature at which test was conducted:	Start: 19,5°C End: 20°C
Incubation temperature:	37°C (± 2°C) for 48-54 hours
Date tested:	Start: 1/12/2006 End: 3/12/2006
Final result: Positive tubes confirmed:	 **2/60**

Determination of carrier load: Total counts done on 6 randomly chosen carriers: (labelled A to F)

Carrier reference:	Individual results on duplicate plates:		Average result for *Pseudomonas aeruginosa:* (limits 1 - 5 x 10^6 organisms per carrier)
	dilutions 10^{-4}	dilutions 10^{-5}	
A	306 x 10^4 316 x 10^4	27 x 10^5 24 x 10^5	2,83 x 10^6
B	252 x 10^4 266 x 10^4	31 x 10^5 28 x 10^5	2,77 x 10^6
C	250 x 10^4 226 x 10^4	40 x 10^5 31 x 10^5	2,97 x 10^6
D	206 x 10^4 232 x 10^4	32 x 10^5 23 x 10^5	2,47 x 10^6
E	188 x 10^4 184 x 10^4	18 x 10^5 25 x 10^5	2,01 x 10^6
F	324 x 10^4 272 x 10^4	36 x 10^5 36 x 10^5	3,29 x 10^6

Average count per carrier: **2,72 x 10^6 cfu/carrier.**

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AOAC Use-dilution test result sheet for: F10SC # 071107

Tubes with positive growth (turbidity) highlighted .

1	2	3	4	5	6	7	8	9	10
11	12	13	14	15	16	17	18	19	20
21	22	23	24	25	26	27	28	29	30
31	32	33	34	35	36	37	38	39	40
41	42	43	44	45	46	47	48	49	50
51	52	53	54	55	56	57	58	59	60

Positive tube confirmation: F10SC # 071107

Tube number	Gram stain after 24 Hours (TSA)	Additional identification done on culture: (incubation period approximately 24 hours at 37°C)
3	Small Gram negative rods	**Cetrimide agar base:** Colonies were creamy-green with yellow-green pigment present in the agar. Fluorescence was observed under UV light. **API 20 NE:** code 1-3-5-4-5-7-5 (excellent ID, 99,9% certainty. Identified as *Pseudomonas aeruginosa* ID done 5/12/2006)
45	Small Gram negative rods	**Cetrimide agar base:** Colonies were creamy-green with yellow-green pigment present in the agar. Fluorescence was observed under UV light. **API 20 NE:** code 1-3-5-4-5-7-5 (excellent ID, 99,9% certainty. Identified as *Pseudomonas aeruginosa* ID done 5/12/2006)

CONCLUSION: For F10SC # 071107: 2 out of a total of 60 tested were confirmed as *Pseudomonas aeruginosa*..

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10.2 Efficacy of F10SC veterinary disinfectant **Batch 080310** against *Pseudomonas aeruginosa* ATCC 15442 on non porous hard surface carriers.

Description of sample:	**F10SC Veterinary disinfectant**
Batch tested and expiry date:	**# 080310**
Recommended dilution:	1/250
Recommended exposure time:	10 minutes
Media used for primary subculture:	10 ml Nutrient broth + inactivator
Inactivator used:	Tryptone Soy Broth containing 3% asolectin and 20 % Tween 80
Volume of inactivator added to 10 ml media	0,1 ml
Preparation of carriers: a) % T of suspension at 580 nm b) drying time	 a) 95,2 % T b) 40 minutes
Temperature at which test was conducted:	Start: 20,5 °C End: 20,5 °C
Incubation temperature:	37°C (± 2°C) for 48-54 hours
Date tested:	Start: 24/11/2006 End: 26/11/2006
Final result: Positive tubes confirmed:	 **2/60**

Determination of carrier load: Total counts done on 6 randomly chosen carriers: (labelled A to F)

