Final Report S-2018-00224 AM

Determination of antibacterial activity of textile products on GERMAGIC (ISO 20743:2013)

Study program: S-2018-00224 AM

Contract n: M3O820170448-03

Client Code N°: 2018-753-121

Sponsor:
CHIAPHUA INDUSTRIES LIMITED
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Study Monitor:
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Cheshire
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Test Facility:
Eurofins Biolab Srl
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Italy

Test item: GERMAGIC

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Study Director: ..... (F. Faccioli) Released on: April 12th 2018

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COMPLIANCE WITH GOOD LABORATORY PRACTICE

I the undersigned declare that the studies described in this report have been conducted under my supervision and in compliance with the following standards of Good Laboratory Practice:

- OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring - OECD principles of Good Laboratory Practice (as revised in 1997) – Environment Directorate – Organisation for Economic Co- Operation and Development , Paris 1998.

- Legislative decree n. 50 of March the 2nd, 2007. Enforcement of Community Directives 2004/9/CE e 2004/10/CE, concerning the inspection and verification of Good Laboratory Practice and the drawing of the legislative, regulatory and administrative dispositions relative to the application of Good Laboratory Practice rules, to the control of their application on the assays performed on the chemical substances (GU n.86 of April the 13th, 2007).

- Certification N. 2017/16 released by the Italian Ministry of Health on May 11th 2017 authorizing Eurofins Biolab S.r.l. to perform analyses in compliance with the principles of good laboratory practices (http://www.eurofins.it).

There were no circumstances that may affect the quality or integrity of the study.

Study Director
(F. Faccioli)

April 12th, 2018
Date

QUALITY ASSURANCE STATEMENT

The study was assessed for compliance with the approved study program and the Standard Operating Procedures of Eurofins Biolab S.r.l.
The study and/or the test facility were periodically inspected by the Quality Assurance unit according to the corresponding SOPs. These inspections and audit were carried out by the Quality Assurance unit, personnel independent of staff involved in the study.

The undersigned hereby certifies the dates on which the inspections have been carried out and reported to the Director of the Study and to Eurofins Biolab's S.r.l. Management:

QAU INSPECTIONS	
PHASE	**DATE**
Experimentation:	
-Audit process-based	November 24th 2017
-Audit study-based	//
Documentation:	
- Study program	February 13th, 2018
- Raw data	April 12th 2018
- Final report	April 12th 2018

This report accurately reflects the raw data.

QA GLP
M.Rossetti

April 12th 2018
Date

SUMMARY

On the test item "GERMAGIC" a study has been conducted in order to determine its antimicrobial effectiveness against bacterial strains, according to ISO 20743:2013 Absorption Method.
To this aim, the Sponsor sent to Eurofins Biolab S.r.L, specimens treated (T) with antibacterial agent "GERMAGIC". Untreated controls without antibacterial agent (Control (C)), have been provided by Eurofins Biolab Srl.
For this purpose the following microorganisms were used to verify antibacterial effectiveness:
Staphylococcus aureus ATCC 6538P
Klebsiella pneumoniae *ATCC 4352 - DSM789*

For each test strain, the test was performed on test item (treated) and on untreated controls (negative control).
Specimens have been prepared with a mass of 0,40 g ± 0,05 g and with suitable size then have been insert into separate sterile specimens containers.
For each test strain: six specimens have been inoculated with test strains, three of untreated controls specimens and three of the test item specimens have been used for t_0, immediately after inoculation and the remaining specimens (three of untreated controls specimens and three of the test item specimens) have been used for the contact time of 24 h.

On the basis of the obtained results, interpreted according to ISO 20743:2013, the test item "GERMAGIC" **has strong antibacterial activity,** because it has an Antibacterial value ≥4 Log for each tested strain in experimental condition adopted.

See Experimental Report S-2018-00224 AM for more details.

