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TEST REPORT

Anti-viral effect of disinfectant against feline calicivirus

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1. Overall summary

In duplicated suspension tests, exposure to GAMA Healthcare Clinell disinfectant at a test concentration of 50%, consistently resulted in a reduction in feline calicivirus titre of at least 4 log units (99.99% reduction).

2. Introduction

The noroviruses (eg. Norwalk virus) are a common cause of human gastroenteritis. They are transmitted by the faecal-oral route and contamination of food. Infection of populations in close contact (eg. cruise ships) can produce mini-epidemics with significant morbidity. Effective cleansing and disinfection is required to prevent and control such instances.

The noroviruses are a group of viruses within the calicivirus family. Feline calicivirus (FCV) is perhaps the best studied of the caliciviruses in part due to its suitability for growth in cell culture. It has been widely used as a surrogate for anti-viral testing against the noroviruses.

In this study the antiviral effect of a test disinfectant (GAMA Healthcare Clinell) on FCV was evaluated.

3. Materials and methods

3.1 Cells and virus

Crandell-Rees feline kidney (CRFK) cells were used for the propagation and titration of virus. Cells were maintained as adherent cultures using standard techniques.

FCV F9 strain was propagated in CRFK cells. To prepare virus stocks, cells were infected at low multiplicity of infection and freeze-thawed upon observation of complete cytopathic effect. Infected cell lysates were clarified by low speed centrifugation and stored frozen at -80°C.

3.2 Test disinfectant

The test disinfectant was “Clinell” supplied by GAMA Healthcare Ltd.

3.3 Anti-viral testing

3.3.1 Assay for tissue culture infectious dose ($TCID_{50}$)

Test samples were diluted in a ten-fold serial dilution series and 0.1 ml of each dilution added to quadruplicate wells of confluent CRFK cells in 96-well plates (final volume 0.2 ml/well) or 24-well plates (final volume 2.1 ml/well). After 2-3 days, wells were inspected for presence of virus as judged by appearance of cytopathic effect. The virus endpoint titre (dilution required to infect 50% of the wells) was determined using the Spearman-Karber formula and expressed as $TCID_{50}$/ml (or as Log $TCID_{50}$/ml).

3.3.2 Suspension test

Virus stock (0.5 mls) was mixed with an equal volume of disinfectant or control solution (phosphate buffered saline, PBS) for 10 minutes at room temperature (22-25°C). The titre of infectious virus in the sample was measured by the tissue culture infectious dose assay.

3.4 Cell culture cytotoxicity test

The test disinfectant (or a 50:50 dilution of disinfectant in PBS) was diluted in a ten-fold serial dilution series and 0.1 ml of each dilution added to quadruplicate wells of confluent CRFK cells in 96-well plates or 24-well plates and cells observed for cytotoxicity.

4. Results

4.1 Preliminary suspension test for anti-viral disinfectant effect

FCV-infected cell lysate was mixed with an equal volume of PBS or test disinfectant for ten minutes and the infectious viral titre assayed by cytopathic effect on CRFK cells in 96-well plates. The titre of the sample mixed with PBS was 6.75 log $TCID_{50}$/ml (5.62 x 10^6 $TCID_{50}$/ml). The titre of the sample mixed with disinfectant was found to be below the detection threshold of the assay, or less than 3.5 log $TCID_{50}$/ml (3.16 x 10^3 $TCID_{50}$/ml).

Exposure to the test disinfectant therefore resulted in a reduction in viral titre of at least 3.25 log $TCID_{50}$/ml.

The detection threshold of the assay was determined by the cytotoxic effect of the disinfectant on the cells. Cytoxicity of the disinfectant was seen at dilutions of less than 3 log units.

4.2 Confirmation of anti-viral disinfectant effect by suspension testing

In order to validate the result of preliminary testing, suspension testing was repeated in triplicate. In addition, the test was modified by the use of larger culture volumes (24-well plate) in the TCID assay to reduce the cytotoxic effect of the disinfectant, thus increasing the sensitivity of the test. With these modifications in place, cytotoxicity was only seen at dilutions of less than 2 log units and the limit of sensitivity of the assay was 2.5 log $TCID_{50}$/ml (<3.16x10^2 $TCID_{50}$/ml).

FCV-infected cell lysate was mixed with an equal volume of PBS for ten minutes and the infectious viral titre assayed by cytopathic effect on CRFK cells in 24-well plates (figure 1 and table 1). The average of triplicate samples was a titre of 6.5 log $TCID_{50}$/ml (3.16x10^6 $TCID_{50}$/ml). After mixing FCV-infected cell lysate with an equal volume of test disinfectant for ten minutes, the titre of triplicate samples were found to be below the detection threshold of the assay, or less than 2.5 log $TCID_{50}$/ml (<3.16x10^2 $TCID_{50}$/ml).

Exposure to the test disinfectant therefore resulted in a reduction in viral titre of at least 4 log $TCID_{50}$/ml or a reduction of 99.99%.