Carrier reference:	Individual results on duplicate plates: dilutions 10^{-4}	dilutions 10^{-5}	Average result for *Pseudomonas aeruginosa:* (limits 1 - 5 x 10^6 organisms per carrier)
A	221 x 10^4 219 x 10^4	22 x 10^5 24 x 10^5	2,25 x 10^6
B	181 x 10^4 218 x 10^4	17 x 10^5 21 x 10^5	1,95 x 10^6
C	189 x 10^4 196 x 10^4	15 x 10^5 22 x 10^5	1,89 x 10^6
D	161 x 10^4 164 x 10^4	11 x 10^5 18 x 10^5	1,54 x 10^6
E	171 x 10^4 320 x 10^4	42 x 10^5 33 x 10^5	3,10 x 10^6
F	282 x 10^4 280 x 10^4	30 x 10^5 24 x 10^5	2,76 x 10^6

Average count per carrier: **2,25 x 10^6 cfu/carrier.**

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AOAC Use-dilution test result sheet for: F10SC # 080310

Tubes with positive growth (turbidity) highlighted .

1	2	3	4	5	6	7	8	9	10
11	12	13	14	15	16	17	18	19	20
21	22	23	24	25	26	27	28	29	30
31	32	33	34	35	36	37	38	39	40
41	42	43	44	45	46	47	48	49	50
51	52	53	54	55	56	57	58	59	60

Positive tube confirmation: F10SC # 080310

Tube number	Gram stain after 24 Hours (TSA)	Additional identification done on culture: (incubation period approximately 24 hours at 37°C)
13	Small Gram negative rods	**Cetrimide agar base:** Colonies were creamy-green with yellow-green pigment present in the agar. Fluorescence was observed under UV light. **API 20 NE:** code 1-3-5-4-5-7-5 (excellent ID, 99,9% certainty. Identified as *Pseudomonas aeruginosa* ID done 29/11/2006)
34	Small Gram negative rods	**Cetrimide agar base:** Colonies were creamy-green with yellow-green pigment present in the agar. Fluorescence was observed under UV light. **API 20 NE:** code 1-3-5-4-5-7-5 (excellent ID, 99,9% certainty. Identified as *Pseudomonas aeruginosa* ID done 29/11/2006)

CONCLUSION: For F10SC # 080310 : 2 out of a total of 60 tested were confirmed as *Pseudomonas aeruginosa*..

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10.3 Efficacy of F10SC veterinary disinfectant **Batch 080511** against *Pseudomonas aeruginosa* ATCC 15442 on non porous hard surface carriers.

Description of sample:	**F10SC Veterinary disinfectant**
Batch tested and expiry date:	**# 080511**
Recommended dilution:	1/250
Recommended exposure time:	10 minutes
Media used for primary subculture:	10 ml Nutrient broth + inactivator
Inactivator used:	Tryptone Soy Broth containing 3% asolectin and 20 % Tween 80
Volume of inactivator added to 10 ml media	0,1 ml
Preparation of carriers: a) % T of suspension at 580 nm b) drying time	 a) 92,9 % T b) 40 minutes
Temperature at which test was conducted:	Start: 20 °C End: 20 °C
Incubation temperature:	37°C (± 2°C) for 48-54 hours
Date tested:	Start: 21/11/2006 End: 23/11/2006
Final result: Positive tubes confirmed:	 **2/60**

Determination of carrier load: Total counts done on 6 randomly chosen carriers: (labelled A to F)

Carrier reference:	Individual results on duplicate plates:		Average result for *Pseudomonas aeruginosa:* (limits 1 - 5 x 10^6 organisms per carrier)
	dilutions 10^{-4}	dilutions 10^{-5}	
A	390 x 10^4 324 x 10^4	10 x 10^5 13 x 10^5	2,36 x 10^6
B	196 x 10^4 344 x 10^4	20 x 10^5 18 x 10^5	2,30 x 10^6
C	248 x 10^4 260 x 10^4	13 x 10^5 17 x 10^5	2,02 x 10^6
D	372 x 10^4 376 x 10^4	24 x 10^5 23 x 10^5	3,05 x 10^6
E	154 x 10^4 218 x 10^4	14 x 10^5 13 x 10^5	1,61 x 10^6
F	222 x 10^4 178 x 10^4	15 x 10^5 14 x 10^5	1,73 x 10^6

Average count per carrier: **2,18 x 10^6 cfu/carrier.**

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AOAC Use-dilution test result sheet for: F10SC # 080511

Tubes with positive growth (turbidity) highlighted .

