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certified according to GMP, cGMP (FDA), GLP
FDA FEI 3002806657 CF numbers 9614423 and 9615669
Etablissement pharmaceutique N° M 07/173
ISO 9001, ISO 17025, ISO 14001, OHSAS 18001

Study report

" MRSA " (STAPHYLOCCUS AUREUS ")

Title **Study of disinfectant efficacy by the microbe carrier test under simulated conditions of use**

Test item Cyber Clean professional

Batch number A: KR 09/014.2

B: KR 09/015

Test facility CONFARMA FRANCE SARL
Zone Industrielle
Rue du Canal d'Alsace
F-68490 HOMBOURG
France

Sponsor Joker AG
Industriezone
Postfach 69
CH 3210 Kerzers

STUDY MRSA

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CONTENTS

1. General information

1.1. Sponsor

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CH 3210 Kerzers

1.2. Study Monitor

Mr. René H. Dietrich
René H. Dietrich Consulting
Seewiesenstrasse 10
Postfach 18
CH- 8597 Landschlacht

1.3. Test facility

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This test facility is licensed to conduct animal trials in accordance with Order No. C 68-144-03 of the French Republic dated October 19th 2009, for the testing of medicines under Number M 07/173 in accordance with the approval from the "Agence Française de Sécurité Sanitaire des Produits de Santé" dated September 20th 2007, and acknowledged as an "acceptable laboratory" by the FDA, C.F. numbers 9614423 and 9615669.

The test facility was classified to comply with the requirements of good laboratory practice, status A (in conformity with GLP) as declared by the certificate from the "Agence Française de Sécurité Sanitaire des Produits de Santé" dated May 6th 2008.

The test facility is certified for testing medical Products by the "Agence Française de Sécurité Sanitaire des Produits de Santé" and is classified to be compliant to the Good Manufacturing Practice as declared by the certificate number HPF/FR/102/2009 and HPF/FR/103/2009 from the "Agence Française de Sécurité Sanitaire des Produits de Santé" dated June 5th 2009.

Furthermore CONFARMA is certified according to ISO 9001, ISO 17025, ISO 14001 and OHSAS 18001, i.e. the norms for Quality, Security, Hygiene and Environment as declared by the certificate number 2008/09/99 from the certification organism "Global Quality Cert - GQC" dated September 19th 2008.

1.4. Report number

The final report number, 229100911 A - B was attributed according to the registration systems described in the corresponding CONFARMA procedures.

1.5. Responsibilities

The study director was responsible for the test system, for the assays performed, the interpretation and the documentation of the results.

Study Director	J. De Geest, Biologist
Test facility Management	R. Holzinger, Microbiologist
Quality Assurance	K. Wechsler, Ph.D., Pharmacist / D. Maire, Pharmacist
Study personnel	R. Ringenbach, technician

1.6. Study methods

The study was conducted according to the indications in the USP, chapter <1072> « Disinfectants and antiseptics » [1] and according to the CONFARMA Protocol number 229100911 A - B [8] which is based on the guideline of the German Society for Hygiene and Microbiology (DGHM) from 1991 [2] and the norms EN 1040 « Chemical disinfectants and antiseptics Basic bactericidal activity Test method and requirements (phase 1) » [3] as well as the norm EN 13697 « Chemical disinfectants and antiseptics — Quantitative non-porous surface test for the evaluation of bactericidal and/or fungicidal activity of chemical disinfectants used in food, industrial, domestic and institutional areas — Test method and requirements without mechanical action (phase 2/step 2) » [4].

1.7. Objective of the study

The objective of the study was to determine the degree of disinfectant efficacy for the test item applied to plastic surfaces under simulated conditions of use in accordance with the USP, chapter <1072> [1], the guideline of the German Society for Hygiene and Microbiology (DGHM) [2] and the norms EN 1040 [3] and EN 13697 [4].
Plastic surfaces were chosen in accordance with the sponsor in order to simulate the surfaces of electronic equipment such as computer keyboards or mobile phones for which the test item should allow an effective disinfection.

1.8. Dates

Study initiation date: October 29th 2009 (the date the test item was booked into the registration system)

Experimental starting date: November 20th 2009

Experimental completing date: November 23rd 2009

Study completion date: the date the study director signed the final study report

1.9. Archiving

The raw data and a copy of the report will be stored in the archives of CONFARMA France for a period of 11 years.
The originals of the final study report will be returned to the sponsor who has the full responsibility for archiving.

