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FDA C. F. numbers 9614423 and 9615669
Etablissement pharmaceutique N° M 07/173
ISO 9001, ISO 17025, ISO 14001, OHSAS 18001

Study report number 222070803

23/02/2009

Study report

Title	Study of disinfectant efficacy by the microbe carrier test under simulated conditions of use
Test item	Cyber Clean ® with Benzalkonium Chloride + Didecyl dimethyl Ammonium Chloride CAS 7173 – 51 - 5
Batch number	KR08/004
Test facility	CONFARMA FRANCE SARL Zone Industrielle Rue du Canal d'Alsace F-68490 HOMBURG France
Sponsor	Joker AG Industriezone Postfach 69 CH 3210 Kerzers

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1. General information

1.1. Sponsor

Joker AG
Industriezone
Postfach 69
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. B 68-144-03 of the French Republic dated November 2nd 2004, 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 was classified to be compliant to the Good Manufacturing Practice as declared by the certificate number HPF/FR/210/2007 from the "Agence Française de Sécurité Sanitaire des Produits de Santé" dated October 1st 2007.

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, 222070803 was attributed according to the registration systems described in the corresponding CONFARMA procedures.

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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 / N. Weber, Microbiologist
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 222070803 [8] which is based on the guideline of the German Society for Hygiene and Microbiology (DGHM) from 1991 [2] the norms EN 1040 « Chemical disinfectants and antiseptics Basic bactericidal activity Test method and requirements (phase 1) » [3] and EN 1275 « Chemical disinfectants and antiseptics : Basic fungicidal activity : test method and requirements (phase 1) » [4] 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) » [5].

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], EN 1275 [4] and EN 13697 [5].

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 : August 4th 2008 (the date the study monitor signed the validation protocol)

Experimental starting date : August 18th 2008

Experimental completing date : December 18th 2008

Study completion date : December 19th 2008

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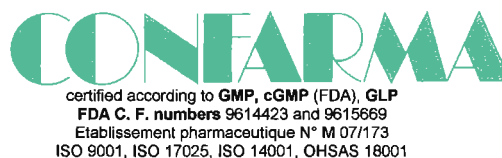
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1.9. Archiving

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 4 log₁₀ reduction with the action time of 1 minute and 5 minutes for living bacteria *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. In this context it has to be mentioned that the norms EN 1040 [3] and EN 13697 [5] recommend an action time of 5 minutes for living bacteria.

For the yeast *Candida albicans*, the test item fulfilled the acceptance criteria after 1 minute and 5 minutes of action time.

For the mould *Aspergillus niger*, which was used within the assay under the form of fungal spores, the test item did not comply with the acceptance criteria after 1 minute and after 5 minutes of action time.

The overall efficacy of the disinfectant is very satisfying for living bacteria as well as yeasts after the action time of 1 minute and 5 minutes, respectively.

For *Aspergillus niger* for which no significant reduction was observed, it can be postulated that this mould, used under the form of fungal spores disposes of a very effective resistance which allows survival even in the presence of the disinfectant.

Therefore the norms EN 1275 [4] and EN 13697 [5] both indicate an action time of 15 minutes in order to allow the disinfectant to be also effective against fungal spores.

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 10⁷ to 10⁸ 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 ® with Benzalkonium Chloride + Didecyl dimethyl Ammonium Chloride CAS 7173 – 51 - 5 arrived at the CONFARMA laboratories on July 22nd 2008 and got the analysis number 222070803 according to the registration systems.

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The test item provided by the sponsor was specified as follows : Cyber Clean ® with Benzalkonium Chloride + Didecyl dimethyl Ammonium Chloride CAS 7173 – 51 – 5, batch number KR08/004.

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 $20 \pm 5^{\circ}\text{C}$).

5. Test system

6 different micro-organisms were employed in order to cover the whole spectrum of typically identified micro-organisms on surfaces.