1	2	3	4	5	6	7	8	9	10
11	12	13	14	15	16	17	18	19	20
21	22	23	24	25	26	27	28	29	30
31	32	33	34	35	36	37	38	39	40
41	42	43	44	45	46	47	48	49	50
51	52	53	54	55	56	57	58	59	60

Positive tube confirmation: F10SC # 080511

Tube number	Gram stain after 24 Hours (TSA)	Additional identification done on culture: (incubation period approximately 24 hours at 37°C)
7	Small Gram negative rods	**Cetrimide agar base:** Colonies were creamy-green with yellow-green pigment present in the agar. Fluorescence was observed under UV light. **API 20 NE:** code 1-3-5-4-5-7-5 (excellent ID, 99,9% certainty. Identified as *Pseudomonas aeruginosa* ID done 29/11/2006)
55	Small Gram negative rods	**Cetrimide agar base:** Colonies were creamy-green with yellow-green pigment present in the agar. Fluorescence was observed under UV light. **API 20 NE:** code 1-3-5-4-5-7-5 (excellent ID, 99,9% certainty. Identified as *Pseudomonas aeruginosa* ID done 29/11/2006)

CONCLUSION: For F10SC # 080511: 2 out of a total of 60 tested were confirmed as *Pseudomonas aeruginosa*..

11. DISCUSSION:

F10SC veterinary disinfectant when tested in accordance with 2000 AOAC International, Official Method 991.49 was effective against *Pseudomonas aeruginosa* ATCC 15442 in a contact time of 10 minutes.

E. LLOYD
MICROBIOLOGY DEPARTMENT

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MANAGER MICROBIOLOGY

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TEST REPORT SABS

PHARMACEUTICAL MICROBIOLOGY 2423

U verw/Your ref: O/No. Ref QF117HealthHyg2006

Ons verw/Our ref: 06-529

Navrae/Enquiries: 428-6269

Datum/Date: 8/8/2006

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Health and Hygiene (Pty) Ltd
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AOAC HARD SURFACE CARRIER TEST ON F10SC VETERINARY DISINFECTANT.

NON POROUS CARRIERS COATED WITH *Salmonella choleraesuis* ATCC 10708.

1. DESCRIPTION OF SAMPLES.

The following three batches of F10SC were supplied by the client:

F10SC batch 071107 manufacturing date 11/2005, expiry date 11/2007.
F10SC batch 080310 manufacturing date 3/2006, expiry date 3/2008.
F10SC batch 080511 manufacturing date 5/2006, expiry date 5/2008.

2. TEST REQUESTED.

To determine the efficacy of the product against a standard bacterial strain of *Salmonella choleraesuis* at a dilution of 1/250 using the AOAC hard surface carrier test method.

3. METHOD OF TEST.

2000 AOAC International, Official Method 991.47. Testing disinfectants against *Salmonella choleraesuis*. Hard surface carrier test method, First action 1991.

Brief summery: The AOAC Use-Dilution test was done with flame polished glass cylinders coated with an exact amount of micro organism. These were dried and exposed to the use-dilution of 1/250 of the disinfectant as indicated by the client for a period of 10 minutes. After neutralization these carriers were cultured to assess the survival of the bacteria.

A single test involved the evaluation of 60 inoculated carriers (one organism) against one batch of the product. Six additional inoculated carriers were used for the determination of the carrier bacterial load. Another set of six additional carriers were prepared at the same time as extras. A total of 72 carriers were required for a single test.

4. MICRO ORGANISM USED AND PREPARATION OF THE CULTURE:

The test organism used was *Salmonella choleraesuis* ATCC No. 10708 supplied by MicroBiologics.

- **Initial preparation of the culture**

The mother culture was opened and prepared according to the AOAC method.
Gram stain reaction after ± 18 hours incubation at 37°C on trypticase soy agar (TSA):
Small gram-negative rods.
Colony morphology on trypticase soy agar: flat, round and opaque colonies were observed.

