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THIS TEST HAS BEEN CARRIED OUT USING A FIO "SUPER CONCENTRATE" SAMPLE

Report of a trial to test the inactivation efficiency of F10 Super Concentrate disinfectant against rabies virus

May 2000

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REPORT OF A TRIAL TO TEST THE INACTIVATION EFFICIENCY OF F10 SUPER CONCENTRATE DISINFECTANT AGAINST RABIES VIRUS

This trial was carried out at the Rabies Unit, Onderstepoort Veterinary Institute.

Introduction

F10 is the tradename of a disinfectant marketed by Health and Hygiene (Pvt) Ltd, Sunninghill, South Africa. F10 Super Concentrate consists of a blend of buffered ampholytic and cationic surfactants and sequesterants/detergents. Label directions recommend using F10 at dilution ranges of 1/125 to 1/500 in clean water. It is recommended against viruses, bacteria and fungi (including spores).

The aim of the trial was to determine whether or not F10 disinfectant will inactivate rabies virus and to determine the approximate dilution range at which it is effective for this purpose.

Materials and methods

The F10 batch number used was BN000206. The rabies virus used was ERA strain grown on BHK-21 tissue cultures. Pre-titration of the virus batch determined that it had a virus concentration of 6.8 $\log_{10}$ median tissue culture infectious doses ($TCID_{50}$) per millilitre

The procedure followed was as given in the protocol appended to this document (see Appendix 1).

Results

The virus titration results of each of the F10 Super Concentrate dilutions are given in Table 1 below. As the volume of test suspension inoculated into the titration test plates was considerably smaller than 1 ml (10 ul at the first dilution), the minimum virus detection threshold was 1.55 (that is, any virus titre of this value or lower would not have been detected by the test). In the stronger F10 concentrations the cells in the first dilution wells were killed off, therefore raising the virus detection threshold of the test, making the test less sensitive. The detection thresholds are given in Table 1.

Table 1. The virus titres (in $\log_{10}$ $TCID_{50}$/ml) of the test suspensions, with the detection threshold (in $\log_{10}$ $TCID_{50}$/ml) for each titration.

	20°C		10°C	
	Titre	Threshold	Titre	Threshold
Virus 1/10 in medium	5.55	-	6.30	
Virus 1/10 in hard water	5.55	-	5.30	
F10 1/125 *	ND	2.55	ND	2.55
F10 1/250	ND	2.55	ND	2.55
F10 1/500	ND	2.55	ND	2.55
F10 1/750	ND	1.55	2.05	
F10 1/1500	2.30	-	3.30	
F10 1/3000	4.05	-	4.05	

ND: No virus detected at lowest dilution range

* Note: the F10 dilutions refer to the dilutions before mixing with virus suspension. Therefore during the incubation stage the dilutions will have been double that indicated.

Conclusions

At a temperature of 20°C and a dilution of 1/750 the F10 reduced the virus concentration by more than 4.0 $\log_{10}$ $TCID_{50}$/ml and at a dilution of 1/1500 the F10 inactivated over 3.25 $\log_{10}$ $TCID_{50}$/ml. At a temperature of 10°C the F10 performed slightly less efficiently, removing 3.25 and 2.0 $\log_{10}$ $TCID_{50}$/ml at dilutions of 1/750 and 1/1500 respectively. This trial shows that the recommended dilution of 1/500 will be sufficient to inactivate rabies virus.

Signed: ..... Date: 6 July 2000

John Bingham BVSc, DPhil
Head, Rabies Unit
Onderstepoort Veterinary Institute

Appendix 1: Protocol for testing of disinfectant

General principles

1. Rabies virus is mixed with dilutions of disinfectant for set periods of time and at pre-determined temperatures.
2. The suspension will be inoculated onto BHK cells that will be tested to detect the presence of rabies virus.

Materials

Virus suspension (ERA)
BHK cells
Cell culture medium (Glascow MEM with 10% foetal calf serum)
Disinfectant
Cell culture tubes
96-well cell culture plates
Distilled water
Calcium chloride $CaCl_2$
Magnesium chloride $MgCl_2$

Procedure

1. Label seven 15 ml cell culture tubes as follows: F10-10, F10-125, F10-250, F10-500, F10-750, F10-1500, F10-3000.

2. Label eight 5 ml cell culture tubes as follows: WV-D125, WV-D250, WV-D500, WV-D750, WV-D1500, WV-D3000, WV, V. (W = hard water; V = virus; D = disinfectant).
3. Make up a 1:10 dilution (1 ml in 9 ml) of the disinfectant under test in hard water in the 15 ml tube labelled F10-10.
4. In the remaining 15 ml tubes make final disinfectant dilutions in hard water of 125, 250, 500, 750, 1500, 3000 (i.e. dilute the 1:10 dilution to 1:12.5, 1:25, 1:50, 1:75, 1:150 and 1:300).
5. Thaw out a vial of virus concentrate under cold running water. Dilute 2 ml of the virus concentrate suspension into 18 ml of cold hard water.
6. Dilute 200 ul of the concentrate virus suspension into 1.8 ml of GMEM cell culture medium.
7. Aliquot 1 ml of the virus suspension in hard water into each of seven 5 ml tubes (labeled WV). Aliquote 1 ml of the virus suspension in GMEM into the eighth tube labelled V.
8. Add 1 ml of each of the disinfectant dilutions to each respective tube of virus suspension. Do not add any disinfectant to the tubes labelled WV and V
9. Place the 8 tubes on a rotary shaker and mix for 30 minutes at 20C
10. Titrate each of the supernatants in 96-well microtitre plates on BHK-21 cells using four replicates for each supernatant sample (refer to SOP-T015).
11. Repeat steps 1 – 8 but incubate (step 9) at 10C.
12. Incubate the plates at 37C in a CO2 incubator for 2-3 days until the cells are confluent. Fix and stain the cells and read the fluorecence using an ultra-violet microscope.

Recipes

Rabies virus concentrate suspension

1. Make up a suspension of ERA rabies virus suspension on BHK-21 cells as described in SOP-T013.
2. Titrate the suspension on BHK-21 cells (SOP-T015). The suspension must contain at least 6.0 $\log_{10}$ $TCID_{50}$/ml.
3. Store the virus suspension at minus 80C

Hard water

1. Add 0.304 g $CaCl_2$ and 0.139 g $MgCl_2$ to one litre of distilled water
2. Autoclave at 121C for 15 minutes
3. Store at 4C
4. Check that the pH is 7.0 before use

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THIS TEST HAS BEEN CARRIED OUT USING A FIÓ "SUPER CONCENTRATE" SAMPLE

John Temperley
Health and Hygiene
Sunninghill, South Africa

4 May 2000

Dear John

Results of the trial to test the inactivation efficiency of F10 super concentrate disinfectant against rabies virus

Herewith the results of the F10 efficacy trial, as we have been discussing. A more complete report will follow in due course.

The virus titration results of each of the F10 dilutions are given in the table below. The detection thresholds are the minimum dilution values at which a test reading could be obtained. Concentration values higher than the thresholds resulted in cell death.

Table. The virus titres (in $\log_{10}$ $TCID_{50}$/ml), of the test suspensions, with the detection threshold (in $\log_{10}$ $TCID_{50}$/ml) for each titration.

	20°C		10°C	
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John Bingham BVSc, DPhil
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