INTRODUCTION

A study was conducted on behalf of CHIAPHUA INDUSTRIES LIMITED, in order to determine the antibacterial activity of the test item, according to ISO 20743:2013.
The study was performed at the Test Facility Eurofins Biolab S.r.l. of Vimodrone (MI) – via B. Buozzi n. 2 (Italy).

EXPERIMENTATION	*START*	*END*	*RESEARCHER*
Determination of antibacterial activity	February 20th, 2018	April 12th, 2018	B. Piazza

REFERENCE

ISO 20743:2013: Textiles — Determination of antibacterial activity of textile products

FILING

The study program, the final report, amendments (if present) and all raw data are filed in the archives of Eurofins Biolab S.r.l. for ten years after the issuing of the final report.
At the end of the study the residual sample has not been kept because it was completely used for the test.
At the end of the conservation period, the Sponsor may request an extension of the conservation of all or part of the documents/products for a further period, or their restitution. A suitable agreement shall be drafted in this case.

PROCEDURES

All procedures used during this study are recorded in the GLP Test Facility Eurofins Biolab S.r.l.

TEST ITEM

The test product consists of a biocide and antimicrobials for HEPA Filter.

Product	GERMAGIC
Active ingredients	Chlorine dioxide / Sodium chlorite
Concentration of Active ingredients	<3%
Stability	1 year
Storage	Room temperature, protected from light

ANALYZED SAMPLE

The test material, representative of the test item, consists of white pad contained into a transparent plastic bag.

Batch	GM15012018
Manufacturing date	15/01/2018
Expiry date	15/01/2019
CoA	Not Provided
Receiving	EUITVI-97849
Date	January 17th, 2018
#ID	ACE-2018-00005259

The test item and the information concerning the test item were provided by the Sponsor.
All data related to the test item are under the responsibility of the Sponsor and have not been verified by the test facility.

Experimental Report S-2018-00224 AM– Determination of antibacterial activity

ASSAY SYSTEM

Identification of the test strains
Cultures of following microorganisms have been used:
Staphylococcus aureus ATCC 6538
Klebsiella pneumoniae *ATCC 4352 - DSM789*

Preparation of test suspension
For each test strain:
Day prior test, from fresh stock culture (not more 7 days aged), a subculture (A) into a 100 ml Erlenmeyer flask(or equivalent container) containing 20 ml of TSB has been prepare then incubated at 37±1°C for 18-24 h with shaking (under dynamic condition).
In day of test, a subculture by adding 0,4 ml (that contains 1-3 x 10^8 cfu/ml, the concentration has been verified by pour plate method) in bacteria from subculture (A), into a 100 ml Erlenmeyer flask (or equivalent container) containing 20 ml of TSB has been prepared then incubated at 37±1°C for 3±1h with shaking (under dynamic condition).

MEDIA AND REAGENTS

The validity of media and reagents have been checked before starting the analyses
- Tryptone soya broth (TSB)
- Tryptone Soy Agar (TSA)
- Peptone water (H2O Peptonata)
- Neutralizing solution (Neu CEN)

EQUIPMENTS

The validity of instruments and equipment have been checked before starting the analyses.
Ordinary microbiology laboratory equipment and in particular:
Thermostat @ 37°±1°C
Balance

SAMPLE PREPARATION

Six test specimens of test item: GERMAGIC (T) and six specimens of the untreated controls (C) with a mass of 0,40 g ± 0,05 g and suitable size have been prepared and insert in appropriate containers.

EXPERIMENTAL DESIGN

Absorption method

Preparation of test inoculum
The test strains suspension have been adjusted at the concentration of 1-3 x 10^5 cfu/ml and verified by pour plate method in TSA at 37±1°C for 18-24 h.

Inoculation of test specimens
For each test strain:
0.2 ml of the inoculum prepared have been accurately distributed by micropipette at several points on the specimens.
Three of the control specimens and three of the test item specimens have been be used for t_0, immediately after inoculation. The remaining specimens have been used for the contact time of 24 h.