- **Additional selective media and biochemical tests used to confirm the genus and species:**

Brilliant Green modified agar: The colour of the media changed from orange-pink to red, colonies were pink to translucent.
XLD (xylose lysine deoxycholate) agar: media did not change colour but stayed red, colonies were reddish pinkish, transparent. No black cores.
MacConkey's agar: The colour of the media changed from light pink to a clear light peach colour. Colonies resembled the colour of the media.
Api 20E from Biomerieux: code obtained 4-3-0-4-5-5-0. Good identification as *Salmonella choleraesuis* 66,2 % certainty.

- **Preparation of the culture used for the coating of the carriers:**

At least 3 consecutively daily transfers were made in synthetic broth and the last 24 hour old broth culture was used to prepare a lawn on TSA plates. These were incubated for 24 ± 4 hours at 37°C. The growth on each plate was gently removed with a sterile swab and 10 ml sterile synthetic broth. The suspensions from individual plates were pooled in a sterile flask and vortexed to break up clumps of bacterial cells.

The suspension was left to stand for approximately 10 minutes at room temperature after which an aliquot was diluted with sterile synthetic broth to the required density and bacterial count according to the previously established standard curve and coating test trial records. The suspension so diluted was used within 30 minutes of preparation to coat the required amount of carriers for exactly 15 minutes according to the method.

The coated carriers were removed from the suspension after 15 minutes and dried on sterile filter paper for 40 minutes at 37°C (± 2°C). Immediately thereafter 6 carriers were used for the determination of the carrier load, using the synthetic broth and the required neutralizer for the initial recovery. Counts were done in duplicate on TSA using sterile distilled water for the serial dilutions.

The sponsor requested no additional organic soiling of the carriers.

5. CARRIERS:

- **Description of carriers:**

Disposable fire-polished borosilicate glass carriers were supplied by the sponsor. Size of carriers: length 10mm (± 1 mm); inside diameter 6mm (± 1 mm) and outside diameter 8 mm (± 1 mm). Only cylinders without visible

cracks and flaws were used for the tests. The cylinders were washed with ethanol and distilled water according to the method and autoclaved. They were cooled and stored at room temperature. Carriers were used once only and discarded after use.

- **Exposure period and transfer of carriers:**

Each carrier was exposed to the use-dilution of the product for exactly 10 minutes.
Three sets of 20 tubes were tested individually. Each set was done with 30 second intervals between transfers. The tubes were not swirled after adding the carriers. Transfer of carriers to disinfectant containing tubes was within ± 5 seconds of the actual time of transfer indicated.

- **Primary subculture and neutralizer:**

The same sequence of transfer was followed for subculturing. Three sets of 20 tubes were tested individually. Each set was done with 30 second intervals between transfer from disinfectant to primary subculture medium. Transfer of carriers to primary subculture tubes was also within ± 5 seconds of the actual time of transfer indicated.

- **Sequence of transfer:**

Each set of 20 tubes was treated as follows:
A calibrated electronic clock-timer was used in 'count up" mode.

Tube/carrier reference	Carrier: Drop time	Carrier: transfer time	Tube/carrier reference	Carrier: Drop time	Carrier: transfer time
1	0 sec	10 min	**11**	5 min	15 min
2	30 sec	10 min 30 sec	**12**	5 min 30 sec	15 min 30 sec
3	1 min	11 min	**13**	6 min	16 min
4	1 min 30 sec	11 min 30 sec	**14**	6 min 30 sec	16 min 30 sec
5	2 min	12 min	**15**	7 min	17 min
6	2 min 30 sec	12 min 30 sec	**16**	7 min 30 sec	17 min 30 sec
7	3 min	13 min	**17**	8 min	18 min
8	3 min 30 sec	13 min 30 sec	**18**	8 min 30 sec	18 min 30 sec
9	4 min	14 min	**19**	9 min	19 min
10	4 min 30 sec	14 min 30 sec	**20**	9 min 30 sec	19 min 30 sec

6. PREPARATION OF THE PRODUCT:

F10SC veterinary disinfectant contains quaternary ammonium and biguanidine compounds (5,8%), non-toxic ampholytic surfactants and sequesterants.
Storage conditions indicated by the manufacturer: below 30°C in dry conditions.

