Humongous Labs
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(A Drug & Cosmetic Dept. Approved, ISO 9001: 2015, 45001:2018 & 14001: 2015 Certified Laboratory)

TEST REPORT

Antimicrobial Efficacy Test for Disinfectant

Date: 22/08/2023

Name of the Costumer	Ecocare Technologies Pvt. Ltd.
Address	Add. - A-176, Sector 83, Noida-201305, India.
Contact Person	Mr. Arvind Verma
Samples	Sample A: EKo Power CF 312 Surface Sample B: EKo Power 7 CD Sample C: EKo Power PAA Sterized Forte 15 Sample D: Hand San
Batch No	N.S.
Date of Sample receipt	22/08/2023
Date of Analysis	22/08/2023
Study Conducted by	Priya Tewari

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Note:
1. The results listed refer only the tested samples and applicable parameter. Endorsement of products is neither inferred nor implied.
2. Sample (s) not drawn by us. Total liability of our analytical division is limited to the sample supplied and/or invoiced amount.
3. This report is not be reproduced wholly or in part and cannot be use as an evidence in the court of law and should not be used in any advertising media without special permission in writing.
4. Sample will be destroyed after one month from the date of issue of test certificate unless otherwise specified.
5. Sample has been tested as per latest/valid version of Standard. In case of MU is having an impact on statement of conformity, decision rule will be applied.

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Title: Antimicrobial Efficacy Test for Disinfectant

1. Information of the study:

1.1 Antimicrobial Efficacy Test for Surface Disinfectant.

1.2 Study number: : HLF/2023822

1.3 Identity of the Customer- Eco care

Regd. Office: Ecocare Technologies Pvt. Ltd.
Add.- A-176, Sector 83, Noida-201305, India.

1.4 Identity of the persons in charge of the study
Manoj Kumar (Person In Charge)
Humongous Labs (OPC) Pvt. Ltd.

1.5 Realization Schedule
Reception's date of samples and supplies: 22/08/2023
Beginning of the study: 22/08/2023
End of the study: 29/08/2023

1.6 Study File

A study file opened under the following Study no.

It encompasses all the study relevant documents: correspondence study procedure, Study procedure, price estimation, uncorrected data, amendments, study report.

The record will be kept in our offices for 2 years.

1.7 Distribution

Two copies are sent to the customer for attribution.

The customer will send the copy marked "Humongous Labs (OPC) Pvt. Ltd" back with his signature.

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2. Background and Objective of Study:

Objectives of Test: To determine the antimicrobial activity of Disinfectant and hand solution

2.1 Name of the Sample:

Contact Time: 05 min and 10 min

Test method:IN House Specification

PRINCIPLE: This antimicrobial efficacy test is carried out for Disinfectant and hand solutionto evaluate the potential reduction of microorganisms after use.

3. Introduction:

Eco Care asks us to Study of Antimicrobial Efficacy Test Disinfectant.

4. Equipment and Materials: -

4.1 Circulating water bath to maintain temperature of 45.5°C±0.2°C.
4.2 Immersion type thermometer -10 to 110°C with 0.1°C subdivisions, certified by National Institute of Standards and technology (NIST).
4.3 Incubator 37±1°C.
4.4 Micropipettes Capacity to measure 100µl to 1000µ1.
4.5 Autoclave L-Spreader (Hi media PW1085) with 45-55 mm spreading area.
4.6 Sterile inoculating loop, 3 mm diameter loop size.
4.7 Sterile graduated pipettes, 1.0 ml and 10 ml capacity.
4.8 Dilution bottle made up of borosilicate glass, with stopper.
4.9 Sterile Erlenmeyer flask with the capacity of 100 ml, 250 ml and 500 ml.
4.10 Measuring cylinder: - capacity of 50 ml to 1000ml for preparation of diutions and complete media.
4.11 Electronic balance with capacity of 220 g and sensitivity of 0.001g.
4.12 S.S Scientific colony counter.
4.13 Presterilized Petri dishes made of plastic of diameter 90 mm.(Hi media)
4.14 pH meter:-accurate to ±0.05 pH unit at 25°C.
4.15 Test tube.
4.16 Oven(dry sterilizer);For equipment, culture media and reagents capable of being maintained at 170°C to 175°C for one hour.
4.17 Autoclave (wet sterilizer):- for equipment, culture media and reagents capable of being maintained at 121°C±1°C
4.18 Stop watch.