2. Summary

As an overall summary of the results obtained it can be concluded that the test item complies with the acceptance criteria of the norm EN 13697 [5] of superior or equal to 5 log10 reduction with the action time of 1 minute and 5 minutes for living *Staphylococcus aureus* MRSA. In this context it has to be mentioned that the norms EN 1040 [3] and EN 13697 [4] recommend an action time of 5 minutes for living bacteria.

3. Introduction

The present study allows to determine the degree of disinfectant efficacy for the disinfectant applied to surfaces under simulated conditions of use.
The method used in this study does not serve to prove the efficacy of wiping a surface with disinfectant as this "mechanical" disinfection technique would already eliminate the majority of the micro-organisms present on the microbe-carriers. Selected test organisms (only standard strains of official culture collections and no micro-organisms isolated in the production rooms during environmental monitoring) at a concentration of 10^7 to 10^8 micro-organisms per carrier are brought onto microbe carriers. The ready-made disinfectant solutions to be examined are then applied "in situ". After the prescribed periods of action, the test organisms are washed off from the microbe carriers using the appropriate inactivator solutions. The number of surviving micro-organisms is determined by the membrane filter technique and compared with the count of the untreated controls.

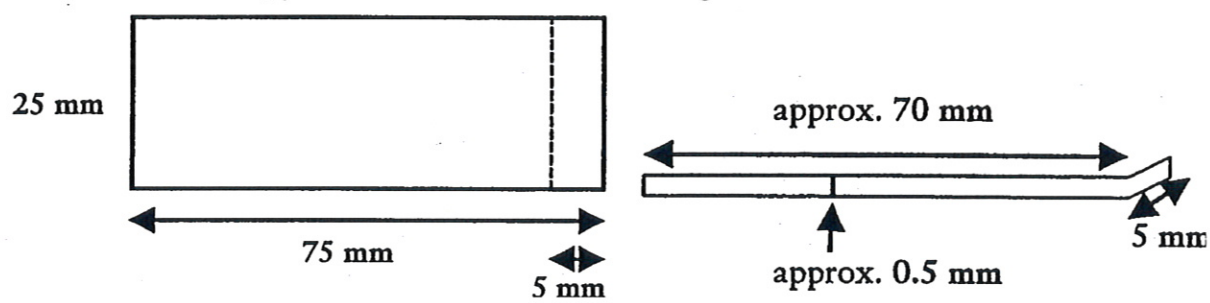
4. Test item

The test item Cyber Clean professional received at the CONFARMA laboratories on October 28th 2008 and got the analysis number 229100911 A - C according to the registration systems.
The test item provided by the sponsor was specified as follows Cyber Clean professional, batch numbers A: KR 09/014.2 and B: KR 09/015.
The identity of the test item will be checked by the appearance. Furthermore the correspondence of the test item is checked according to the registration systems of CONFARMA.
The test substance has to be stored at room temperature (definition according to the European Pharmacopoeia 20 ± 5°C).

5. Test system

1 micro-organism was employed, *Staphylococcus aureus* MRSA Gram-positive, Catalase positive cocci.

The ATCC (American type culture collection) reference micro-organisms were supplied by AES (manufacturer Micro-biologics) as lyophilisates. Quality, purity and identity control on the micro-organisms and the preparation of the spore suspension was performed according to CONFARMA SOP M 26.
The maximum of 5 passages starting from the lyophilisate required by the official compendia, was respected for every ATCC micro-organism in compliance to the CONFARMA SOP M 26.
As microbe carriers, plastic material of the following dimensions were used :



6. Assay procedure

6.1. Preparation of microbe-carriers

The microbe-carriers, made of plastic material, with dimensions of approximately 25 mm x 75 mm were provided by CONFARMA. They were chosen to simulate the conditions of routine use of the disinfectant, i.e. the use of the test item on plastic surfaces .
Microbe carriers which have already been used were washed and autoclaved. The carriers were taken by the edge and placed in separate plastic bags. The dishes were sterilized in a steam autoclave at 121°C for 15 minutes (carrier material which is deformed should be smoothed after the treatment, e.g. by applying pressure).

6.2. Preparation of the suspensions of test organisms

6.2.1. Bacteria

Using an inoculation loop material from the bacterial cultures was spread in dense streaks onto TSA and a SDA plate followed by incubation at 25 to 30°C for 24 hours.
The microbes grown on the plate were harvested by means of a inoculation loop and suspended in approx. 9 ml of PBPS resulting in a bacterial suspension of an optical density of approx. 1.
Normally this yields a microbial concentration of 10^7 to10^8 micro-organisms per ml..