Among the 5 micro-organisms, 3 living bacteria were used

Escherichia coli : Gram-negative, Oxidase negative rods

Pseudomonas aeruginosa : Gram-negative, Oxidase positive rods

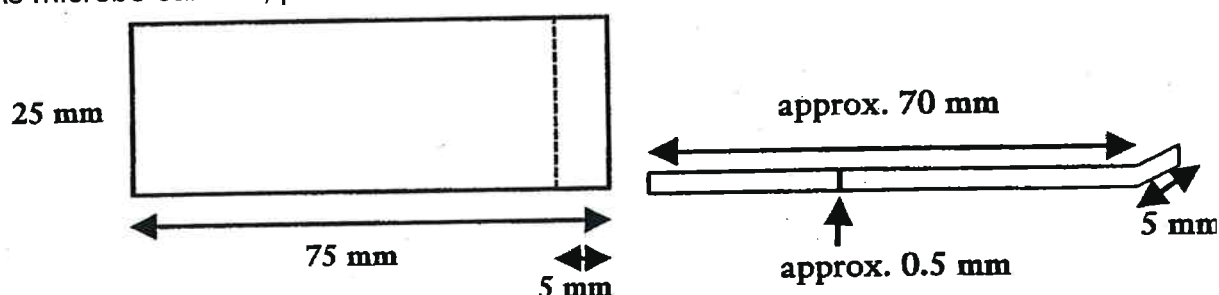
Staphylococcus aureus Gram-positive, Catalase positive cocci

In order to cover yeasts *Candida albicans* was employed and for moulds *Aspergillus niger* spores were used.

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).

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6.2. Preparation of the suspensions of test organisms

6.2.1. Bacteria and yeasts

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 for bacteria and 48h for yeasts. 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 to 10^8 micro-organisms per ml.

6.2.2. Suspensions of fungal spores

In order to prepare the fungal suspension, material from the stock culture was inoculated onto SDA and incubated at room temperature for 7 - 10 days.

The fungal culture was washed off into a test tube using approx. 5 ml of NaCl solution with 0.1 % Tween and the mixture was centrifuged at 3000 rpm for 10 minutes. The supernatant solution was discarded except for approx. 1 ml.

The concentration of the spore suspension is approx. 10^9 to 10^{10} fungal spores per ml.

6.3. Determination of microbial growth (control 1)

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 and SDA for the yeasts and moulds.

The plates were incubated for 2 days at 30 - 35°C, and for 2 – 5 days for the yeasts and moulds at 20 - 25°C.

6.4. Control of inactivation solutions (control 2)

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 shaken 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 and SDA for the yeasts and moulds.

The plates were incubated for 2 days at 30 - 35°C, and for 2 – 5 days for the yeasts and moulds at 20 - 25°C.

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

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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 x 10⁴).

1 ml of dilution 1 was added to 9 ml of PBPS solution and mixed thoroughly (= dilution 2 : 1 x 10⁵).

1 ml of dilution 2 was added to 9 ml of PBPS solution and mixed thoroughly (= dilution 3 : 1 x 10⁶).

From each of the 3 dilutions two times 100 µl were removed and applied to Petri dishes containing TSA for the bacteria and SDA for the yeasts and moulds.

The plates were incubated for 2 days at 30 - 35°C, and for 2 - 5 days for the yeasts and moulds at 20 - 25°C.

6.6. Determination of the microbial count after disinfection

2 specified periods to act were tested: 1 and 5 minutes.

Two microbe carriers (after autoclaving) were inoculated with 10 µl quantities of each microbial suspensions 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.

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 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, and for 2 - 5 days for the yeasts and moulds at 20 - 25°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

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6.7. Documentation of results

According to the USP, chapter <1227> preferably counts between 25 and 250 CFU for bacterial colonies (between 10 and 100 fungal 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 (10 + 90 ml) 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 4×10^7 organisms/microbe-carrier

Thus the initial microbial count amounts to 3×10^7 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 and 15 minutes for yeasts and moulds according to the norms EN 1040 [3] and EN 1275 [4], the microbial reduction regarding

- Vegetative bacteria must be superior or equal to 5 log₁₀ units according to the norm EN 1040 [3]
- Vegetative cells of yeasts and fungal spores must be superior or equal to 4 log₁₀ units according to the norm EN 1275 [4]
- Unless a disinfectant is declared to have a sporicidal effect on fungal spores, the results for *Aspergillus niger* serve for information purposes only.

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 [5]

- 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.
- After the specified action period, which is 15 minutes for yeasts and moulds according to the norm EN 13697 [5], the microbial reduction regarding yeasts and moulds must be superior or equal to 3 log₁₀ units.

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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 spore suspension was performed as indicated in chapter 6. The concentration of vegetative cells and of the spore 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 or SDA, respectively.