4.1.1 Media and diluents: -

1 Soyabean casein digest agar (Hi media M290)
2 0.3%w/v solution of neutralizing fluid
3 0.9%w/v solution of saline
4 Nutrient Agar (M001)
5 Soyabean casein Digest Medium (M011)

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4.1.2 Test organisms: -

Table 1: Challenge Organisms

Sl. No.	Challenge Organisms	Name of Activity
01	*Escherichia Coli*	Bactericide
02	*Staphylococcus Aureus*	Bactericide
03	*Pseudomonas aeruginosa*	Bactericide
04	*Aspergillus Brasiliensis*	Fungicide
05	*Candida albicans*	Fungicide
06	*Clostridium Sporogenes*	Sporocide
07	*Bacillus Subtilis*	Sporocide

4.1.3 PROCEDURE:

A. Preparation and harvesting of challenge Micro – organisms:

The inoculum level of bacterial cultures of about 10^8 cfu/ml was prepared and harvested

Preparation of Serial dilution of culture suspension (10^{-1} to 10^{-8}):

(Serial dilutions will be made only all test Microorganism)

Preparation of sterile tubes for serial dilutions:

Prepare n number of tubes as required containing 9.0 ml sodium chloride solution. Then sterile all tube in autoclave for 15 minutes.

1. **Serial dilution 10^{-1}(1^{st}):**

Take 1.0 ml of culture suspension from Reference Culture Suspension in one sterile tube & mix the culture over the vertex mixer.

2 **Serial dilution 10^{-2}(2^{nd}):**

Take 1.0 ml of culture suspension from Serial Dilution 1^{st} in one sterile tube & mix the culture over the vertex mixer.

3 **Serial dilution 10^{-3}(3^{rd}):**

Take 1.0 ml of culture suspension from Serial Dilution 2^{nd} in one sterile tube & mix the culture over the vertex mixer.

4 **Serial dilution 10^{-4}(4^{th}):**

Take 1.0 ml of culture suspension from Serial Dilution 3^{rd} in one sterile tube & mix the culture over the vertex mixer.

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5 **Serial dilution 10^{-5}(5^{th}):**

Take 1.0 ml of culture suspension from Serial Dilution 4^{th} in one sterile tube & mix the culture over the vertex mixer.

- **Procedure for inoculum: (Pour Plate Method)**

Inoculate 1ml from each serial dilution in separate Petri plate & incubate all Petri plates. On the completion of incubation period take observations and find out the suitable serial dilution.

- **Observation:**

On the completion of incubation time take observation for each Petri dish for each serial dilution.

Table 1: Initial inoculum level of Challenge Organisms

Sl. No.	Challenge Organisms	Initial inoculum level
01	*Escherichia Coli*	$2.5x10^5$
02	*Staphylococcus Aureus*	$3.0x10^5$
03	*Pseudomonas aeruginosa*	$2.7x10^5$
04	*Aspergillus Brasiliensis*	$3.2x10^5$
05	*Candida albicans*	$3.0x10^5$
06	*Clostridium Sporogenes*	$3.6x10^5$
07	*Bacillus Subtilis*	$3.8x10^5$

- **Sample preparation:**

Sample is directly taken from the original container and use to determine its ability to kill the above-mentioned gram negative and gram-positive bacteria. At the same time its residual activity.after 5 min days and 10 min days were determined by using the following method.

- **Antimicrobial efficacy test:-**

• 0.1 ml of the challenge suspension (test organism) was transferred to a vial containing 10 ml of test solution.

• The challenge suspension was exposed to the test solution for the contact time (5min, 10 min).

• Upon elapse of the exposure time, an appropriate aliquot was transferred from the above vial to a bottle containing suitable neutralizing broth (so as to obtain 10^{-2} dilution) and mixed thoroughly.

• Additional ten-fold dilutions were prepared in the neutralizing broth, mixing thoroughly between dilutions.

• Duplicate 1.0 mL and/or 0.1 mL aliquots of the working test solution/neutralizing broth/challenge suspension were pour-plated using suitable agar with product neutralizers, producing plated dilutions of 10^{-2}, 10^{-3} , 10^{-4} , and 10^{-5} .

• The plates SCDA were incubated at 35°C ± 2ºC for 24-48 hours. And SDA 25°C ± 2ºC for 120 hours.