Exposure to the test disinfectant reduced the virus titre to below the detection threshold of the assay as a result of the cytotoxicity of the test disinfectant. The modification to the TCID assay (described above) improved the sensitivity of the test and allowed the demonstration of a 4 log reduction in viral titre by the disinfectant. However, the stated figure for reduction in viral titre remains a minimum figure. The absolute effectiveness of the disinfectant against feline calicivirus may well be significantly greater than the figures obtained in this study.

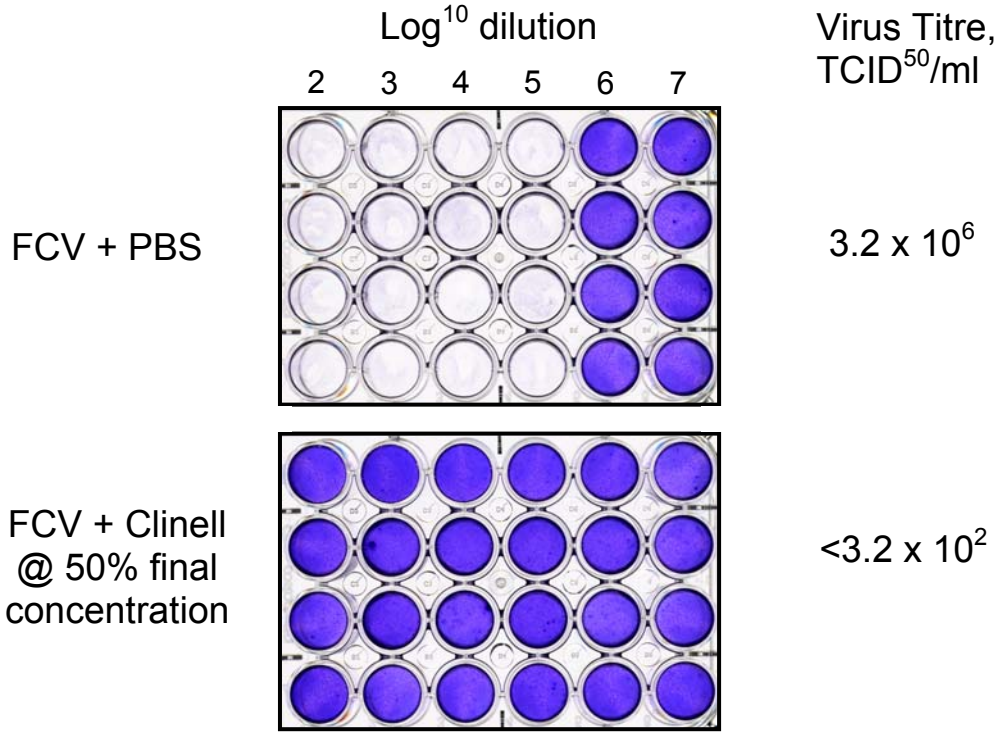

Figure 1. Clinell disinfectant reduces the infectious titre of feline calicivirus. Appearance of representative assay showing no viral CPE in disinfectant treated samples (lower panel) at log dilutions of 2 or more.

Table 1.

			TCID50/ml	logTCID50/ml	Average logTCID50/ml	log reduction	% reduction
Suspension Test	1	+Clinell	≤3.162E+02*	≤2.5*	≤2.5*	≥4.0#	≥99.99#
	2		≤3.162E+02*	≤2.5*			
	3		≤3.162E+02*	≤2.5*			
	4	+PBS	3.162E+06	6.5	6.5		
	5		3.162E+06	6.5			
	6		3.162E+06	6.5			

* Maximum potential titre. Absolute titre obscured by disinfectant toxicity.
# Minimum reduction. Absolute figure obscured by disinfectant toxicity.

5. Conclusions

The effect of a test disinfectant (GAMA Healthcare Clinell) against feline calicivirus (surrogate for norovirus) was investigated using suspension tests.

In a preliminary test, exposure to Clinell disinfectant at a test concentration of 50% for ten minutes resulted in a reduction in feline calicivirus titre of at least 3.25 log units.

In further duplicated tests, a modified protocol to increase the sensitivity of the test was used to confirm the disinfectant activity of Clinell. In these tests, exposure to Clinell disinfectant at a test concentration of 50% for ten minutes resulted in a reduction in feline calicivirus titre of at least 4 log units (99.99%).

GAMA Healthcare Clinell is effective at reducing the infectivity of feline calicivirus to the extent described.

In all tests carried out, exposure to the test disinfectant reduced the virus titre to below the detection threshold of the assay as a result of the cytotoxicity of the test disinfectant. The stated figures for reduction in viral titre are therefore minimum figures. The absolute effectiveness of the disinfectant against feline calicivirus may well be significantly greater than the figures obtained in this study.

6. Additional Information

This study was carried out by Dr Tuthill in the virology laboratory of Professor Rowlands and Professor Killington in the Faculty of Biological Sciences at the University of Leeds.

This report does not constitute an endorsement of the tested article by the author or by the University of Leeds.

The data contained in this report is genuine and derived from experimental work carried out using good standards of laboratory practice.

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