Evaluating the Efficacy of Sigma-MM™ Transport Medium for the Inactivation of Mpox Virus

Dominic Wooding[1], Anushri Somasundaran[1], Nadia Kontogianni[1], Alessandra Romero Ramirez[1], Thomas Edwards[1], Ana Cubas Atienzar[1]

Background

- There is a need to render specimens safe for transportation and testing in locations with limited biological containment facilities
- Sigma-MM™ has been shown to be effective at eliminating various microorganisms from specimens, including RNA viruses such as Influenza and SARS-CoV-2, while maintaining the nucleic acid intact for identification
- We aimed to test the medium for the inactivation of Mpox virus to enable safe transportation of samples for testing via qPCR, following the outbreak in non-endemic countries in 2022
- Orthopoxviruses are large DNA viruses known to be more resistant than other enveloped viruses to certain disinfectants, and have long-term environmental stability

Methods

1

- For **viral inactivation**, three lots of Sigma-MM™ medium were mixed with Mpox virus (approx. concentration 1x10e6PFU/mL) and incubated at room temperature. PBS was used as a control.
- The 3 lots represented different stages of the product shelf life, including one at expiry date.
- Two volumes of virus and two incubation times were used for a total of 12 experimental conditions plus two controls, as shown in **Figure 1** (right)

2

- Following inactivation, the cytotoxic component of the medium was removed using the **PEG-8000 precipitation method**
- PEG-8000 was added to the virus-medium solution to a final concentration of 30% and incubated at 4°C overnight
- Virus was pelleted by centrifugation at 1500rpm for 1 hour. Pellets were washed twice with 500µL PBS and centrifugation at 1500rpm for 10 mins
- Pellets were resuspended in 500µL DMEM + 2% FBS

3

- Concentration of live virus was quantified using a **Plaque Assay**
- Each sample was serially diluted from 1 in 10 to 1 in 10,000
- 10µL of each dilution was added to 190µL DMEM + 2% FBS on individual wells of a confluent 24-well cell culture plate of Vero E6 cells
- Plates were incubated for 1 hour at 37°C
- 500µL of overlay solution (50/50 Cellulose solution/DMEM + 4% FBS) was added to each well
- Plates were incubated for 72 hours at 37°C
- Cells were fixed with formaldehyde solution and stained with crystal violet. An example plaque assay plate is shown in **Image 1**

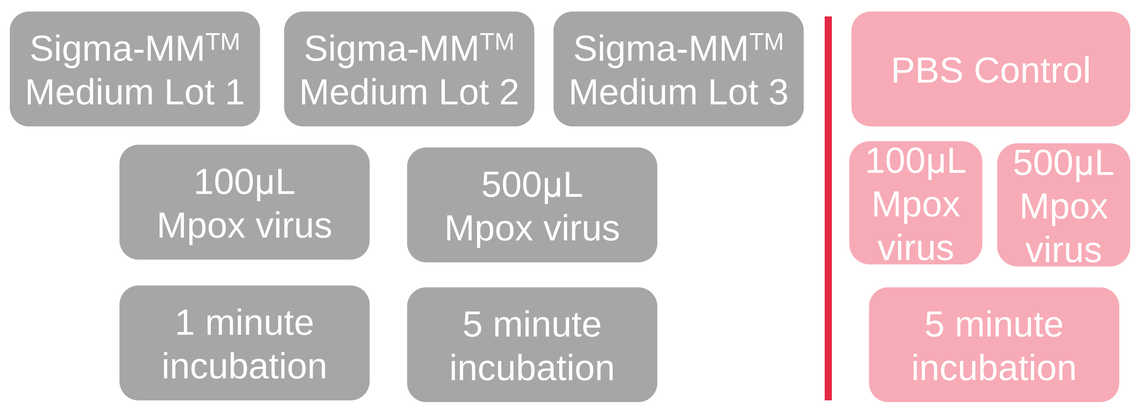

Figure 1: Experimental conditions tested. Each lot of Sigma-MM™ medium was combined with either 100µL or 500µL of Mpox virus and incubated for either 1 or 5 minutes. PBS was used as a control and combined with either 100µL or 500µL of Mpox virus and incubated for 5 minutes