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➢ **Control Preparation:**

• 0.1 ml of the challenge suspension (test organism) was transferred to a vial containing 10 ml of saline fluid and the solution.

• The challenge suspension was exposed to the test solution for the contact time (5min, 10 min).

• Upon elapse of the exposure time, an appropriate aliquot was transferred from the above vial to a bottle containing suitable neutralizing broth (so as to obtain 10^{-2} dilution) and mixed thoroughly.

• Additional ten-fold dilutions were prepared in the neutralizing broth, mixing thoroughly between dilutions.

• Duplicate 1.0 mL and/or 0.1 mL aliquots of the working test solution/neutralizing broth/challenge suspension were pour-plated using suitable agar with product neutralizers, producing plated dilutions of 10^{-2}, 10^{-3} , 10^{-4} , and 10^{-5} .

• The plates were incubated at 35°C ± 2°C for 24-48 hours.
The Log10 Reduction for the challenge suspension attributable to the test solution at the timed exposure was calculated for each replicate as follows:

• Log10 Reduction = Log10 Average (NC) - Log10 Average (PEX)

• Where:
• NC = Numbers Control Population (CFU/mL)
• PEX= Post-Exposure Population (CFU/mL)

➢ **Neutralizer efficiency test:-**

The efficiency of the neutralizer and their ability to recover the inoculated microorganisms from the material was analyzed as follows:

A low level of 100cfu inoculums was added to diluent fluid as well as sample with diluents fluid separately and made plate count to confirm the recovery of the inoculated microorganism.

➢ **Test for inhibitory residue on glassware and plasticware: -**

A set of plasticware as well as glassware used for the above test was subjected to demonstrate their absence of antimicrobial inhibitory residual activity.
One set of glassware was thoroughly washed and rinsed 12 times with reagent grade water and designate as Group A.

For presterilized glassware, set up six plastic Petri dishes and designate them as Group B, A known amount of culture suspension of Bacillus Subtilis (100-150 cfu/ml) was inoculated in each set of glassware as well as plastic ware.

Presterilized, molten cooled 15-20 ml of Plate count agar was added to the petriplates.
Allowed to solidify it and incubate at 37°C for 24 hour

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Sample B: EKo Power 7 CD **Report No: HLF/2023822/0002**

S. No	PARAMETERS	RESULTS		SPECIFICATION			
	Description	Clear colourless viscous liquid					
	TEST STRAINS	SAMPLE VOLUME	INOCULUM STRENGTH (CFU/ml)	CONTACT TIME PERCENTAGE REDUCTION AFTER Contact Time %		CONTACT TIME log REDUCTION	
				05 min	10 Min	05 min	10 min
BACTERICIDAL EFFICAC (4 % Solution)							
1	E.Coli	9ml	$2.5x10^5$	99.99	99.99	4	4
2	S.aureus	9 ml	$3.0x10^5$	99.99	99.99	4	4

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3	P.aeruginosa	9 ml	$2.7X10^5$	99.99	99.99	4	4
FUNGICIDAL EFFICACY 4 % Solution							
1	Candida albicans	9ml	$3.0x10^5$	99.99	99.99	4	4
2	A.brasiliensis	9ml	$3.2x10^5$	99.99	99.99	4	4
SPORICIDAL EFFICACY 4 % Solution							
1	Bacillus subtilis	9 ml	$3.8X10^5$	99.99	99.99	4	4
2	Clostridium Sporogenes	9 ml	$3.6X10^5$	99.99	99.99	4	4
3	**Heavy Metals**						
a.	Arsenic (As As)	BDL					
b.	Lead (As Pb)	BDL					
c.	Cadmium (As Cd)	BDL					
d.	Mercury (As Hg)	BDL					
Test Method :- USP-Disinfectant and Antiseptics -2023							

6. Conclusion: -

The above-performed study concluded the following: -

(i) 1 ml of original concentration of Disinfectant and Hand San.

(ii) Disinfectant mixed with 0.1 ml cultures suspension of Bactericide, Fungicide and Sporocide Showed 99.98 and 99.99 percentage population reduction at 5-min contact time and 10 min contact time.

(iii) Finally, it has been confirmed that the Disinfectant and Hand San contain antimicrobial activity up to5 min and 10 min in a controlled environment condition.

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