6.3. Determination of microbial growth (control 1)

A suspension containing 10^7 - 10^8 CFU / ml was prepared.
10 µl of the microbial suspension (10 to 10^8 / 10 µl) were added to 100 ml of the inactivation solution and shaken for 1 minute and process further within 15 minutes (= dilution 1; 1:10^4)

1 ml of dilution 1 was added to 9 ml of PBPS solution and mixed thoroughly (= dilution 2; 1: 10^5).
1 ml of dilution 2 was added to 9 ml of PBPS solution and mixed thoroughly (= dilution 3; 1:10^6).
From each of the 3 dilutions two times 100 µl were removed and applied to Petri dishes containing TSA for the bacteria.
The plates were incubated for bacteria for 2 days at 30 - 35°C.

6.4. Control of inactivation solutions (control 2)

A suspension containing 10^7 - 10^8 CFU / ml was prepared.
10 µl of each microbial suspension and one piece of the test item (about 20 mm x 55 mm, who will be weigh before use) were added in separate 100 ml of inactivation solution and homogenized in a stomacher for 5 minutes and processed further within 15 minutes (= dilution 1; 1:10^4).
1 ml of dilution 1 was added to 9 ml of PBPS solution and mixed thoroughly (= dilution 2; 1: 10^5).
1 ml of dilution 2 was added to 9 ml of PBPS solution and mixed thoroughly (= dilution 3 1: 10^6)..
From each of the 3 dilutions two times 100 µl were removed and applied to Petri dishes containing TSA for the bacteria.
The plates were incubated for bacteria for 2 days at 30 - 35°C.

6.5. Determination of the initial count of organisms (control 3)

A suspension containing 10^7 - 10^8 CFU / ml was prepared.
A microbe carrier (after autoclaving) was inoculated with 10 µl quantities of each microbial suspension separately.
The drop was spread on the carrier using an inoculating loop.
Care was taken not to smear the microbial suspension onto the edge of the microbe carrier.
The microbe carriers were allowed to dry in the air for maximum 30 minutes.
The prepared microbe-carrier were placed in a bottle containing 100 ml of inactivation solution (= dilution 1) and let stand for 15 minutes.
Then they were shaken vigorously for 1 minute and processed further within 15 minutes (= dilution 1; 1:10^4)..
1 ml of dilution 1 was added to 9 ml of PBPS solution and mixed thoroughly (= dilution 2; 1:10^5).).
1 ml of dilution 2 was added to 9 ml of PBPS solution and mixed thoroughly (= dilution 3; 1:10^6).
From each of the 3 dilutions two times 100 µl were removed and applied to Petri dishes containing TSA for the bacteria.
The plates were incubated for bacteria for 2 days at 30 - 35°C.

6.6. Determination of the microbial count after disinfection

A suspension containing 10^7 - 10^8 CFU / ml was prepared
2 specified periods to act were tested: 1 and 5 minutes.
Two microbe carriers (after autoclaving) were inoculated with 10 µl quantities.
The drop was spread on the carrier using an inoculating loop.
Care was taken not to smear the microbial suspension onto the edge of the microbe carrier.
The microbe carriers were allowed to dry in the air for maximum 30 minutes.
For each period of action to be tested, the microbe carriers were dapped with one piece of the test item (about 20 mm x 55 mm, who will be weigh before use) about 5 - 10 times throughout the whole surface and let act for 1 and 5 minutes respectively.
When the period of action was over, the disinfected microbe carriers were placed in separate 100 ml quantities of inactivation solution and let stand for 15 minutes, which corresponds to **assay A**.
The piece of the test item was also placed in separate 100 ml quantities of inactivation solution respectively and homogenized in a stomacher for 5 minutes let stand for 10 minutes, which corresponds to **assay B**, serving as an additional control. The assays A and B were shaken vigorously for 1 minute and processed further within 15 minutes.
From all inactivation solutions separately 1 ml, 10 ml and 89 ml (for assay B 89 ml could not be filtered) quantities were removed and filtered through separate 0.45 µm membrane filters.
Then the filters, with the contaminated side facing upwards, were placed on the appropriate nutrient media plates, taking care that no air bubbles are formed between the filter and the surface.
The plates were incubated with the bacteria for 2 days at 30 - 35°C.
After incubation the number of colonies were counted on each plate. For the result of the total viable count the arithmetical average of the two plates was calculated. The number of CFU was calculated according to the following formula:
Total viable count [CFU/10 µl] = Count [CFU] / Volume plated [10 µl] x dilution used

6.7. Documentation of results

According to the USP, chapter <1227> preferably counts between 25 and 250 CFU for bacterial colonies were taken in account for the calculation to obtain a statistically reliable result.
For less CFU the statistically possible error would be too elevated, as indicated in the table 2 of the chapter <1227>.