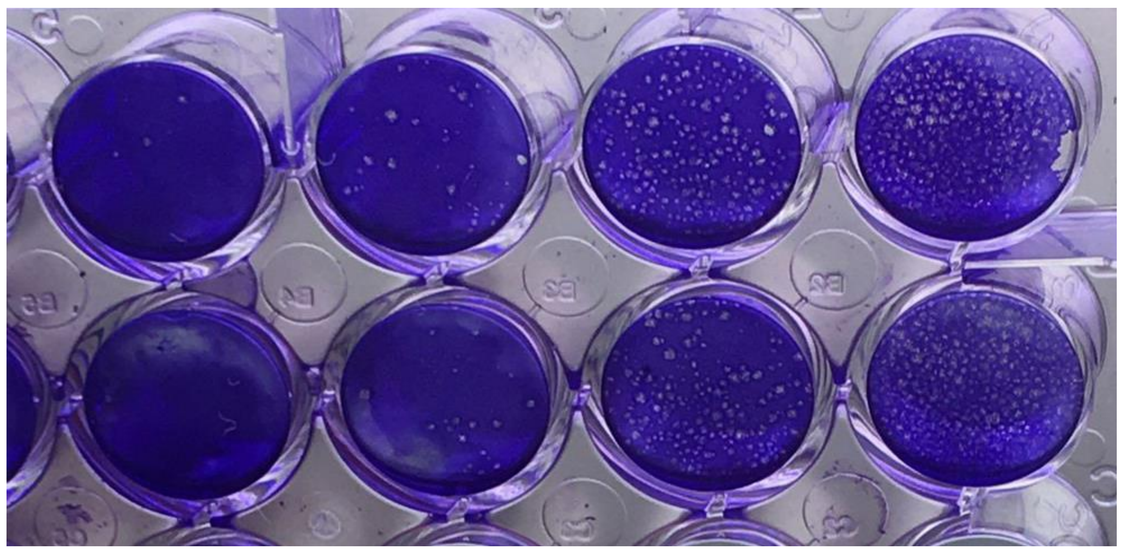

Image 1: A plaque assay plate following staining. The rightmost wells are the undiluted samples, with the serial dilution moving from right to left.

Condition	Buffer		Virus volume (µL)	Inactivation time (min)	PFU/mL			
	Buffer	Buffer volume (mL)			Replicate 1	Replicate 2	Replicate 3	Mean
1	1	1.5	100	1	0	0	0	0
2	1	1.5	100	5	0	0	0	0
3	1	1.5	500	1	0	0	0	0
4	1	1.5	500	5	0	0	0	0
5	2	1.5	100	1	0	0	0	0
6	2	1.5	100	5	0	0	0	0
7	2	1.5	500	1	0	0	0	0
8	2	1.5	500	5	0	0	0	0
9	3	1.5	100	1	0	0	0	0
10	3	1.5	100	5	0	0	0	0
11	3	1.5	500	1	0	0	0	0
12	3	1.5	500	5	0	0	0	0
13	PBS	1.5	100	5	2.8x10e2	6.4x10e2	6x10e2	5.06x10e2
14	PBS	1.5	500	5	1x10e3	1.72x10e3	1.24x10e3	1.32x10e3

Table 1: Concentration of virus present in each sample following incubation with Sigma-MM™ medium at various conditions. Concentration is calculated as plaque-forming units/mL (PFU/mL)

Results

- All three lots of Sigma MM™ at all four conditions had no plaques present in any of the serial dilutions or undiluted resuspended pellet (**Table 1**)
- We calculated an average of 5.06x10e2 and 1.32x10e3 PFU/mL for the controls with 100µL and 500µL of virus respectively
- The above is therefore the titre reduction we were able to calculate for all three buffers at these conditions

Conclusion

- We demonstrated that Sigma–MM™ medium is effective at inactivation (killing) of Mpox virus

[1] Liverpool School of Tropical Medicine, Centre for Drugs and Diagnostics, Liverpool, UK

This research was supported by MWE