UK Health Security Agency

Monkeypox Virus Inactivation Testing Report

Report identifier	HCM/MPx/001/v2
Report date	5 July 2022
Testing laboratory	High Containment Microbiology, UK Health Security Agency (UKHSA)

Product details	
Product name	Cobas PCR Media (from Cobas PCR Media Dual Swab Sample Packet)
Product code	Cobas PCR Media: 08042969001 Cobas PCR Media Dual Swab Sample Packet: 07958021190
Batch number and expiry date (if available)	Cobas PCR Media: F26663; expiry date 30 Nov 2021 N.B. Only expired product available at time of testing
Manufacturer	Roche
Storage conditions	Ambient temperature
Active substances and concentrations (if known)	≤40% (w/w) guanidine hydrochloride
Instructions for use	Swab collected directly into tube containing 4.3mL Cobas PCR media

Experimental conditions	
Period of analysis	28 May 2022 – 3 June 2022
Product test concentrations	10 volumes product to 1 volume test sample
Test temperature	Ambient temperature
Treatment times tested	30 minutes; 60 minutes; 120 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	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 Spin 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. 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 $\log_{10}$ FFU/mL for treated conditions and the PBS control.

Table of results				
Treatment condition	**Mean virus titre in FFU/mL**	**Mean virus titre in log_{10} FFU/mL [95% CI]**	**Titre reduction in log_{10} FFU/mL [95% CI]**	**% reduction in virus titre**
PBS-treated	$4.6x10^5$	5.7 [5.4-6.0]	-	-
30-minute treatment	$1.2x10^3$	3.1 [2.7-3.5]	2.6 [2.2-3.0]	99.738%
60-minute treatment	≤53*	≤1.7†*	≥3.9 [3.6-4.2]	≥99.988%
120-minute treatment	≤93*	≤2.0†*	≥3.7 [3.4-4.0]	≥99.980%

Mean titres are reported as ≤ when at least one replicate was below the limit of detection
*Limit of detection varied between replicates due to differences in buffer toxicity
†95% confidence interval cannot be calculated

Results interpretation and limitations

Treatment with Cobas PCR media for 60 minutes or more reduced monkeypox virus titre to below the limit of detection of the titration assay. This equates to a ≥3.9 $\log_{10}$ reduction in virus titre, or a reduction of ≥99.988%.

Titres were also reduced by 2.6 $\log_{10}$ reduction, or 99.738%, following a shorter 30-minute treatment although virus was readily detectable after this treatment time.

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 4.6 $\log_{10}$ (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
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Summary of revisions
Version 1: New document Version 2: Minor edits to disclaimer