- **Use-dilution of product indicated and preparation thereof:**

The recommended dilution of 1/250 was used for the test. A total of 4 ml of the super concentrate was diluted with sterile distilled water at room temperature to a total volume of 1 L using "A" grade volumetric glassware. This preparation was dispensed in 10 ml portions into 60 (plus a few extra) tubes and placed into a water bath at 20°C (± 0,5°C) approximately 30 minutes before the test started. Testing started once the contents of the tubes had reached water bath temperature. The disinfectant at use-dilution was tested within less than 3 hours of preparation.

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First set:

Tube selected:	Initial result: (in synthetic broth with inactivator)	Spiked with the 24 hour broth culture of the test organism (1 ml of a 10^{-8} dilution was added to each tube.) Result after 48 hours:
3	Negative	Positive
6	Negative	Positive
10	Negative	Positive
15	Negative	Positive
20	Negative	Positive
36	Negative	Positive

Plate count results (TSA) done in triplicate: 8, 7 and 16 cfu/plate (equal to 1 ml).
(Average of 10,3 within the stated limits of 5-100 cells per tube)

Second set:

Tube selected:	Initial result: (in synthetic broth with inactivator)	Spiked with the 24 hour broth culture of the test organism (1 ml of a 10^{-8} dilution was added to each tube.) Result after 48 hours:
12	Negative	Positive
22	Negative	Positive
26	Negative	Positive
35	Negative	Positive
38	Negative	Positive
47	Negative	Positive

Plate count results (TSA) done in triplicate: 10, 6 and 9 cfu/plate (equal to 1 ml).
(Average of 8,3 within the stated limits of 5-100 cells per tube)

All the positive tubes were cultured on TSA for ± 24 hours at 37°C for Gram staining.
All were small Gram negative rods resembling the mother culture.

All the positive tubes were also confirmed using the selective media previously indicated. Colour and biochemical reactions resembled those of the mother culture.

XLD agar: media did not change colour but stayed red, colonies were reddish pinkish, transparent. No black cores.
Brilliant green modified agar: The colour of the media changed from orange-pink to red, colonies were pink to translucent.
MacConkey's agar: The colour of the media changed from light pink to a clear light peach colour. Colonies resembled the colour of the media.

7. MEDIA USED:

Description of media	**Supplier**	**Batch No:**
AOAC Synthetic broth	Difco	1131006
Brilliant Green modified agar	Scharlau	14751
XLD agar (xylose lysine deoxycholate)	Merck	1023822
MacConkey agar	Biolab	102116
Trypticase soy agar (TSA)	Biolab	1026752

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10.1 Efficacy of F10SC veterinary disinfectant **Batch 071107** against ***Salmonella choleraesuis*** ATCC 10708 on non porous hard surface carriers.

Description of sample:	**F10SC Veterinary disinfectant**
Batch tested and expiry date:	**# 071107**
Recommended dilution:	1/250
Recommended exposure time:	10 minutes
Media used for primary subculture:	10 ml AOAC Synthetic broth + glucose + inactivator
Inactivator used:	Tryptone Soy Broth containing 3% asolectin and 20 % Tween 80
Volume of inactivator added to 10 ml media	0,1 ml
Preparation of carriers: a) % T of suspension at 580 nm b) drying time	 a) 33,5 % T b) 40 minutes
Temperature at which test was conducted:	Start: 19,5°C End: 19,5°C
Incubation temperature:	37°C (± 2°C) for 48-54 hours
Date tested:	Start: 21/7/2006 End: 23/7/2006
Final result: Positive tubes confirmed:	 **1/60**

Determination of carrier load: Total counts done on 6 randomly chosen carriers: (labelled A to F)