6.8. Calculation of the reduction in the microbial count after disinfection

The value from control 3 served as the initial count before disinfection. By comparing this initial count with the microbial count from the total volume of inactivation solution from the disinfected microbe-carriers, the reduction in the count of organisms (stated in log cycles) was obtained.

Here an example:
For organism B => Control 3 yields 4 x 10^7 organisms/microbe-carrier
Thus the initial microbial count amounts to 3 x 10^7 organisms/microbe-carrier.
After disinfection (15 minutes) the count determined is 117 organisms in 100 ml.
Hence the reduction in the microbial count is > 5 log cycles.

6.9. Acceptance criteria for the validation

For a disinfectant to be regarded as sufficiently effective under simulated conditions of use, the following requirements must be fulfilled

Requirements and testing frequencies for suspension tests (for information purpose)
After the specified action period, which is 5 minutes for living bacteria according to the norms EN 1040 [3] the microbial reduction regarding

- Vegetative bacteria must be superior or equal to 5 log10 units according to the norm EN 1040 [3]

For a disinfectant to be regarded as sufficiently effective under simulated conditions of use, the following requirements must be fulfilled according to the norm EN 13697 [4]

- The initial microbial count should be sufficient high in order to demonstrate the required log10 reduction (approx. 10^5 to 10^6 CFU per 10 µl, which corresponds to a TCO of 10^7 to 10^8 CFU / ml)
- After the specified action period, which is 5 minutes for living bacteria according to the norm EN 13697 [5], the microbial reduction regarding living bacteria must be superior or equal to 4 log10 units.

7. Study Results

Tables and figures of results are shown in the corresponding ANNEXES.

The assays of disinfectant efficacy were performed as indicated in chapter 6.

The results for the enumeration of CFU by the filtration method during the assays on the test item are shown in ANNEX 2 in the tables 2 to 6 for the used micro-organisms.

The preparation of the suspension was performed as indicated in chapter 6. The concentration of vegetative cells suspensions was measured using 100 µl of the dilutions of 10^{-4}, 10^{-5} and 10^{-6} which were spread over the surface of each time two Petri-dishes containing TSA.

According to the USP, chapter <1227> preferably counts between 25 and 250 CFU for bacterial colonies are taken in account for the calculation to obtain a statistically reliable result.

For less CFU the statistically possible error would be too elevated, as indicated in the table 2 of the chapter <1227>.

For the calculation of the results, the number of CFU of assay A was added to the number of CFU of assay B. The calculation of the log10 reduction factor was therefore performed on the sum of CFU of assay A and assay B.

The interpretation of the results in terms of log10 reduction was based on rounded values to one figure in order to have the same rounding as specified by the acceptance criteria.

8. Discussion

The overall efficacy of the disinfectant is very satisfying for living *Staphylococcus aureus* MRSA after the action time of 1 minute and 5 minutes
The requirement of superior or equal to 5 log units was fulfilled.

9. Conclusion

As an overall summary of the results obtained it can be concluded that the 2 batches of the test item complies with the acceptance criteria of the norm EN 13697 [5] of superior or equal to 5 log10 reduction with the action time of 1 minute and 5 minutes for living bacteria *Staphylococcus aureus* MRSA.
In this context it has to be mentioned that the norms EN 1040 [3] and EN 13697 [4] recommend an action time of 5 minutes for living bacteria

10. Material and equipment

Any material and equipment used during the study is mentioned in table 14 with the corresponding supplier.

11. References

[1] USP chapter <1072> « Disinfectants and antiseptics »
[2] Guideline of the German Society for Hygiene and Microbiology (DGHM) of 1991
[3] EN 1040 « Chemical disinfectants and antiseptics : Basic bactericidal activity Test method and requirements (phase 1) » (1997)
[4] EN 13697 « Chemical disinfectants and antiseptics — Quantitative non-porous surface test for the evaluation of bactericidal and/or fungicidal activity of chemical disinfectants used in food, industrial, domestic and institutional areas — Test method and requirements without mechanical action (phase 2/step 2) » (2001)
[5] IES Contamination Control Division Recommended Practice IES RP CC023.1 »Microorganisms in Cleanrooms » (1993)
[6] PIC Document PE 002 2 « Recommendation on the Validation of Aseptic Processes (April 2000)
[7] CONFARMA Protocol number 222070803
[8] CONFARMA SOP M 26 « Handling of micro-organisms »
[9] CONFARMA SOP M 37 « Preparation of culture media »
[10] CONFARMA SOP M 38 « Quality control on culture media »
[11] CONFARMA SOP M 56 « Identification of bacteria »

