GUI – Gesellschaft für Umwelt- und Innenraumanalytik mbH

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ANALYSIS REPORT

Report number:	**100219-01**
Customer:	Joker AG/ SA Industriezone CH-3210 Kerzers
Test item:	Versa Clean 1 Package, 140 g
Contract date:	2010-02-19
Sample arrival date:	2010-02-19
Test period:	2010-02-19 to 2010-03-29

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1. Task

We were commissioned to test the substance “Versa Clean“, declared as a “High-Tech Cleaning Compound“, regarding its efficacy as a desinfectant towards different microorganisms. The test war performed on the basis of Study Report Number 222070803 by Confarma France, describing the testing of “Cyber Clean® with Benzalkonium Chloride + Didecyl demethyl Ammonium Chloride CAS 7173-51-5“.

2. Test Item

One package containing 140 g “Versa Clean“ (Distribution: NMCI International, Mandaluyong City, Philippines) was provided by the customer.

3. Material and Methods

3.1 Test Organisms

	ATCC-Number	Lot Number	Date of Expiry (Manufacturer)	Internal Date of Expiry
Aspergillus niger	-	-	-	-
Candida albicans	10231	443633	2 / 2011	2 / 2012
Escherichia coli	8739	483463	12 / 2010	12 / 2011
Pseudomonas aeruginosa	10145	416947	3 / 2011	3 / 2012
Staphylococcus aureus	6538	485941	4 / 2009	4 / 2010

Aspergillus niger was taken from the GUI stock culture collection. The strain was originally obtained in the course of an interlaboratory test.

3.2 Cultivation of Test Organisms

Aspergillus niger was cultivated in an agar slant with malt extract agar (MEA, Merck No. 1.05398.0500) for seven days at 25℃.

Candida albicans was streaked on Petri dishes with MEA (MEA with Chloramphenicol and Gentamycin, Merck No. 1.13423.0480) and cultivated for five days at 25℃.

Escherichia coli, *Pseudomonas aeruginosa* and *Staphylococcus aureus* were grown in sterile culture tubes filled with liquide growth medium for 16 h at 37℃. The liquide growth medium had the following composition: 17 g/l Trypton (Oxoid No. LP 0042); 3 g/l Phyton-Pepton (Merck No. 1.07212.0500); 5 g/l NaCl; 2.5 g/l K_2HPO_4; 2.5 g/l D-Glucose.

3.3 Preparation of suspensions of the test organisms

3.3.1 Spore Suspension of *Aspergillus niger*

To an agar slant with an *Aspergillus niger* culture, 5 ml suspension buffer (0.9 % (w/v) NaCl and 0.02 % (w/v) TWEEN 80) were added. The tube was shaken for one minute using a Vortex mixer. Afterwards, 3 ml of the spore suspension obtained were transferred into a sterile test tube and used for the tests within 1 h.

3.3.2 Suspension of *Candida albicans*

Candida albicans was transferred from a Petri dish culture into a test tube with 10 ml suspension buffer (see 3.3.1) using an inoculation loop. Yeast cells from a 2 cm^2 surface area were harvested during this step. The suspension was homogenized for 1 min. using a Vortex mixer and used for the tests within 1 h.

3.3.3 Suspensions of the three bacterial strains

Of each of the three bacterial cultures (*Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*), 1 ml was transferred into a sterile test tube with 3 ml suspension buffer (see 3.3.1). The suspensions were homogenized for 1 min. using a Vortex mixer and used for the tests within 1 h.

3.4 Determination of the initial titer of the suspensions

To determine the titer of colony forming units (CFU) in the microorganism suspensions, 10 µl of each suspension were pipetted into separate 250 ml flasks, each containing 100 ml of inactivation solution, resulting in suspensions diluted 1:10^4.

Composition of the inactivation solution: 30 g/l Tween 80 (Merck No. 822187); 5 g/l Sodium thioulfat (Sigma No. 72049-250G); 3 g/l L-α-Phosphatidylcholine (Sigma No. 61755-25G); 1.1 g/l L-Histidine (Merck No. 1.04351.0100).

Suspensions were diluted further using sterile dilution buffer PBS (2.9 g/l Na_2HPO_4 x 12 H_2O; 0.2 g/l KH_2PO_4; 0.2 g/l KCl and 8 g/l NaCl) to obtain dilutions 1:10^5 and 1:10^6 in fresh test tubes. For each of the five test organisms, two times 100 µl of the dilutions 1:10^4, 1:10^5 and 1:10^6 were plated on Petri dishes with the growth media listed and counted after incubation as indicated below:

Test organism	Growth media	Culture conditions
Aspergillus niger	MEA with Chloramphenicol and Gentamycin Merck No. 1.13423.0480	96 h at 25℃
Candida albicans	MEA with Chloramphenicol and Gentamycin Merck No. 1.13423.0480	48 h at 25℃
Escherichia coli	CASO-Agar Merck No. 1.13582	24 h at 37℃
Pseudomonas aeruginosa	*Pseudomonas*-Cetrimide-Agar Oxoid No. PO5076A	48 h at 37℃
Staphylococcus aureus	CASO-Agar Merck No. 1.13582	28 h at 37℃

3.5 Control of inactivating solution

The inactivation solution is used to neutralize the desinfecting activity of the test item. To check the effectivity of the inactivation solution, 5 g of the test item “Versa Clean“ were added to each of the five 1:10^4 diluted suspensions obtained before (see 3.4). The bottles

with the diluted suspensions and the pieces of “Versa Clean“ were shaken for 1 min. and then diluted (1:10^5 and 1:10^6) and plated as described above (see 3.4).

