Abstract: P1793

Study on the antiviral efficacy of Citrofresh®, a flavonoid based organic acid complex sanitizer

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Objective: Determine the antiviral efficacy of this organic sanitizer against enveloped and non-enveloped viruses using a carrier based method. Seeking registration for Citrofresh® in Australia and in the EU as a Hospital Grade antiviral sanitizer.
Methods: The study was performed according to the American Society of Testing and Materials (ASTM) Designation (E 1053-85) recommended by the Australian Therapeutic Goods Administration (TGA) to determine the efficacy of a disinfectant intended to use on inanimate, environmental surfaces. We tested Citrofresh® (diluted in standard hard water) in three different concentrations: 1%, 2% and 4% on adherent cell lines (PK-15, MRC-5, MDCK, A549, L929) in four replicates against five different viruses including: Porcine Parvovirus (non-enveloped, high resistant against sanitizer); Human Rhinovirus-16 (non-enveloped, high resistant against sanitizer); Human Adenovirus-4 (non-enveloped, moderate resistant against sanitizer); Human Influenza Type A (H3N2) virus (enveloped, moderate resistant against sanitizer); Human Herpes simplex virus Type 1 (enveloped, low resistant against sanitizers). Prior to the viral testings, acute toxicity assay was carried out to determine the adherent cells viability against Citrofresh®.
Results: Cell lines exhibited >80% viability after exposure to all three concentration. Herpes simplex Type 1, Human Influenza Type A and Human Adenovirus-4 exhibited the most significant viral log reduction of log10 4 to 5 at 4% concentration of Citrofresh® followed by the Human Rhinovirus-16 and Porcine Parvovirus log10 4 reduction at 4% concentration. The reduction of viable virus load was exhibited after 1 minute exposure time to Citrofresh®, which means no time-dependant activity. Citrofresh® clearly exhibited concentration and pH dependent viral load reduction activity against Influenza Type A and the Human Adenovirus -4 and Human Herpes simplex Type 1 virus. The reduction in viral titre for Porcine Parvovirus and Human Rhinovirus-16 is probably pH dependent (the pH of 1% Citrofresh® is 6.5, 2% is 4.5 and 4% is 3.5).
Conclusion: Our investigation shows that Citrofresh® is an effective disinfectant on environmental surfaces, eliminating enveloped and non-enveloped viruses and sufficient to achieve the minimum 4-log reduction with complete viral inactivation which is prerequisite for registration.

STUDY ON THE ANTIVIRAL EFFICACY OF CITROFRESH®, A FLAVONOID BASED ORGANIC ACID COMPLEX SANITIZER

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ABSTRACT

Objective:

To determine the antiviral efficacy of Citrofresh®, a new organic acid sanitizer against enveloped and non-enveloped viruses

Methods:

Citrofresh® was diluted in PBS and RPMI-1640 at 1.0%, 2.5% and 5% and tested against adherent cell lines (PK-15, MRC-5, MDCK, A549/88, L929, PK-15/45), infected with five different viruses.

1. Porcine Parvovirus (non-enveloped, high resistance against disinfectants)
2. Human Rhinovirus-16 (non-enveloped, high resistance against disinfectants)
3. Human Adenovirus-4 (non-enveloped, moderate resistance against disinfectants)
4. Human Influenza Type A (H3N2) virus (enveloped, moderate resistance against disinfectants)
5. Human Herpes simplex virus type 2 (enveloped, low resistance against disinfectants)

Results:

Cell lines exhibited >80% viability after exposure to all three concentration of Citrofresh®

Herpes simplex type 2, Human Influenza type A and Human Adenovirus-4 exhibited a 4-5 $\log_{10}$ reduction in viability at 5% concentration of Citrofresh®

Human Rhinovirus-16 and Porcine Parvovirus showed a 4-fold $\log_{10}$ reduction at 5% concentration.

For Human Rhinovirus-16, Porcine Parvovirus and Human Herpes simplex virus type 2, the inactivation due to Citrofresh® was shown to be both concentration and pH dependent.

Conclusion:

We demonstrated that Citrofresh® was an effective antiviral disinfectant on environmental surfaces, reducing the viability of enveloped and non-enveloped viruses by a minimum 4-fold $\log_{10}$ at 5% concentration.

INTRODUCTION AND PURPOSE

Citrofresh® is a flavonoid based, organic acid sanitizer, under development as a virucidal agent.

The purpose of this investigation was to determine its virucidal efficacy on surfaces, against enveloped and non-enveloped viruses.

The following viruses were tested:

1. Porcine Parvovirus (non-enveloped, DNA virus, high resistance against disinfectants)
2. Human Rhinovirus-16 (non-enveloped, RNA virus, high resistance against disinfectants)
3. Human Adenovirus-4 (non-enveloped DNA virus, moderate resistance against disinfectants)
4. Human Influenza Type A (H3N2) virus (enveloped, RNA virus, moderately resistance against disinfectants)
5. Human Herpes simplex virus Type 2 (enveloped, DNA virus, low resistance against disinfectants)

Small, non-enveloped, viruses show greater resistant towards disinfectants than larger enveloped viruses, probably due to the simple structure of the virion.

The biochemical basis of the action of flavonoids-organic acids based sanitizers on viruses is uncertain.

