

Analytical MicroLabs

Industrial Microbiologists

NATA Accredited Laboratory: 14917

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Citrofresh Bactericidal Efficacy testing

Certificate number: 020905
Batch number: Citrofresh 360332
Production date: March 2005
Date or period of testing: 01/09/05-31/09/05

Organism tested:

1. <i>Listeria monocytogenes</i>	NCTC 11994
2. <i>Listeria ivanovii</i>	NCTC 11846
3. <i>Listeria innocua</i>	NCTC 11288

Kelsey- Maurer Capacity test (TGA Disinfectant test, Appendix 54, 54A, TGA Protocol)

(The above test was validated, using "Virkon S" registered disinfectant)

Summary of results: Day 1

Concentration of Citrofresh	8%	
Exposure time	8 min.	18 min.
Organism tested		
<i>Listeria monocytogenes</i> NCTC 11994	0/5Growth	2/5Growth
<i>Listeria ivanovii</i> NCTC 11846	1/5Growth	2/5Growth
<i>Listeria innocua</i> NCTC 11288	0/5Growth	2/5Growth

0, 1, 2, 3, 4, 5/5 growth represent the positive broth (visible growth) out of 5 Nutrient broth No. 2

Initial bacterial load:

(Day 1)

1. <i>Listeria monocytogenes</i>	8.0 x 10 ⁸ orgs/ml
2. <i>Listeria ivanovii</i>	2.4 x 10 ⁹ orgs/ml
3. <i>Listeria innocua</i>	1.5 x 10 ⁹ orgs/ml

Controls:

(Day 1)

1. Recovery Broth contamination	No growth
2. Disinfectant contamination	No growth
3. Fertility test	Growth
4. Inactivator Efficacy	Growth

Summary of results: Day 3

Concentration of Citrofresh	8%	
Exposure time	8 min.	18 min.
Organism tested		
Listeria monocytogenes NCTC 11994	1/5Growth	2/5Growth
Listeria ivanovii NCTC 11846	1/5Growth	2/5Growth
Listeria innocua NCTC 11288	1/5Growth	1/5Growth

0, 1, 2, 3, 4, 5/5 growth represent the positive broth (visible growth) out of 5 Nutrient broth No. 2

**Initial bacterial load:
(Day 3)**

1. <i>Listeria monocytogenes</i>	1.5×10^9 orgs/ml
2. <i>Listeria ivanovii</i>	8.5×10^8 orgs/ml
3. <i>Listeria innocua</i>	9.0×10^8 orgs/ml

**Controls:
(Day 3)**

1. Recovery Broth contamination	No growth
2. Disinfectant contamination	No growth
3. Fertility test	Growth
4. Inactivator Efficacy	Growth

Summary of results: Day 5

Concentration of Citrofresh	8%	
Exposure time	8 min.	18 min.
Organism tested		
Listeria monocytogenes NCTC 11994	2/5Growth	2/5Growth
Listeria ivanovii NCTC 11846	1/5Growth	2/5Growth
Listeria innocua NCTC 11288	0/5Growth	2/5Growth

0, 1, 2, 3, 4, 5/5 growth represent the positive broth (visible growth) out of 5 Nutrient broth No. 2

**Initial bacterial load:
(Day 5)**

1. <i>Listeria monocytogenes</i>	7.0×10^8 orgs/ml
2. <i>Listeria ivanovii</i>	8.0×10^8 orgs/ml
3. <i>Listeria innocua</i>	6.5×10^8 orgs/ml

**Controls:
(Day 5)**

1. Recovery Broth contamination	No growth
2. Disinfectant contamination	No growth
3. Fertility test	Growth
4. Inactivator Efficacy	Growth

Number of challenges: Two (2) after 8 and 18 min.

Origin of Test Organisms: The University of Queensland, Australia

Enumeration of initial bacterial load: HBA in duplicate-surface spread technique

Incubation temperature and time: as per TGA Appendix 54, 54A, page 12 to 17.
(37 degree Celsius, 48 hrs)

Medium for growth of Test Organisms: Wright- Mundy Dextrose medium (Difco)

Recovery Medium: Nutrient Broth No. 2 (Oxoid)

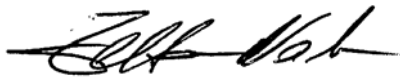
Diluent for Citrofresh 14P and 14W: Sterile Hard Water (Melbourne University)

Resuspension of centrifuged organisms: dirty condition- 5% yeast suspension

Temperature during testing: 20 degree Celsius in air-conditioned room

(Note for “Inactivator Efficacy”: Dilution of the disinfectant, after exposure to the bacteria, was always sufficient to stop the activity of the disinfectant.

Inactivator Efficacy test gave no different result using neutralizer: 0.1% peptone, 0.1% sodium thiosulphate, 0.5% Tween 80 and 0.07% lecithin, dissolved in distilled water)



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