Test report

Bactericidal efficacy test against spores by "ALTANT"

2017.9.14

Kitasato Research Center for Environmental Science

1. The Purpose

The bactericidal effect on spores by the "Altant" provided by your company was investigated. In this test, as a test system to investigate the basic bactericidal effect of the specimen, a method of inoculating the specimen directly with the fungus solution was used.

2. Requester

name: E · Tech Co., Ltd.

location: Hyogo Prefecture Kobe City Chuo-ku Minatojima 9-1 Kobe Incubation Office 316

3. Laboratory

name: Kitasato Research Center for Environmental Science

location: 252-0329Kanagawa prefecture Sagamihara-shi Minami-ku Kitasato 1-15-1

Responsible: Microbial Biotechnology Division

4. Test period

2017.9.4～2017.9.6

5. Test item (provided by your company)

1) 「ALTANT」

2) IPA (Control article)

Each stock sample was subjected to the test.

6. Test condition

1) Working time: 0(Initial, control only), 15seconds,1minute, 5minutes

2) Working temperature : 25±2℃

7. Used medium and reagent

1) Culture medium

① Tryptic Soy Agar(Difco, Hereinafter referred to as TSA)

② SCDLP broth medium(EIKEN CHEMICAL, Hereinafter referred to as SCDLP)

2) reagent

① Sodium chloride(Wako Pure Chemical Industries、 Hereinafter, the 0.85% solution is described as physiological saline)

② sodium thiosulfate (Wako Pure Chemical Industries、First grade)

8. Preparation of test bacteria and test bacteria

1) Test fungus

Bacillus subtilis ATCC6633

(Spore fluid manufactured by CROSSTEX、 Lot.SB009,, 1.4×10^9CFU/mL)

2) Preparation of test bacteria solution

Spore solution was diluted with sterilized ion-exchanged water to be about 10^8 CFU / mL, and this was used as a test bacterial solution.

9. Test method

1) Bactericidal efficacy test

10 ml of the test sample was collected in a centrifuge tube having a volume of 50 ml.

0.1 ml of the test bacterial solution was added thereto, mixed with a test tube mixer, and allowed to act at 0 (initial), 15 seconds, 1 minute, 5 minutes at 25 ± 2 ℃.

After a predetermined period of action, 9 mL of the test article was added to the inactivating agent * 1 to stop the bactericidal action on the test bacteria, which was used as a sample solution for measuring the number of bacteria.

For working time 0 (initial) and control, sterile physiological saline was used instead of the specimen.

* 1: Sodium thiosulfate added SCDLP (hereinafter referred to as SCDLP with reagent added) confirmed its effectiveness was used.

Confirmation of the effectiveness of the tested product as an inactivating agent The test procedure and the test are shown on the last page.

2) Measurement of bacterial count

A 10-fold serial dilution series was prepared with a physiological saline solution as a stock solution, and 1 ml each of the sample stock solution and dilution solution was transferred to a petri dish, mixed with about 20 ml TSA, and solidified to obtain 36 ± 2 ℃ for 42 hours. The number of test bacteria per 1 ml of the test sample was counted by counting the growth colonies after cultivation (lower limit of determination: 10 CFU).

3) Calculation of bacterial count log reduction value

Based on the initial number of control bacteria and the number of test bacteria in the test product, the bacterial number log reduction value (= LRV: log reduction value) was calculated using the following formula. In addition, LRV is represented by one digit after the decimal point (the second digit after the decimal point is rounded down).

LRV (bacterial count log reduction value): $\log_{10}$ (initial number of control ÷ number of bacteria after test article action)

10. Test results

The test results are shown in Table 1.

The test results showed that "Altant" was less than the lower limit of quantification (<10 CFU / ml, LRV> 5.2) at a time of action of 5 minutes, 6.8×10^4 CFU / ml (LRV 1.3) against Bacillus subtilis spores, The number of bacteria decreased.

On the other hand, the control product, IPA, did not change from the initial number of bacteria even at the action time of 5 minutes.