Shake-out after inoculation (t_0)
For each test strain:
20 ml of appropriate neutralizing solution have been added to three control specimens containers and to three testing specimens, then shake out by vortex mixer for 5 s × 5 cycles.
The viable bacterial count has been verified by proper serial ten-fold dilution in Peptone Water by inclusion method in TSA at 37°C±1°C for 24-48h.
After incubation, the colonies in Petri dishes have been counted and number recorded.

Incubation
The remaining specimens have been incubated at 37°C±1°C for 24h.

Shake-out after incubation (t_{24})
At the end of the contact time (t_{24}), the specimens have been processed like t_0 specimens.

ASSAY VALIDITY CRITERIA

When conditions are satisfied, the test is judged to be effective.
a) The inoculum concentration shall be 1×10^5 cfu/ml to 3×10^5 cfu/ml;
b) The difference in common logarithm in extremes of the number of bacteria, for the three control specimens immediately after inoculation and after incubation, respectively, shall be <1;
c) The growth value (F) obtained shall be ≥1,0 in the plate count method.

$$F = lgCt - lgC0$$

Where:
F is the growth value on the control specimen;
lg Ct is the common logarithm of arithmetic average of the numbers of bacteria, obtained from three control specimens after incubation;
lg Co is the common logarithm of arithmetic average of the numbers of bacteria, obtained from three control specimens immediately after inoculation.

To validate the test, it is necessary **verify the efficacy of neutralizer** as the difference in logarithm of extremes for the specimens of the treated sample that shall be <2 after inoculation and incubation.

The test has been judged to be effective.

CALCULATION AND EXPRESSION OF THE RESULTS

The **bacteria concentration (cfu/ml)** in the solution has been calculated according to the following formula:

$$C_B = Z \times R$$

where
C_B is the bacteria concentration, in Colony Forming Units per millilitre (cfu/ml);
Z is the average value of two Petri dishes in cfu/ml of inoculum;
R is the dilution rate.

The **bacteria concentration (cfu/specimen)** has been calculated according to the following formula:

$$M = C_B \times 20$$

where
M is the number of bacteria per specimen;
C_B is the bacteria concentration obtained;
20 is the volume of the shake-out solution, in millilitres (ml).

To obtain the **antibacterial activity value** according to the following formula, in case of $C_0 > T_0$, substitute C_0 for T_0.

$$A = (\lg C_t - \lg C_0) - (\lg T_t - \lg T_0) = F - G$$

where
A is the antibacterial activity value;
F is the growth value on the control specimen ($F = \lg C_t - \lg C_0$);
G is the growth value on the antibacterial testing specimen ($G = \lg T_t - \lg T_0$)
$\lg T_t$ is the common logarithm of arithmetic average of the numbers of bacteria, obtained from three antibacterial testing specimens after an 18 h to 24 h incubation;
$\lg T_0$ is the common logarithm of arithmetic average of the numbers of bacteria, obtained from three antibacterial testing specimens immediately after inoculation.

INTERPRETATION OF RESULTS

Efficacy of Antibacterial Activity
From the testing result, the efficacy of antibacterial property of the test item can be considered as shown in Table:

Efficacy of antibacterial property	Antibacterial value: A
Significant	$2 \leq A < 3$
Strong	$A \geq 3$

RESULTS

The assay validity criteria were satisfied.

Contact time **24h**	***Staphylococcus aureus*** **ATCC 6538P**	***Klebsiella pneumonie*** ***ATCC 4352 - DSM789***
Inoculum (cfu/ml)	1.8×10^5	2.9×10^5
Growth value of control specimens (**F**)	1.7	1.3
Growth value of test item specimens (**G**)	-2.6	-2.7
Antibacterial Activity value (**A**) (A = F - G)	4.3	4.0

DEVIATION

No deviation has been recorded from study program.

CONCLUSIONS

On the basis of the obtained results, interpreted according to ISO 20743:2013, the test item "GERMAGIC" **has strong antibacterial activity,** because it has an Antibacterial value ≥4 Log for each tested strain in experimental condition adopted.

ADDENDA

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