According to the USP, chapter <1227> preferably counts between 25 and 250 CFU for bacterial colonies (between 10 and 100 fungal 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

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 4 log10 reduction with the action time of 1 minute and 5 minutes for living bacteria *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. In this context it has to be mentioned that the norms EN 1040 [3] and EN 13697 [5] recommend an action time of 5 minutes for living bacteria.

For the yeast *Candida albicans*, the test item fulfilled the acceptance criteria after 1 minute and 5 minutes of action time.

For the mould *Aspergillus niger*, which was used within the assay under the form of fungal spores, the test item did not comply with the acceptance criteria after 1 minute and after 5 minutes of action time.

During the assays, an insufficient penetration of the disinfectant contained in the matrix of the test item onto the surfaces of the microbe carriers was observed.

Therefore it is recommended to raise the penetration of the disinfectant on the surfaces by either a higher concentration of alcohol and/or a higher concentration of surfactants contained in the test item.

9. Conclusion

The overall efficacy of the disinfectant is very satisfying for living bacteria as well as yeasts after the action time of 1 minute and 5 minutes, respectively.

For *Aspergillus niger* for which no significant reduction was observed, it can be postulated

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that this mould, used under the form of fungal spores disposes of a very effective resistance which allows survival even in the presence of the disinfectant.
Therefore the norms EN 1275 [4] and EN 13697 [5] both indicate an action time of 15 minutes in order to allow the disinfectant to be also effective against fungal spores.

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 1275 « Chemical disinfectants and antiseptics : Basic fungicidal activity Test method and requirements (phase 1) » (1997)
- [5] 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)
- [6] IES Contamination Control Division Recommended Practice IES RP CC023.1 « Microorganisms in Cleanrooms » (1993)
- [7] PIC Document PE 002 2 « Recommendation on the Validation of Aseptic Processes (April 2000)
- [8] CONFARMA Protocol number 222070803
- [9] CONFARMA SOP M 26 « Handling of micro-organisms »
- [10] CONFARMA SOP M 37 « Preparation of culture media »
- [11] CONFARMA SOP M 38 « Quality control on culture media »
- [12] 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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Study report number 222070803

23/02/2009

13. Signatures

Issued by: (Date/signature)

27. 02. 2009

Report issued by: (Date/signature)
Director of study

23. 02 2009

Approved by: (Date/signature)
Test facility management

27.02.2009

Approved by : (Date/signature)
Quality assurance

22. 02. 2009

N. WEBER

Approved by: (Date/signature)
Study Monitor



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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
<i>Escherichia coli</i>	8739	483344	5 th June 2008	June 2010
<i>Pseudomonas aeruginosa</i>	9027	484942	21 st July 2008	June 2010
<i>Staphylococcus aureus</i>	6538	485982	21 st July 2008	June 2010
<i>Candida albicans</i>	10231	443152	10 th June 2008	June 2010
<i>Aspergillus niger</i> spores	16404	392722	10 th June 2008	June 2010

Annex 2 : Results for the test item

Table 2 : Results of the enumeration of CFU of *Staphylococcus aureus*

<i>Staphylococcus aureus</i>	Dilutions				TCO / 10 µl	
	10 ⁻⁴	10 ⁻⁵		10 ⁻⁶		
Control 1	TNTC / TNTC	186 / 171		18 / 21	1.79 x 10 ⁶	
Control 2	TNTC / TNTC	169 / 163		17 / 15	1.66 x 10 ⁶	
Control 3	TNTC / TNTC	68 / 67		7 / 10	6.75 x 10 ⁵	
	10 ml	90 ml		100 ml	Log reduction (A+B)	Con-clusion
		1 ml	89 ml			
Assay A 1 minutes	2	NG	NG	2	5.53	C
Assay A 5 minutes	NG	NG	NG	< 1	> 5.83	C
Assay B 1 minutes	NG	NG	NG	< 1	-	-
Assay B 5 minutes	NG	NG	NG	< 1	-	-

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Table 3 : Results of the enumeration of CFU of *Pseudomonas aeruginosa*