As a reference, the culture medium after the culture is shown in Photo 1. In the photographs, the petri dish after culturing the sample stock solution was photographed.

11. comment

In this test, the bactericidal efficacy against spores of your "Altant" was evaluated. In the evaluation test method of the disinfectant of the 17 th revised Japanese Pharmacopoeia, the bacterial count log reduction value before and after disinfectant action is defined as "efficacious" with the LRV of 2 or more in the spores.

Therefore, in this test, it was judged that there was a bactericidal effect when LRV was 2 or more. As a result of the test, by acting "altant", the number of bacteria decreased depending on the action time, LRV became> 5.2 when the action time was 5 minutes, and the bactericidal effect was Admitted.

This test is a test system for examining the basic bactericidal effect of the tested product, which is a condition without load of organic matter. Ordinarily, microorganisms are present together with organic substances (nutrient organic substances such as carbohydrates, fats and oils, proteins, saliva, nasal discharge, feces, vomitus, etc.).

Therefore, as a future test, it is desirable to verify the effectiveness test based on EN 1276: 2009 loaded with organic matter and the effect on real life use model.

12. Reference Test · Reference

1) 17 th revised Japanese Pharmacopoeia, Reference information disinfection method and decontamination method, 2414-2416.

2) EN 1276:2009

Table 1. Bactericidal effect of test article on Bacillus subtilis (spores)

Test condition	Working time				LRV *2		
	0(initial)	15sec	1min	5min	15sec	1min	5min
Control (physiological saline)	1.7×10^{6}	1.6×10^{6}	1.5×10^{6}	1.4×10^{6}	-	-	-
① Altant		4.8×10^{5}	6.8×10^{4}	<10	0.5	1.3	>5.2
② IPA(control item)		1.5×10^{6}	1.4×10^{6}	1.5×10^{6}	-	-	-

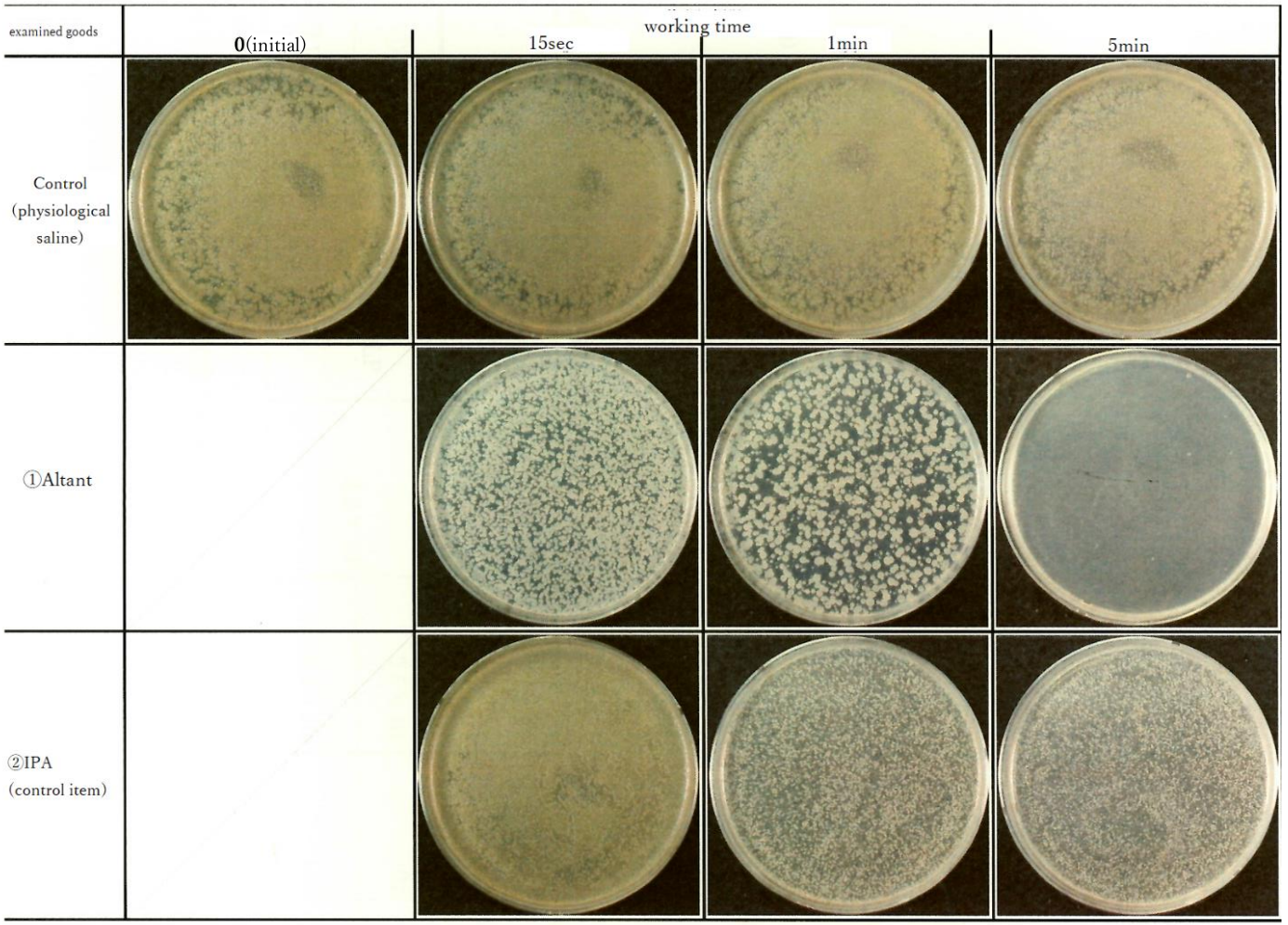
Test bacteria: Bacillus subtilis ATCC 6633