12. Abbreviations used

DGHM : Germany Society for Hygiene and Microbiology
DIS : Disinfectant
TCO : Total count of organisms
TNTC: Too numerous to count
NG: No growth
CFU : Colony forming units
C : Complies
NC : Does not comply
ATCC : American type culture collection

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13. Signatures

Study performed by: (Date/signature)
Study personnel 05.01.2010

Report issued by: (Date/signature)
Study director 04.01.2010

Approved by : (Date/signature)
Quality assurance 05.01.2010

Approved by: (Date/signature)
Study Monitor 4/01/2010

14. Annexes

Annex 1 : Used micro-organisms

Table 1 : Used ATCC reference micro-organisms

Micro-organism	ATCC number	Batch number	Expiry date of the manufacturer	Internal expiry date
Staphylococcus aureus (MRSA)	ATCC 43300	852313	April 2011	November 2011

Annex 2 : Results for the test item

Table 2 : Results of the enumeration of CFU of *Staphylococcus aureus* MRSA for batch A

Staphylococcus aureus	**Dilutions**			**TCO / 10 µl**	
	10^{-4}	**10^{-5}**	**10^{-6}**		
Control 1	TNTC / TNTC	57 /50	3 / 4	5.35×10^5	
Control 2	TNTC / TNTC	43 / 52	7/ 2	4.75×10^5	
Control 3	TNTC / TNTC	40 / 44	3/ 9	4.20×10^5	
	10 ml	**90 ml**		**100 ml**	**Log reduction (A+B)**	**Con-clusion**
		1 ml	**89 ml**			
Assay A 1 minutes	NG	NG	2	2	5.32	C
Assay A 5 minutes	NG	NG	NG	0	5.62	C
Assay B 1 minutes	NG	NG	*	< 1	-	-
Assay B 5 minutes	NG	NG	*	< 1	-	-

* This volume could not be filtered.

Table 3 : Results of the enumeration of CFU of *Staphylococcus aureus* MRSA for batch B

Staphylococcus aureus	**Dilutions**				**TCO / 10 µl**	
	10^{-4}	**10^{-5}**		**10^{-6}**		
Control 1	TNTC / TNTC	57 /50		3 / 4	5.35×10^5	
Control 2	TNTC / TNTC	45 / 36		3 / 6	4.05×10^5	
Control 3	TNTC / TNTC	40 / 44		3 / 9	4.20×10^5	
	10 ml	**90 ml**		**100 ml**	**Log reduction (A+B)**	**Con-clusion**
		1 ml	**89 ml**			
Assay A 1 minutes	NG	NG	1	1	5.62	C
Assay A 5 minutes	1	NG	2	3	5.14	C
Assay B 1 minutes	NG	NG	*	< 1	-	-
Assay B 5 minutes	NG	NG	*	< 1	-	-

* This volume could not be filtered.

Annex 3 : Material and equipment

Table 6 : Material used during the study

Instruments :	**Manufacturer**	**Internal number**
-80°C deep freezer	Dairei	735
Laminar Flow hood ISO 14644 class 5	Woetho	1
Autoclave	Fedegari FVA3/AKL	636
pH meter	Mettler-Toledo	271
Vortex	VWR	921
Incubator	Gallenkamp Fisher Scientific	137 / 352
Pipettes	**Manufacturer**	**Internal number**
Micropipettes	Gilson / Ependorf	278 / 326 / 739
Culture media:	**Manufacturer**	**Article number**
Casein Soybean Digest Agar	Merck	1.05458

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Buffer:	**Manufacturer**	**Article number**
Stock solution:		
KH2PO4	LPCR	1.04873.0250
NaOH	LPCR	31627290
Stock solution diluted to 1 : 80 with 0.05% Tween 80	Acros	278630010
Inactivation solution:	**Manufacturer**	**Article number**
3 g %Tween 80,	Acros	278630010
0.3 g% Lecithin,	Fluka	61755
0.11 g %L-Histidine,	Merck	4351
0.5 g% Na-thiosulfat	Merck	65125