Analytical MicroLabs Industrial Microbiologists

NATA Accredited Laboratory: 14917

Douglas Hocking Research Institute, The Geelong Hospital, Barwon Health, Ryrie St. Geelong, VIC. 3220 Phone: 1300 724 961 Fax: 03-52603183 ABN 55 167 987 554

Citrofresh Bactericidal Efficacy testing

Certificate number: 010905

Batch number: Citrofresh 360332

Production date: March 2005

Date or period of testing: 01/09/05-31/09/05

Organism tested: 1. Salmonella salford IMVS 1710

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 14P >	8%	
Exposure time >	8 min.	18 min.
Organism tested		
Salmonella salford	1/5Growth	2/5Growth
IMVS 1710		

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

Initial bacterial load: Salmonella salford 4.0×10^8 orgs/ml

(Day 1)

Controls: 1. Recovery Broth contamination No growth (Day 1) 2. Disinfectant contamination No growth

3. Fertility test Growth

4. Inactivator Efficacy Growth

Summary of results: Day 3

Concentration of Citrofresh 14P >	4%	
Exposure time >	8 min.	18 min.
Organism tested		
Salmonella salford IMVS 1710	2/5Growth	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:

Salmonella salford 9.0 x 10⁸ orgs/ml

(Day 3)

Controls: (Day 3)

1. Recovery Broth contamination No growth 2. Disinfectant contamination No growth Growth

3. Fertility test

4. Inactivator Efficacy Growth

Summary of results: Day 5

Concentration of Citrofresh 14P >	4%	
Exposure time >	8 min.	18 min.
Organism tested		
Salmonella salford IMVS 1710	2/5Growth	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:

Salmonella salford 1.5 x 10⁹ orgs/ml

(Day 5)

Controls: (Day 5)

1. Recovery Broth contamination 2. Disinfectant contamination

3. Fertility test

4. Inactivator Efficacy

No growth No growth

Growth 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: PCA in duplicate-pour-plate 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 1W: 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)

Zoltan Nack MSc. MBA. MASM

Biologist, Senior Scientist, Analytical MicroLabs

Of Jah