(spore fluid, manufactured by CROSSTEX, Lot. SB009, 1.4×10^{9} CFU / ml)

Unit: CFU / ml

Lower limit of determination: 10 CFU / ml

* 2: LRV (bacterial log log reduction) = log 10 (initial number of control ÷ number of bacteria after test article action) LRV truncates the second digit after the decimal point, and when "LRV" is 0 or less, it is expressed as "-".



Picture 1. Bactericidal effect on specimen Bacillus subtilis (spore) (sample solution stock solution)

13. Efficacy confirmation test of deactivator

1)the purpose

The effectiveness of the inactivating agent used for stopping the bactericidal action on the test bacteria by the tested product was confirmed.

2) Method

1 ml of the test sample was added to 9 ml of deactivator (reagent added SCDLP) and mixed. To this was inoculated 0.1 ml of a bacterial solution of about 10^3 to 10^4 CFU / ml and allowed to act at room temperature for 20 minutes, then the number of bacteria in this mixed solution was measured.

As a control, sterilized water was used instead of the specimen. The effectiveness of the deactivating agent was judged according to the following judgment criteria in accordance with 17 th revised Japanese Pharmacopoeia 4.05 - I - 3.5.

Judgment criteria: B (number of bacteria after deactivating agent treatment) / A (control bacterial count × 100 = within 50 to 200%

3) results

The test results are shown in Table 2. Since the ratio with the number of control bacteria was 92 to 116% and it was within the judgment criteria shown in 13.2), the deactivator was judged as effective for the test.

表-2. Effectiveness of inactivating agent on specimen

examined goods	Inactivating agent used (dilution ratio of test sample)	Number of bacteria (CFU / ml)		A ratio *3 (%)	Evaluation result of effectiveness *4
		Control (A)	The deactivator (B)		
① Altant	Reagent added SCDLP (10 times)	3.8×10^1	3.5×10^1	92	Effectiveness
② IPA (control item)			4.4×10^1	116	Effectiveness

* 3: B / A × 100

* 4: Judged as valid when the ratio is within 50 to 200% (No. 17 Japanese Pharmacopoeia)