The literature suggests that the antiviral action is multifocal and probably based on:

1. structural changes of the virion (viral capsid), resulting in the loss of a capsid protein- loss of infectivity similar to uncoating
2. conformational changes of the lipid envelope
3. oxidization-fatty acid degradation of enveloped viruses
4. the effect of lowering pH

Due to the low toxicity of organic acids (ascorbic acid, citric acid, etc.) and flavonoids present in bitter orange extract (rutin, hesperidin), Citrofresh® appears to be a good alternative to chlorine, iodine, peroxide or aldehydes based sanitizers.

METHODS

Human Influenza type A (H3N2) virus was tested on Madin-Darbyn canine kidney (MDCK) adherent cell line

Human Rhinovirus-16 was tested on human foetal lung fibroblast (MRC-5) adherent cell line

Porcine Parvovirus was tested on Porcine Kidney (PK-15/45) adherent cell line

Human Adenovirus type 4 was tested on human lung carcinoma (A 549/88) adherent cell line

Herpes simplex type 2 virus was tested on adherent, monkey kidney cells (VERO)

TWO METHODS USED:

1. SUSPENSION TEST

Summary of test method:

- Viral titre was determined, using 10-fold dilution to achieve at least 10^4 or 10^5 viral unit per ml
- Virus suspension with greater than 10^4 viral units per ml was exposed to 1 and 5 minutes to 1.0%, 2.5% and 5% Citrofresh®
- After 1 and 5 minutes contact time samples were taken and the reaction was terminated with the addition of excess maintenance media
- Host cells were seeded with the diluted reaction media and incubated for 5 days at 37 °C, 5% CO_2 incubator
- CPE s were scored daily

2. CARRIER TEST

Summary of test method:

- Viral titre was determined, using 10-fold dilution to achieve at least 10^4-10^5 TCID viral units per ml
- Virus suspension was dried onto nonporous surface
- 2 ml of Citrofresh® disinfectant was added to the dried film and exposed for 5 minutes at room temperature
- Virus - disinfectant mixture was double diluted in PBS and 50 µl aliquots were seeded onto appropriate host cells and incubated at 37 C° for 1 hour
- Maintenance media was replaced and plates were incubated at 37 °C in 5% CO_2 for up to 5 days
- CPE's were scored daily

Controls included: cytotoxicity control; host cells with maintenance media only; host cells and virus suspension ($TCID_{50}$)

RESULTS

TABLE 1.

PH OF DIFFERENT WORKING CONCENTRATIONS OF CITROFRESH®

Concentration of Citrofresh®	1%	2.5%	5%
pH in RPMI-1640	6.5	5.4	3.4
pH in PBS	6.5	5.4	3.6

pH of Citrofresh® was determined in three different concentration and in two different diluents in order to help to evaluate the effect of the antiviral action of organic acids by lowering pH.

TABLE 2.

CYTOTOXICITY OF CITROFRESH®

Cell lines	Percentage cell survival (%) at 20 minutes: Citrofresh® concentration (% v/v) 1.0	2.5	5
MRC-5	90	85	80
MDCK	100	100	80
A 549/88	100	95	95
PK-15/45	100	95	95
VERO	100	95	95

CONCLUSION:Even at 5% concentration of Citrofresh®, cell monolayers were between 80-95% viable, rendering them suitable for viral viability studies

TABLE 3.

REDUCTION IN VIRAL LOAD (LOG_{10}) FOLLOWING EXPOSURE OF FIVE DIFFERENT VIRUSES TO CITROFRESH® AT THREE CONCENTRATIONS

	Porcine Parvovirus	Human Rhinovirus-16	Human Adenovirus-4	Human Influenza A (H3N2)	Herpes simplex -2
Concentration of Citrofresh®					
1%	N/A	N/A	N/A	1 min: 2.8 5 min: 2.8	N/A
2.5%	5 min: 3.0	1 min: 2.5 5 min: 2.5	5 min: 3.0	1 min: 2.8 5 min: 2.8	5 min: 3.0
5%	5 min: 4.0	1 min: 3.0 5 min: >3.5	5 min: 5.0	5 min: 4.0	5 min: 5.0

CONCLUSION: At 5% concentration and 5 minutes exposure, Citrofresh® caused a 3.5-5.0 log10 reduction in virus viability

CONCLUSION

1. Citrofresh® is exhibited concentration dependent viral load reduction activity against Porcine Parvovirus, Human Rhinovirus-16, Human Adenovirus and Herpes simplex type 2.
2. Citrofresh® at 2.5% concentration and 1 minute exposure is able to remove light or medium viral contamination from surfaces (equal or less than 10^3 viral units per ml.
3. Citrofresh® at 5% concentration and 5 minutes exposure is able to remove heavy viral contamination from surfaces (10^4-10^5 viral units per ml)
4. There were no significant differences in the ability of Citrofresh® to eliminate enveloped or non-enveloped viruses.
5. Herpes simplex type 2 and Human Adenovirus-4 were most sensitive to Citrofresh®, exhibiting a 5-fold $\log_{10}$ reduction in viability with 5% Citrofresh®.
6. Human Rhinovirus-16 and Porcine Parvovirus had a3.5-4-fold $\log_{10}$ reduction.
7. Concentration or pH dependent viral log reduction against Human Influenza A requires further investigation, but it is probably pH dependent.

Citrofresh® is an effective disinfectant on environmental surfaces, eliminating enveloped and non-enveloped viruses to achieve a 4-fold $\log_{10}$ reduction in viability.