Carrier reference:	Individual results on duplicate plates:		Average result for *Salmonella choleraesuis* : (limits 0,5 - 2 x 10^6 organisms per carrier)
	dilutions 10^{-4}	dilutions 10^{-5}	
A	110 x 10^4 102 x 10^4	9 x 10^5 14 x 10^5	1,1 x 10^6
B	166 x 10^4 199 x 10^4	22 x 10^5 23 x 10^5	2,04 x 10^6
C	528 x 10^4 384 x 10^4	66 x 10^5 69 x 10^5	5,66 x 10^6
D	324 x 10^4 332 x 10^4	28 x 10^5 35 x 10^5	3,21 x 10^6
E	768 x 10^4 744 x 10^4	104 x 10^5 76 x 10^5	8,28 x 10^6
F	760 x 10^4 952 x 10^4	64 x 10^5 70 x 10^5	7,6 x 10^6

Average count per carrier: **4,6 x 10^6 cfu/carrier**. (Higher than the maximum limit but sample result was less than 2/60 positive.)

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10.2 Efficacy of F10SC veterinary disinfectant **Batch 080310** against ***Salmonella choleraesuis*** ATCC 10708 on non porous hard surface carriers.

Description of sample:	**F10SC Veterinary disinfectant**
Batch tested and expiry date:	**# 080310**
Recommended dilution:	1/250
Recommended exposure time:	10 minutes
Media used for primary subculture:	10 ml AOAC Synthetic broth + glucose + inactivator
Inactivator used:	Tryptone Soy Broth containing 3% asolectin and 20 % Tween 80
Volume of inactivator added to 10 ml media	0,1 ml
Preparation of carriers: a) % T of suspension at 580 nm b) drying time	 a) 35,5 % T b) 40 minutes
Temperature at which test was conducted:	Start: 19,5°C End: 19,5°C
Incubation temperature:	37°C (± 2°C) for 48-54 hours
Date tested:	Start: 26/7/2006 End: 28/7/2006
Final result: Positive tubes confirmed:	 **1/60**

Determination of carrier load: Total counts done on 6 randomly chosen carriers: (labelled A to F)

Carrier reference:	Individual results on duplicate plates:		Average result for *Salmonella choleraesuis* : (limits 0,5 - 2 x 10^6 organisms per carrier)
	dilutions 10^{-4}	dilutions 10^{-5}	
A	105 x 10^4 117 x 10^4	11 x 10^5 13 x 10^5	1,16 x 10^6
B	568 x 10^4 904 x 10^4	80 x 10^5 81 x 10^5	7,7 x 10^6
C	143 x 10^4 145 x 10^4	14 x 10^5 10 x 10^5	1,32 x 10^6
D	71 x 10^4 85 x 10^4	7 x 10^5 9 x 10^5	0,79 x 10^6
E	276 x 10^4 368 x 10^4	33 x 10^5 25 x 10^5	3,06 x 10^6
F	140 x 10^4 103 x 10^4	10 x 10^5 12 x 10^5	1,16 x 10^6

Average count per carrier: **2,53 x 10^6 cfu/carrier.** (Higher than the maximum limit but sample result was less than 2/60 positive.)

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AOAC Use-dilution test result sheet for: F10SC # 080310

Tubes with positive growth (turbidity) highlighted.

1	2	3	4	5	6	7	8	9	10
11	12	13	14	15	16	17	18	19	20
21	22	23	24	25	26	27	28	29	30
31	32	33	34	35	36	37	38	39	40
41	42	43	44	45	46	47	48	49	50
51	52	53	54	55	56	57	58	59	60

Positive tube confirmation: F10SC # 080310

Tube number	Gram stain after 24 Hours (TSA)	Additional identification done on culture: (incubation period approximately 24 hours at 37°C)
29	Small Gram negative rods	**XLD agar:** media did not change colour but stayed red, colonies were reddish pinkish, transparent. No black cores. **Brilliant green modified agar:** The colour of the media changed from orange-pink to red, colonies were pink to translucent. **MacConkey's agar:** The colour of the media changed from light pink to a clear light peach colour. Colonies resembled the colour of the media. **API 20E:** code 4-3-0-4-5-5-0 (Very good ID, 66,2 % certainty. Identified as *Salmonella choleraesuis*, ID done 3/8/2006)

CONCLUSION: For F10SC # 080310 only 1 tube out of a total of 60 tested was confirmed as *Salmonella choleraesuis.*

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10.3 Efficacy of F10SC veterinary disinfectant **Batch 080511** against ***Salmonella choleraesuis*** ATCC 10708 on non porous hard surface carriers.