3.6 Preparation of microbe-carriers

Polystyrene weighing dishes (45 x 45 mm; bottom area aprox. 30 x 30 mm) were used as carriers for the microorganisms. Prior to use, the weighing dishes were sterilized by UV irradiation of both sites for 2x 15 min.

Three carriers were prepared for each test organism, using the concentrated suspensions described above (see 3.3). For this purpose, 10 µl of the suspensions were pipetted on the bottom area of the weighing dishes and spread with the pipett tip. The carriers were drying in the air within 10 min. and thereafter were used for the cleaning tests within 10 minutes.

3.7 Determination of the initial count of organisms on the carriers

One of the three carriers prepared for each test organism was transferred into a 250 ml flask containing 100 ml of inactivation solution and let stand for 15 minutes. Then the bottles were shaken vigorously by hand for 1 minute. The resulting suspensions corresponded to the 1:10^4 dilutions mentioned above and were diluted and plated as described (see 3.4).

3.8 Investigation of the cleaning and desinfecting activity of the test item (Assay A)

Ten pieces of the test item “Versa Clean“ with a weight of 5 ± 0.1 g each were prepared. Each carrier was treated with one piece of the test item. For this, the bottom areas of the carriers were first dabed with pieces of the test item 10 times within about 10 seconds.

Then the pieces of test item were pressed towards the bottom areas of the carriers, using another sterile weighing dish to ensure a close and even contact between carriers and test item. The times of action were 1 minute (Assay A1) and 5 minutes (Assay A5), respectively.

After treatment, the carriers were transferred into 250 ml flasks containing 100 ml of inactivation solution and let stand for 15 minutes. Then the bottles were shaken vigorously by hand for 1 minute. From the microorganism suspensions obtained, 1 ml, 10 ml and 89 ml were filtrated separately using sterile membrane filters (Mixed cellulose ester, pore size 45 µm, Ø 50 mm, Whatman Ref.-No. 10406872). The filters were placed on Petri dishes with the appropriate growth media with the contaminated site facing upwards. Incubation was as described above (see 3.4).

3.9 Data collection and interpretation

Petri dishes with up to 1000 colonies of bacteria or *Candida* were countable. In the case of *Aspergillus* not more than 100 CFU per Petri dish could be counted. On membrane filters, up to 300 CFU (bacteria and *Candida*) and up to 30 CFU (*Aspergillus*) were countable.

The results of the titer determinations are given in CFU / ml. For the carriers, the numbers of CFU which could be washed off is given for before and after cleaning. The ratio between these values is taken as measure for the reduction of microbial surface contamination.

In accordance with Study Report Number 222070803 (Confarma France), the efficacy of a desinfectant is considered as sufficient if bacterial surface contaminations are reduced by a factor of 10^4 or higher under simulated conditions of use. For yeasts and moulds, a reduction by a factor of 10^3 or higher is required.

In contrast to the procedure described in Study Report Number 222070803 (Confarma France), the number of CFU within the test item after cleaning the carriers was not determined and taken into account for the assessment of the desinfectant effectivity.

4. Results [1]

Aspergillus niger	Dilution			Result
	$1 : 10^4$	$1 : 10^5$	$1 : 10^6$	
Determination of initial titer (Control 1)	not countable	29 / 34	(2 / 5)*	$3{,}2 \times 10^7$ CFU/ml
Control of inactivating solution (Control 2)	not countable	29 / 31	(3 / 5)*	$3{,}0 \times 10^7$ CFU/ml
Initial count of organisms on the carriers (Control 3)	not countable	28 / 35	(0 / 5)*	$3{,}2 \times 10^5$ CFU / carrier (washed off)
	1 ml	10 ml	89 ml	
Count of organisms after cleaning carriers for 1 min. (Assay A1)	1	7	not countable	72 CFU / carrier reduction: $4{,}4 \times 10^3$
Count of organisms after cleaning carrieres for 5 min. (Assay A5)	3	8	not countable	100 CFU / carrier reduction: $3{,}2 \times 10^3$

