

**Determination of the Antimicrobial Activity
Of A Disinfectant Solution Based on EN BS 1276:1997**

Test Product: Trio 100 Product Code 201
Silliker Reference No. 0402 0573

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1. **Scope**

The following report gives details of the method used to determine the antimicrobial activity of disinfectants used in Food Hygiene and is based on British Standard EN 1276:1997.

2. **Test Products**

2.1 Trio 100 Product Code 201 Silliker Reference No. 0402 0573

3. **Test Organisms**

3.1	<i>Staphylococcus aureus</i>	NCTC 10788	Silliker Ref S3
3.2	<i>Enterococcus hirae</i>	ATCC 8043	Silliker Ref E10
3.3	<i>Pseudomonas aeruginosa</i>	NCIMB 10421	Silliker Ref P2
3.4	<i>Escherichia coli</i>	ATCC 10536	Silliker Ref E9

4. **Equipment / Materials**

- 4.1 Diluent
- 4.2 Bovine albumin solution 0.3g/100ml
- 4.3 Water of Standard Hardness (WSH)
- 4.4 Inactivation Liquid
- 4.5 Tryptone Soya Agar (TSA)

All above prepared as per EN 1276:1997

- 4.6 Incubator at 36°C +/- 1°C
- 4.7 Sterile 1ml Pipettes
- 4.8 Sterile 10ml Pipettes
- 4.9 Sterile 90mm Petri Dishes
- 4.10 Sterile 30ml plastic universals
- 4.11 Incubator at 37°C ± 1°C

5. **Preparation of Test Microbial Suspensions**

- 5.1 For each organism, inoculate the surface of Tryptone Soya Agar plates from a stock culture. Incubate the plates at 37°C +/- 1°C for 18 – 24 hours.
- 5.2 Make a suspension containing approximately 1.5×10^8 to 5×10^8 cfu/ml for each microorganism. Serially dilute the suspension down in diluent to obtain a count of between 6.0×10^2 and 3×10^3 cfu/ml. Pour with TSA and incubate at 36°C +/- 1°C for 48 hours. This is used as a control count.

6. Preparation of Test Disinfectant Solutions

A volumetric solution 1.25 times stronger than the required concentration of test product was prepared in WSH.

7. Determination of the Microbial Effect of the Disinfectant Solutions

7.1 Clean Conditions (Organic Load 0.03% Bovine Albumin)

7.1.1 1ml of 0.3g/100ml bovine albumin solution was added to each clearly labelled sterile 30ml universal. 1ml of the appropriate microbial suspension was added to each container and mixed. After 2 minutes incubation at $20^{\circ}\text{C} \pm 1^{\circ}\text{C}$. 8mls of product solution was added to each container and mixed. After 5 minutes \pm 10 seconds (contact time) at $20^{\circ}\text{C} \pm 1^{\circ}\text{C}$, 1ml of liquid from each container was transferred into separate 8ml aliquots of inactivation liquid and 1ml of WSH and mixed. After a neutralisation time of 5 minutes \pm 10s, 1ml of the test solution was serially diluted out in diluent and plated out into duplicate petri dishes.

Approximately 15mls of TSA melted and tempered to $45^{\circ}\text{C} \pm 1^{\circ}\text{C}$ was added to the plates, mixed and allowed to set before incubating at $36^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 48 hours.

7.1.2 This procedure was repeated for each of the test microbial suspensions. And for each product dilution.

8. Assay Validation

To ensure that the experimental conditions do not interfere with the test, the following was done:

Temperature Validation

A suspension containing between 6×10^2 and 3×10^3 of each test organism was prepared in diluent. 1ml of the interfering substance and 1ml of the bacterial suspension was added to a sterile universal and mixed. After an incubation time of 2 min \pm 10s at $20^{\circ}\text{C} \pm 1^{\circ}\text{C}$. 8.0mls of hard water was added and the suspension mixed. After an incubation time of 5 min \pm 10s at $20^{\circ}\text{C} \pm 1^{\circ}\text{C}$, the test solution was plated out into duplicate petri dishes. The above procedure was carried out for each organism at the highest product concentration. The plates were poured with 15ml of TSA and incubated at $36^{\circ}\text{C} \pm 1^{\circ}\text{C}$.

Neutraliser Validation

A suspension containing between 6×10^2 and 3×10^3 of each test organism was prepared in diluent. 1ml of the bacterial suspension was added to a universal already containing 8ml of neutraliser and 1ml of hard water and mixed. After a contact time of 5 min \pm 10s at $20^{\circ}\text{C} \pm 1^{\circ}\text{C}$, 1ml of the test solution was plated out into duplicate petri dishes. The above procedure was carried out for each organism at the highest product concentration. The plates were poured with 15ml of TSA and incubated at $36^{\circ}\text{C} \pm 1^{\circ}\text{C}$.

Dilution / Neutralisation Validation

1ml of the interfering substance and 1ml of diluent was added to a sterile universal and mixed. After starting a stopwatch, 1ml of the product dilution was added and mixed. After a contact time of 5 min \pm 10s at 20°C \pm 1°C, 1ml of the test mixture was added to 8ml of neutraliser for 5 min \pm 10s. 1ml of the bacterial suspension containing between 6×10^2 and 3×10^3 cfu/ml was added to the test mixture and left for a contact time of 30min \pm 10s at 20°C \pm 1°C. After the contact time, 1ml of the test solution was plated out into duplicate petri dishes. The above procedure was carried out for each organism at the highest product concentration. The plates were poured with 15ml of TSA and incubated at 36°C \pm 1°C.

Control Counts

Organism	Count on Plate
P2	1.2×10^3
S3	1.4×10^3
E9	9.9×10^2
E10	6.9×10^2

Validation of Neutraliser

Organism	Count on Plate			
	1	2	Mean	
P2	101	110	1.1×10^3	Valid
S3	116	108	1.1×10^3	Valid
E9	96	104	1.0×10^3	Valid
E10	60	65	6.3×10^2	Valid

Temperature Validation

Organism	Count on Plate			
	1	2	Mean	
P2	109	116	1.3×10^3	Valid
S3	109	100	1.1×10^3	Valid
E9	90	98	9.4×10^2	Valid
E10	86	74	8.0×10^2	Valid

Validation – Dilution / Neutralisation

Sample	Organism	Count on Plate			
		1	2	Mean	
0402 0573	P2	106	98	102	Valid
0402 0573	S3	111	121	116	Valid
0402 0573	E9	71	74	73	Valid
0402 0573	E10	74	79	77	Valid

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Organisms	Initial Inoculum	1:120			1:100			1:80			1:50		
		1	2	Mean	1	2	Mean	1	2	Mean	1	2	Mean
P2	1.4 x 10 ⁹	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
S3	1.5 x 10 ⁹	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
E9	1.4 x 10 ⁹	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
E10	3.0 x 10 ⁸	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10

Conclusion

A 5 log reduction from the initial count must be achieved for the product to pass the set criteria.

Trio 100 Product Code 201 Silliker Reference 0402 0573 passed the criteria for all test dilutions and micro-organisms tested.