Description of sample:	**F10SC Veterinary disinfectant**
Batch tested and expiry date:	**# 080511**
Recommended dilution:	1/250
Recommended exposure time:	10 minutes
Media used for primary subculture:	10 ml AOAC Synthetic broth + glucose + inactivator
Inactivator used:	Tryptone Soy Broth containing 3% asolectin and 20 % Tween 80
Volume of inactivator added to 10 ml media	0,1 ml
Preparation of carriers: a) % T of suspension at 580 nm b) drying time	 a) 34,2 % T b) 40 minutes
Temperature at which test was conducted:	Start: 19,5°C End: 19,5°C
Incubation temperature:	37°C (± 2°C) for 48-54 hours
Date tested:	Start: 15/6/2006 End: 17/6/2006
Final result: Positive tubes confirmed:	 **1/60**

Determination of carrier load: Total counts done on 6 randomly chosen carriers: (labelled A to F)

Carrier reference:	Individual results on duplicate plates:		Average result for *Salmonella choleraesuis* : (limits 0,5 - 2 x 10^6 organisms per carrier)
	dilutions 10^{-4}	dilutions 10^{-5}	
A	126 x 10^4 80 x 10^4	14 x 10^5 8 x 10^5	1,07 x 10^6
B	63 x 10^4 57 x 10^4	4 x 10^5 3 x 10^5	0,48 x 10^6
C	142 x 10^4 146 x 10^4	12 x 10^5 9 x 10^5	1,25 x 10^6
D	128 x 10^4 118 x 10^4	12 x 10^5 4 x 10^5	1,02 x 10^6
E	208 x 10^4 168 x 10^4	19 x 10^5 10 x 10^5	1,67 x 10^6
F	206 x 10^4 178 x 10^4	20 x 10^5 11 x 10^5	1,74 x 10^6

Average count per carrier: **1,21 x 10^6 cfu/carrier.**

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AOAC Use-dilution test result sheet for: F10SC # 080511

Tubes with positive growth (turbidity) highlighted.

1	2	3	4	5	6	7	8	9	10
11	12	13	14	15	16	17	18	19	20
21	22	23	24	25	26	27	28	29	30
31	32	33	34	35	36	37	38	39	40
41	42	43	44	45	46	47	48	49	50
51	52	53	54	55	56	57	58	59	60

Positive tube confirmation: F10SC # 080511

Tube number	Gram stain after 24 Hours (TSA)	Additional identification done on culture: (incubation period approximately 24 hours at 37°C)
35	Small Gram negative rods	**XLD agar:** media did not change colour but stayed red, colonies were reddish pinkish, transparent. No black cores. **Brilliant green modified agar:** The colour of the media changed from orange-pink to red, colonies were pink to translucent. **MacConkey's agar:** The colour of the media changed from light pink to a clear light peach colour. Colonies resembled the colour of the media. **API 20E:** code 4-3-0-4-5-5-0 (Very good ID, 66,2 % certainty. Identified as *Salmonella choleraesuis*, ID done 20/6/2006)

CONCLUSION: For F10SC # 080511 only 1 tube out of a total of 60 tested was confirmed as *Salmonella choleraesuis*.

11. DISCUSSION:

F10SC veterinary disinfectant when tested in accordance with 2000 AOAC International, Official Method 991.47 was effective against *Salmonella choleraesuis* ATCC 10708 in a contact time of 10 minutes.

E. LLOYD
MICROBIOLOGY DEPARTMENT

R A ROOS
MANAGER MICROBIOLOGY

Fax no. (011) 474 1670

TEST REPORT

SABS

PHARMACEUTICAL MICROBIOLOGY 2423

U verw/Your ref: O/No. Ref QF117HealthHyg2006

Ons verw/Our ref: 06-529

Navrae/Enquiries: 428-6269

Datum/Date: 28/9/2006

2423/529/A101797 B

Bladsy/page 1 van/of 12

Health and Hygiene (Pty) Ltd
Attention: Mr. J Temperley
PO Box 347
SUNNINGHILL
2157
ZA

AOAC HARD SURFACE CARRIER TEST ON F10SC VETERINARY DISINFECTANT.

