BRITISH ANALYTICAL CONTROL
CONSULTANT CHEMICAL AND MICROBIOLOGICAL ANALYSTS

Report Number: 970086
Revision Number: 1

Challenge Testing

Test Product:	Solo Concentrate
BAC Reference Number:	97092252
Client:	Swan Chemicals Ltd Mintsfeet Road South Kendal Cumbria LA9 6ND
Date Received:	23 September 1997
Date Commenced:	24 September 1997
Date Completed:	26 September 1997
Author:	J R Dobbs

Authorised By: ..... Date: 26.9.97
Mrs Rachel Goff BSc (Hons), CBiol, MIBiol, AIBMS, MRSH
Head of the Department of Microbiology

Circulation List

Original: Swan Chemicals Ltd
Copy 1: British Analytical Control Archives

1. **Scope**

The experiment was carried out to assess the biocidal activity of a sample F44 at two concentrations against *Pseudomonas aeruginosa* at various contact intervals.

2. **Test Product**

2.1 Sample F44 at 1% Concentration in Sterile Distilled Water
BAC Reference 97092252A

2.2 Sample F44 at 5% Concentration in Sterile Distilled Water
BAC Reference 97092252B

3. **Test Organism**

3.1 *Pseudomonas aeruginosa* NCIMB 8626 BAC Ref P1.

4. **Preparation of Inoculum**

4.1 A Stock Culture of the test bacteria was streaked onto the surface of a Tryptone Soya Agar (TSA) plate and incubated overnight at 37°C ± 1°C. The resulting growth was used to prepare a bacterial suspension of approximately 1.0×10^8 colony forming units per millilitre (cfu/ml) in Sterile Maximal Recovery diluent (MRD).

4.2 Further serial dilutions were prepared to establish the size of inoculum being used in the test procedure.

5. **Test Procedure**

1ml and 5ml amounts of the sample were placed into sterile vessels and made up to 100ml using Sterile Distilled Water and mixed. 1ml of the bacterial suspension was added and mixed. After 2, 5 and 10 minute contact time intervals, 1ml aliquots were removed and placed into 9ml of inactivation liquid (prepared according to BS DD177:1988) and left for 10-15 minutes to inactivate. After this time, serial dilutions were prepared and plated out in duplicate, poured with TSA and incubated for 48 hours ± 2 hours at 35°C ± 1°C. After incubation, the plates were examined for the presence of colonies and results recorded.

6. **Validation**

The bacterial suspension was diluted to a level of approximately 1.0 x 10^4 cfu/ml. The two concentrations of the sample were prepared as above and 1ml was transferred to 9ml of inactivation liquid. Also 1ml of water was added to 9ml of inactivation liquid to act as a control. After 10-15 minutes inactivation period, 0.1ml of the 1.0 x 10^4 cfu/ml suspension was added to each inactivation mixture and mixed. 1ml was then plated out in duplicate and poured and incubated as before. After incubation, the plates were examined and colony numbers recorded. The test is considered valid if the count returned by the test plate is within ± 50% of the count returned by the control plates.

7. **Results**

7.1 Number of Organisms at the start of the tests - 1.1 x 10^6 cfu/ml.

7.2 Number of Organisms recovered after the contact times.

	Contact Time		
Concentration	**2**	**5**	**10**
1%	<5	<5	<5
5%	<5	<5	<5

7.3 Validation

Concentration of Sample	Count With Sample	Count Without Sample	Valid
1%	90	112	Yes
5%	109	112	Yes