To Mr Breuil

Test Report

Measurement of antibacterial activity on one reference according to adapted standard ISO 22196:2011= JIS E2801.

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This document contains 6 pages

1. Introduction

the client requests CONIDIA to test the antibacterial efficacy of one reference. The ISO22196:2011 standard (=JIS Z2801) on 3 strains of bacteria: *Escherichia coli* (Gram-), *Staphylococcus aureus* (Gram+) and *Staphylococcus aureus MRSA* (Gram+) is recommended to quantify the antibacterial activity.

CONIDIA is an **ISO9001: 2015** certified laboratory working on the relationships between microorganisms and materials. Many resistance tests are carried out in our laboratory (ISO846, ASTM D3273…). In addition, CONIDIA is an accredited CIR laboratory for the period 2019-2021.

2. Samples

The sample was received at the laboratory the 26.07.2021 under the reference O126. The references of the samples are:

Customer Reference	Conidia Reference
Decochoc – Decoclean - Decosmic- Decowood- Decotrend	DECO

3. Material and Method

3.1. Lab works

The analyzes were carried out from 08/03/2021 to 08/09/2021and from 09/05/2021 to 09/17/2021.

The protocol is carried out with 3 strains of bacteria (*Escherichia coli*, *Staphylococcus aureus* and *Staphylococcus aureus* MRSA). These strains exhibit the characteristics described below.

3.2. Strains reference

CONIDIA reference	International reference	Strain	Gram
B011	CIP 54.127	*Escherichia coli*	negative
B025	CIP 4.83	*Staphylococcus aureus*	positive
B294	DSM 11 729	*Staphylococcus aureus MRSA*	positive

3.3. *Procedure*

Test is performed according to standard ISO22196:2011:

-Pre-culture of both bacteria on slant culture medium at 30°C during 48 to 72 hours.

-Preparation of specimens by cleaning with ethanol 70%.

-Preparation of inoculum by dilution in 1/500 Nutrient Broth (NB) to obtain between 2.5 10^5 and 1.0 10^6 bacteria/ml.

-Inoculation of test specimens (0.4 ml for each specimen). Each test is tripled.

-Covering of the specimen with a PET cover film.

-Incubation during (24h ±1) hours at (35 ± 1°C) and not less that 90% of humidity.

-Recovery of bacteria in 30 ml of SCLPD broth.

-Suspension-dilution in phosphate buffer and place 0.1 ml of each suspension-dilution in Petri dish filled with 15 ml of PCA.

-Quantification of bacteria and determination of their viability.

The same test is carried out in parallel on a stainless steel control and the results make it possible to quantify the possible contribution of the antibacterial treatment to bacterial viability.

4. *Results*

4.1. Results of tests with *Escherichia coli*

After culture, the results show that the solution of *Escherichia coli* used for this test is calibrated at 9.3 10^5 CFU/ml.

Sample	Number of CFU/sample	
	Initial contamination	*After 24 hours of contact*
DECO	5.8 10^5	<17
Control	4.5 10^5	8.8 10^6

Table 1: Results of culture of *Escherichia coli* before and after contact with test specimens. The results are the averages of 3 trials.

4.2 Results of tests with *Staphylococcus aureus*

After culture, the results show that the solution of *Staphylococcus aureus* used for this test is calibrated at 6.3 10^5 CFU/ml.

Sample	Number of CFU/sample	
	Initial contamination	*After 24 hours of contact*
DECO	2.7 10^5	9.7 10^4
Control	3.3 10^5	2.5 10^6

Table 2: Results of culture of *Escherichia coli* before and after contact with test specimens. The results are the averages of 3 trials.

4.3 Results of tests with *Staphylococcus aureus MRSA*

After culture, the results show that the solution of *Staphylococcus aureus MRSA* used for this test is calibrated at 1.4 10^6 CFU/ml.

Sample	Number of CFU/sample	
	Initial contamination	*After 24 hours of contact*
DECO	4.2 10^5	2.2 10^5
Control	4.3 10^5	6.0 10^6

Table 3: Results of culture of *Staphylococcus aureus MRSA* before and after contact with test specimens. The results are the averages of 3 trials.

All the results obtained make it possible to validate the test. Among the criteria to be verified:

- The number of bacteria deposited on the untreated sample must be between 1.10^5 and 4.10^5 bacteria for both species. In the case of our test, the number of bacteria deposited is slightly higher with 1.10^6 CFU/sample for both bacteria. Nevertheless, this number of deposited bacteria greater than that recommended do not challenge the test validity.

- The logarithm of the number of bacteria recovered immediately after inoculation on the untreated control should satisfy the following requirement:

$$(L_{max} - L_{min})/L_{mean} \leq 0.2$$

Where

Lmax is the common logarithm (i.e. base 10 logarithm) of the maximum number of viable bacteria found on a specimen;
Lmin is the common logarithm of the minimum number of viable bacteria found on a specimen;
Lmean is the common logarithm of the mean number of viable bacteria found on the specimens.

Results are compiled in the table below:

	Escherichia coli	*Staphylococcus aureus*	*Staphylococcus aureus MRSA*
Control	L=0.01	L=0.03	L=0.01

Table 4: Value of L for the different combinations of bacteria and untreated material.

The values of L available for this test are valid.

- Finally, the number of bacteria after 24 hours incubation should not be < 1.10^3 on the untreated sample (**Control**). In the case of this test, the number of bacteria after 24 hours of incubation is around 2.5 10^6 CFU/sample for *Staphylococcus aureus*, 6.0 10^6 CFU/sample for *Staphylococcus aureus* MRSA and 8.8 10^6 CFU/sample for *Escherichia coli*, respectively. These two values are much greater than 1.0 10^3 validating the test.

The calculations of the antibacterial efficiencies are given according to the following formula:

$$R = (U_t - U_0) - (A_t - U_0) = U_t - A_t$$

Where

R is the antibacterial activity;

U0 is the average of the common logarithm of the number of viable bacteria, in cells/sample, recovered from the untreated test specimens immediately after inoculation;

Ut is the average of the common logarithm of the number of viable bacteria, in cells/sample, recovered from the untreated test specimens after 24 h;

At is the average of the common logarithm of the number of viable bacteria, in cells/sample, recovered from the treated test specimens after 24 h.

The R results are given on the table below:

	Escherichia coli	*Staphylococcus aureus*	*Staphylococcus aureus MRSA*
DECO	>5.7	1.4	1.4

Table 5: R values on the specimen after 24 hours of incubation.

5. Conclusions et interprétations.

The tests were carried out according to the protocol of the ISO22196:2011 standard. The results are only valid for the references tested.

As part of the test with the DECO reference, a slight difference in bacterial concentration at T+24h is visible between this reference and the control (stainless steel disc) on the strains *Staphylococcus aureus* and *Staphylococcus aureus* MRSA. This confirms a bacteriostatic activity of the DECO material towards these two strains.

On the other hand, during tests with the DECO reference, a reduction in the viable bacterial flora is visible on *Escherichia coli* confirming bactericidal activity (R>5.7) on this strain.

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