<i>Pseudomonas aeruginosa</i>	Dilutions			TCO / 10 µl		
	10 ⁻⁴	10 ⁻⁵	10 ⁻⁶			
Control 1	TNTC / TNTC	TNTC / TNTC	251/236	2.43 x 10 ⁷		
Control 2	TNTC / TNTC	TNTC / TNTC	312/336	3.24 x 10 ⁷		
Control 3	TNTC / TNTC	TNTC / TNTC	47/50	4.85 x 10 ⁶		
	10 ml	90 ml		100 ml	Log reduction (A+B)	Con-clusion
		1 ml	89 ml			
Assay A 1 minutes	NG	NG	NG	< 1	> 6.69	C
Assay A 5 minutes	NG	NG	NG	< 1	> 6.69	C
Assay B 1 minutes	NG	NG	NG	< 1	-	-
Assay B 5 minutes	NG	NG	NG	< 1	-	-

Table 4 : Results of the enumeration of CFU of *Escherichia coli*

<i>Escherichia coli</i>	Dilutions			TCO / 10 µl		
	10 ⁻⁴	10 ⁻⁵	10 ⁻⁶			
Control 1	TNTC / TNTC	TNTC / TNTC	199/198	1.99 x 10 ⁷		
Control 2	TNTC / TNTC	TNTC / TNTC	283/259	2.71 x 10 ⁷		
Control 3	TNTC / TNTC	TNTC / TNTC	112/118	1.15 x 10 ⁷		
	10 ml	90 ml		100 ml	Log reduction (A+B)	Con-clusion
		1 ml	89 ml			
Assay A 1 minutes	NG	NG	NG	< 1	> 7.06	C
Assay A 5 minutes	NG	NG	NG	< 1	> 7.06	C
Assay B 1 minutes	NG	NG	NG	< 1	-	-
Assay B 5 minutes	NG	NG	NG	< 1	-	-

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Table 5 : Results of the enumeration of CFU of *Candida albicans*

<i>Candida albicans</i>	Dilutions			TCO / 10 µl		
	10 ⁻⁴	10 ⁻⁵	10 ⁻⁶			
Control 1	TNTC / TNTC	76 / 52	3 / 1	6.40 x 10 ⁵		
Control 2	TNTC / TNTC	63 / 48	6/ 3	5.55 x 10 ⁵		
Control 3	TNTC / TNTC	33 / 41	3 / 6	3.70 x 10 ⁵		
	10 ml	90 ml		100 ml	Log reduction (A+B)	Con- clusion
		1 ml	89 ml			
Assay A 1 minutes	NG	NG	NG	< 1	> 5.57	C
Assay A 5 minutes	NG	NG	NG	< 1	> 5.57	C
Assay B 1 minutes	NG	NG	NG	< 1	-	-
Assay B 5 minutes	NG	NG	NG	< 1	-	-

Table 6 : Results of the enumeration of CFU of *Aspergillus niger*

<i>Aspergillus niger</i>	Dilutions			TCO / 10 µl		
	10 ⁻⁴	10 ⁻⁵	10 ⁻⁶			
Control 1	TNTC / TNTC	33 / 18	6 / 3	2.55 x 10 ⁵		
Control 2	TNTC / TNTC	34 / 36	4/ 3	3.50 x 10 ⁵		
Control 3	TNTC / TNTC	34 / 26	4/ 3	3.00 x 10 ⁵		
	10 ml	90 ml		100 ml	Log reduction (A+B)	Con- clusion
		1 ml	89 ml			
Assay A 1 minutes	20	3	101	124	1.59	NC
Assay A 5 minutes	TNTC	34	TNTC	≅ 3.40 x 10 ³	1.94	NC
Assay B 1 minutes	TNTC	77	TNTC	≅ 7.70 x 10 ³	-	-
Assay B 5 minutes	TNTC	5	37	66	-	-

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Annex 3 : Material and equipment

Table 7 : 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 / 285 / 10 352
Pipettes	Manufacturer	Internal number
Micropipettes	Gilson / Ependorf	278 / 326 / 739
Culture media:	Manufacturer	Article number
Casein Soybean Digest Agar	Merck	1.05458
Sabouraud 4% Dextrose Agar	Merck	1.05438
Buffer:	Manufacturer	Article number
Stock solution: KH ₂ PO ₄ NaOH	LPCR LPCR	1.04873.0250 31627290
Stock solution diluted to 1 : 80 with 0.05% Tween 80	Acros	278630010
Inactivation solution:	Manufacturer	Article number
3 g %Tween 80, 0,3 g% Lecithin, 0,11 g %L-Histidine, 0,5 g% Na-thiosulfat	Acros Fluka Merck Merck	278630010 61755 4351 65125