* Values in brackets were not taken into account for calculating the results

Candida albicans	Dilution			Result
	$1 : 10^4$	$1 : 10^5$	$1 : 10^6$	
Determination of initial titer (Control 1)	382 / 370	39 / 41	(4 / 8)*	$3{,}9 \times 10^7$ CFU/ml
Control of inactivating solution (Control 2)	408 / 391	35 / 58	(6 / 7)*	$4{,}3 \times 10^7$ CFU/ml
Initial count of organisms on the carriers (Control 3)	20 / 24	(0 / 4)*	(0 / 0)*	$2{,}2 \times 10^4$ CFU / carrier (washed off)
	1 ml	10 ml	89 ml	
Count of organisms after cleaning carriers for 1 min. (Assay A1)	1	1	25	27 CFU / carrier reduction: $0{,}8 \times 10^3$
Count of organisms after cleaning carriers for 5 min. (Assay A5)	11	63	437	511 CFU / carrier reduction: $0{,}4 \times 10^2$

* Values in brackets were not taken into account for calculating the results

Escherichia coli	Dilution			Result
	$1 : 10^4$	$1 : 10^5$	$1 : 10^6$	
Determination of initial titer (Control 1)	not countable	137 / 144	23 / 24	$1{,}9 \times 10^8$ CFU/ml
Control of inactivating solution (Control 2)	not countable	125 / 144	19 / 19	$1{,}6 \times 10^8$ CFU/ml
Initial count of organisms on the carriers (Control 3)	5 / 6	1 / 2	0 / 0	10000 CFU / carrier (washed off)
	1 ml	10 ml	89 ml	
Count of organisms after cleaning carriers for 1 min. (Assay A1)	1	3	15	19 CFU / carrier reduction: $0{,}5 \times 10^3$
Count of organisms after cleaning carriers for 5 min. (Assay A5)	0	1	7	8 CFU / carrier reduction: $1{,}3 \times 10^3$

Pseudomonas aeruginosa	Dilution			Result
	$1 : 10^4$	$1 : 10^5$	$1 : 10^6$	
Determination of initial titer (Control 1)	not countable	316 / 247	22 / 19	$2{,}4 \times 10^8$ CFU/ml
Control of inactivating solution (Control 2)	not countable	278 / 325	24 / 21	$2{,}6 \times 10^8$ CFU/ml
Initial count of organisms on the carriers (Control 3)	10 / 4	1 / 1	0 / 0	8500 CFU / carrier (washed off)
	1 ml	10 ml	89 ml	
Count of organisms after cleaning carriers for 1 min. (Assay A1)	0	1	16	17 CFU / carrier reduction: $0{,}5 \times 10^3$
Count of organisms after cleaning carriers for 5 min. (Assay A5)	0	0	1	1 CFU / carrier reduction: $8{,}5 \times 10^3$

[1] The results solely refer to the tested samples.

Staphylococcus aureus	Dilution			Result
	$1 : 10^4$	$1 : 10^5$	$1 : 10^6$	
Determination of initial titer (Control 1)	not countable	496 / 494	69 / 53	$5{,}5 \times 10^8$ CFU/ml
Control of inactivating solution (Control 2)	not countable	437 / 413	30 / 102	$5{,}4 \times 10^8$ CFU/ml
Initial count of organisms on the carriers (Control 3)	not countable	392 / 191	23 / 25	$2{,}7 \times 10^6$ CFU / carrier (washed off)
	1 ml	10 ml	89 ml	
Count of organisms after cleaning carriers for 1 min. (Assay A1)	18	139	not countable	1400 CFU / carrier reduction: $1{,}9 \times 10^3$
Count of organisms after cleaning carriers for 5 min. (Assay A5)	17	177	not countable	1800 CFU / carrier reduction: $1{,}5 \times 10^3$

Mönchengladbach, 2010-03-29

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- Managing Director -

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Assessment and recommendation[2]

to Report No.: 100219-01

The test item “Versa Clean“, declared as a “High-Tech Cleaning Compound“, reduces surface contaminations by bacteria, yeasts and moulds very moderate.

However, a reduction by the factor 10^4, required to certify a sufficient efficacy of the desinfectant, was not reached in the tests with bacteria (*Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*). The cleaning and desinfection efficacy of “Versa Clean“ concerning bacteria is not sufficient.

So the tested product did not match the requirements.

In the test with *Candida albicans*, the results were slightly below the limit in Assay A1 and clearly below the limit in Assay A5. Even for yeasts, the cleaning and desinfection efficacy is not sufficient.

Also in this test “Versa Clean” is not useful.

Remarkably, the increased time of action in Assay A5 resulted only in the cases of *Escherichia coli* and *Pseudomonas aeruginosa* in a better cleaning effect. For *Aspergillus niger* and *Staphylococcus aureus*, the results were slightly worse and in case of *Candida albicans* clearly worse after 5 minutes of action.

This indicates that “Versa Clean” has just a physical effect in removing germs but no effect as a disinfectant.

If you use it in case of a contaminated surface there is a big risk to transport the germs to the next surface like using a duster.

The expectation of the user to remove different types of germs successful and for longer periods from surfaces is not fulfilled.

GUI – Gesellschaft für Umwelt- und Innenraumanalytik
Mönchengladbach, 2010-03-29

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[2] The assessment and recommendation are exclusively based on the presented test item.