



Agri-food & Veterinary Authority of Singapore

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Our Reference : M003/09/2002
Payment No. : CVL 3199

Mr Wong Mun Chiang
Vetpharm Laboratories (S) Pte Ltd
Block 27, Tuas Ave 13
#01-25
Singapore 638993

LABORATORY REPORT

Type of examination : Bacteriological
Specimen description : Liquid disinfectant "Germicide 21st Century"
Received from : Vetpharm Laboratories (S) Pte Ltd
Date received : August 2002

Report :

The above sample was tested according to the method in Annex A.

The results obtained are as follows :

Test micro-organism	Initial count of test micro- organism per ml of test mixture	Average count of surviving test micro-organism per ml of test mixture	Percentage of test micro- organism inactivated
<i>Salmonella</i> Enteritidis	60,000,000	0	100%
<i>Salmonella</i> Typhimurium	165,000,000	25	>99.9%
<i>Escherichia coli</i> O157:H7	355,000	0	100%
<i>Actinobacillus pleuropneumoniae</i>	2,700,000	0	100%

Date : 02.09.02

Toh Say Ling
Laboratory Microbiologist

Method

1. The test bacterial strains used were :
Salmonella Enteritidis phage type 4 (poultry isolate);
Salmonella Typhimurium (poultry isolate);
Escherichia coli O157:H7 (cattle isolate);
Actinobacillus pleuropneumoniae (swine isolate).
2. The active chlorine concentration used was 40 mg/L (1:200 dilution of product).
3. Duplicates of 0.1 ml of each bacterial inoculum were each added to 20 ml of diluted disinfectant of active chlorine concentration of 40 mg/L (at least 10^4 cells per ml of mixture - the initial cell count was determined). The mixture was allowed to stand for 20 minutes at room temperature (25 ± 2 °C).
4. 1 ml of each mixture was transferred to 9 ml of D/E Neutralising Broth (Difco 281910). 0.1 ml aliquot was used for bacterial count by the spread plate technique.



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Case Ref. No.: M03/09/02

Payment no.: CVL3199

Attn: Mr Wong Mun Chia 1g
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LABORATORY REPORT

Type of examination : Virological
Specimen Description : Liquid disinfectant "Germicide 21st Century"
Received from : Vetpharm Laboratories (S) Pte Ltd
Date received : August 2002


Report:

The disinfectant was tested according to the method in Annex B.

Please refer to the attached sheet for results of the virucidal activity of the disinfectant against Aujeszky's Disease virus and Infectious Bursal Disease virus.

Tests for virucidal activity against Infectious Bronchitis virus and Classical Swine Fever virus are in progress.

16/09/02


Dr Lim Chee Wee
Laboratory Veterinarian

M03/09/02

Test Results:

Table 1: Effects of Germicide 21st Century on Aujeszky's Disease Virus following a 10 and 20 minutes exposure at dilutions 1:20, 1:40 and 1:80

Dilution of Disinfectant	1:20*		1:40*		1:80*		Virus Control (TCID ₅₀ /25µl)
Exposure Time	Virus Titre (TCID ₅₀ /25µl)	% Reduction	Virus Titre (TCID ₅₀ /25µl)	% Reduction	Virus Titre (TCID ₅₀ /25µl)	% Reduction	
10 min	≤10 ^{1.5}	≥99.999	10 ^{3.9}	99.84	10 ^{6.5}	36.90	10 ^{6.7}
20 min	≤10 ^{1.5}	≥99.999	10 ^{2.8}	99.99	10 ^{6.0}	80.05	

* - Toxic effect at 10⁻¹ viral dilution

Table 2: Effects of Germicide 21st Century on Infectious Bursal Disease Virus following a 15 and 30 minutes exposure at dilutions 1:20, 1:40 and 1:80

Dilution of Disinfectant	1:20		1:40		1:80		Virus Control (TCID ₅₀ /25µl)
Exposure Time	Virus Titre (TCID ₅₀ /25µl)	% Reduction	Virus Titre (TCID ₅₀ /25µl)	% Reduction	Virus Titre (TCID ₅₀ /25µl)	% Reduction	
15 min	10 ^{2.3}	99.98	10 ^{5.1}	87.41	10 ^{5.5}	68.38	10 ^{6.0}
30 min	10 ^{0.5}	99.999	10 ^{4.7}	94.99	10 ^{5.6}	60.19	

