



## Monkeypox Virus Inactivation Testing Report

<b>Report identifier</b>	HCM/MPx/004/v2
<b>Report date</b>	5 July 2022
<b>Testing laboratory</b>	High Containment Microbiology, UK Health Security Agency (UKHSA)

<b>Product details</b>	
Product name	70% Ethanol (Prepared from ethanol absolute (99.8+%) diluted in deionised water)
Product code	E/0650DF
Batch number	2216043
Manufacturer	Fisher Chemical
Storage conditions	Ambient temperature
Active substances and concentrations (if known)	70% ethanol
Instructions for use	Not applicable

Report identifier and version number: HCM/MPx/004/v2

Report date: 5 July 2022

Page 1 of 5

**UNCONTROLLED WHEN PRINTED**

<b>Experimental conditions</b>	
Period of analysis	6 June – 13 June 2022
Product test concentrations	10 volumes product to 1 volume test sample
Test temperature	Ambient temperature
Treatment times tested	10 minutes
Sample type tested and virus details	Monkeypox virus stock: monkeypox virus isolate UK2 (GenBank entry MT903344), in tissue culture fluid containing 5% foetal bovine serum
Description of test	<p>Triplicate samples of monkeypox virus tissue culture fluid were treated with product at the indicated test concentration for indicated contact times. Mock-treatments were carried out in triplicate using an equivalent volume of phosphate-buffered saline (PBS) instead of product. After treatment, all samples were subjected to a filtration step to reduce cytotoxic buffer components, using Pierce Detergent Removal Columns in accordance with the manufacturer's instructions. PBS-treated samples were subjected to the same filtration procedure in parallel. All samples were immediately titrated on Vero E6 cells and plates immunostained using an anti-vaccinia virus antibody to establish virus titre. Product only controls (purified and unpurified) were additionally titrated to determine product cytotoxicity before and after filtration.</p> <p>This test is quantitative and reports the virus titre for each treatment condition in focus forming units (FFU)/mL. Reduction in virus titre following treatment is given as the difference between the mean <math>\log_{10}</math> FFU/mL for treated conditions and the PBS control.</p>

<b>Table of results</b>				
<b>Treatment condition</b>	<b>Mean virus titre in FFU/mL</b>	<b>Mean virus titre in log<sub>10</sub> FFU/mL [95% CI]</b>	<b>Titre reduction in log<sub>10</sub> FFU/mL [95% CI]</b>	<b>% reduction in virus titre</b>
<b>PBS-treated</b>	4.5x10 <sup>5</sup>	5.7 [5.6-5.7]	-	-
<b>10-minute treatment</b>	13	≤1.1 <sup>†</sup>	≥4.5 [4.4-4.6]	99.997%

Mean titres are reported as ≤ when at least one replicate was below the limit of detection  
<sup>†</sup>95% confidence interval cannot be calculated

## Results interpretation and limitations

Treatment with 70% Ethanol for 10 minutes reduced virus titre to below the limit of detection of the titration assay. This equates to a  $\geq 4.5 \log_{10}$  reduction in virus titre, or a reduction of  $\geq 99.997\%$ .

Demonstrating complete inactivation is dependent on the starting titre of virus used for testing. Complete inactivation may occur if samples contain lower levels of infectious virus than those tested here, but sample treatments that inactivate virus effectively in these tests may fail to inactivate samples containing higher levels of virus than those evaluated in this study.

This test has been performed using tissue culture fluid. The effectiveness of this treatment against monkeypox virus may vary when used to inactivate clinical samples or other types of sample matrix.

Nucleic acid stability in this product has not been examined, nor has the suitability of this product for inactivation of other pathogens been evaluated in this study. The effectiveness of this product against SARS-CoV-2 has previously been assessed by this laboratory and a treatment time of 10 minutes or more reduced virus titre by  $\geq 6.0 \log_{10}$  (Welch SR et al. (2020). Analysis of Inactivation of SARS-CoV-2 by Specimen Transport Media, Nucleic Acid Extraction Reagents, Detergents, and Fixatives. *Journal of Clinical Microbiology* 58(11):e01713-20. doi: 10.1128/JCM.01713-20).

## **Disclaimer**

UKHSA does not in any way recommend any particular product for virus inactivation; and UKHSA shall not be responsible for the choice of product or treatment for virus inactivation, and it is the responsibility of users of the product to ensure that any such product or treatment implemented has undergone the necessary verification and validation; and UKHSA shall not be liable, to the greatest extent possible under any applicable law, for any claim, loss or damage arising out of or connected with use of this and related reports and choice of virus inactivation products or treatments.

UKHSA is an executive agency of the Department of Health and Social Care.  
Unauthorised use of the UKHSA name and/or logo is prohibited.

## **Summary of revisions**

Version 1: New document

Version 2: Minor edits to disclaimer