NON POROUS CARRIERS COATED WITH *Staphylococcus aureus* ATCC 6538.

1. DESCRIPTION OF SAMPLES.

The following three batches of F10SC were supplied by the client:

F10SC batch 071107 manufacturing date 11/2005, expiry date 11/2007.
F10SC batch 080310 manufacturing date 3/2006, expiry date 3/2008.
F10SC batch 080511 manufacturing date 5/2006, expiry date 5/2008.

2. TEST REQUESTED.

To determine the efficacy of the product against a standard bacterial strain of *Staphylococcus aureus* at a dilution of 1/250 using the AOAC hard surface carrier test method.

3. METHOD OF TEST.

2000 AOAC International, Official Method 991.48. Testing disinfectants against *Staphylococcus aureus*. Hard surface carrier test method, First action 1991.

Brief summery: The AOAC Use-Dilution test was done with flame polished glass cylinders coated with an exact amount of micro organism. These were dried and exposed to the use-dilution of 1/250 of the disinfectant as indicated by the client for a period of 10 minutes. After neutralization these carriers were cultured to assess the survival of the bacteria.

A single test involved the evaluation of 60 inoculated carriers (one organism) against one batch of the product. Six additional inoculated carriers were used for the determination of the carrier bacterial load. Another set of six additional carriers were prepared at the same time as extras. A total of 72 carriers were required for a single test.

4. MICRO ORGANISM USED AND PREPARATION OF THE CULTURE:

The test organism used was *Staphylococcus aureus* ATCC No. 6538. (SABS type culture collection ref Sta 10)

- **Initial preparation of the culture**

The mother culture was opened and prepared according to the AOAC method.
Gram stain reaction after ± 18 hours incubation at 37°C on trypticase soy agar (TSA):
Gram positive cocci.
Colony morphology on trypticase soy agar: flat, round, shiny light orange-yellow colonies were observed.

- **Additional selective media and biochemical tests used to confirm the genus and species:**

Baird Parker agar (with added volumes of 1% potassium tellurite solution and 50 % egg yolk emulsion as stipulated by the manufacturer): Colonies are small, shiny and pitch black with a white margin surrounded by a clear zone/halo.
DNase agar: Creamy coloured colonies are surrounded by a clear zone on the addition of 1 N HCl. The rest of the plate remains turbid.
Mannitol Salts agar: The colour of the media changes from reddish pink to yellow. Colonies are orange-yellow.

- **Preparation of the culture used for the coating of the carriers:**

At least 3 consecutively daily transfers were made in synthetic broth and the last 24 hour old broth culture was used to prepare a lawn on TSA plates. These were incubated for 24 ± 4 hours at 37°C. The growth on each plate was gently removed with a sterile swab and 10 ml sterile synthetic broth. The suspensions from individual plates were pooled in a sterile flask and vortexed to break up clumps of bacterial cells.

The suspension was left to stand for approximately 10 minutes at room temperature after which an aliquot was diluted with sterile synthetic broth to the required density and bacterial count according to the previously established standard curve and coating test trial records. The suspension so diluted was used within 30 minutes of preparation to coat the required amount of carriers for exactly 15 minutes according to the method.

The coated carriers were removed from the suspension after 15 minutes and dried on sterile filter paper for 40 minutes at 37°C (± 2°C). Immediately thereafter 6 carriers were used for the determination of the carrier load, using the synthetic broth and the required neutralizer for the initial recovery. Counts were done in duplicate on TSA using sterile distilled water for the serial dilutions.

The sponsor requested no additional organic soiling of the carriers.

5. CARRIERS:

- **Description of carriers:**

Disposable fire-polished borosilicate glass carriers were supplied by the sponsor. Size of carriers: length 10mm (± 1 mm); inside diameter 6mm (± 1 mm) and outside diameter 8 mm (± 1 mm). Only cylinders without visible cracks and flaws were used for the tests. The cylinders were washed with ethanol and distilled water according