Annex B

- I. Test substance: Germicide 21st Century, Vetpharm Lab (S) Pte Ltd
- II. Dilution: 1:20, 1:40, 1:80
- III. Virus: a) Aujeszky's Disease virus (ADV)
b) Infectious Bursal Disease (IBDV)
- IV. Exposure Time: a) ADV - 10 and 20 minutes
b) IBDV - 15 and 20 minutes
- V. Exposure Temperature: Room temperature (28°C, +/- 1°C)
- VI. Culture: a) ADV - PK15 cell-line
b) IBDV - CEF primary culture
- VII. Neutralising solution (NS): Sodium Thiosulfate (0.057%)
- VIII. Method:
1. The disinfectant was diluted to 1:10 with deionised, distilled water and sterilized by filtration through a 0.2µm filter membrane on the day of test. The filtrate was further diluted to 1:20 and 1:40.
 2. 1 ml of each of the diluted test substance was added to an equal volume of virus. Final dilution of test substance: 1:20, 1:40 and 1:80.
 3. At the end of each exposure time, 0.5ml of the virus-disinfectant mixture was added to 2ml of the neutralising solution.
 4. The mixtures were then serially diluted up to 10⁻⁷.
 5. Include virus (virus + NS), cytotoxicity (diluted disinfectant + NS), neutralisation (diluted disinfectant + NS + low titre virus) and negative (virus diluent only) controls.
 6. 50µl of each of the virus dilution was added to their respective wells on a 96-well tissue culture microplate. An equal volume of cell suspension was added to all wells.
 7. The plates were incubated at 37°C, 5% CO₂ for 48 hours (ADV) and 4 days (IBDV).
 8. At the end of the incubation period, the cells were observed under microscope for the presence of cytopathic effect.
 9. The virus titre was calculated using the Karber's method.

Annex C

- I. **Test substance:** Germicide 21st Century, Vetpharm Lab (S) Pte Ltd
- II. **Dilution:** 1:100, 1:200, 1:400
- III. **Virus:** Infectious Bronchitis virus (IBV)
- IV. **Exposure Time:** 15 and 30 minutes
- V. **Exposure Temperature:** Room temperature (28°C, +/- 1°C)
- VI. **Host:** 10-day-old Specific-Pathogen-Free chicken embryonated eggs (5 eggs per dilution per exposure time)
- VII. **Neutralising solution (NS):** Lethen broth (2x) + 0.1% Sodium Thiosulfate
- VIII. **Method:**
1. The disinfectant was diluted to 1:50 with deionised, distilled water and sterilized by filtration through a 0.2µm filter membrane on the day of test. The filtrate was further diluted to 1:100 and 1:200.
 2. 1 ml of each of the diluted test substance was added to an equal volume of virus. Final dilution of test substance: 1:100, 1:200 and 1:400.
 3. At the end of each exposure time, 0.5ml of the virus-disinfectant mixture was added to 2ml of the neutralising solution.
 4. The mixtures were then serially diluted up to 10⁻⁸.
 5. Include virus (virus + NS), cytotoxicity (diluted disinfectant + NS), neutralisation (diluted disinfectant + NS + low titre virus) and negative (virus diluent only) controls.
 6. 0.2ml of each of the suspension was inoculated into the eggs.
 7. The eggs were incubated at 37°C for 4 days and candled daily to check for embryo death.
 8. Upon embryo death or at the end of the incubation period, the embryos were examined for symptoms of IBV infection.
 9. The virus titre was calculated using the Karber's method.

M44/11/02

Test Results:

Table 1: Effects of Germicide 21st Century on Infectious Bronchitis Virus following a 15 and 30 minutes exposure at dilutions 1:100, 1:200 and 1:400

Dilution of Disinfectant	1:100		1:200		1:400		Virus Control (EID ₅₀ /0.2ml)
Exposure Time	Virus Titre (EID ₅₀ /0.2ml)	% Reduction	Virus Titre (EID ₅₀ /0.2ml)	% Reduction	Virus Titre (EID ₅₀ /0.2ml)	% Reduction	
15 min	$\leq 10^{0.5}$	≥ 99.999	$\leq 10^{0.5}$	≥ 99.999	$\leq 10^{0.5}$	≥ 99.999	$10^{7.7}$
30 min	$\leq 10^{0.5}$	≥ 99.999	$\leq 10^{0.5}$	≥ 99.999	$\leq 10^{0.5}$	≥ 99.999	