Campden BRI
food and drink innovation

Bacteriophage Phi6 As A Surrogate For Coronavirus To Demonstrate The Efficacy Of An Inactivation Medium For Safe Specimen Handling And Transportation

Authors: Annette Sansom & Alice Foxall

Introduction:

PCR analysis has become widely used for the diagnosis and surveillance of viruses, especially those viruses associated with the covid 19 pandemic.

As these types of diagnostic tests do not require the virus to be infectious at the point of testing, use of an effective inactivation medium can render the specimen safe for transportation and analysis. An effective inactivation medium will render the virus non-infectious by rapidly denaturing all viral and host proteins, including nucleases, whilst preserving the genomic nucleic acid.

Σ–MM™ has been on the market for a number of years, during the pandemic it was demonstrated to be compatible with most PCR platforms for detection of SARS-CoV-2 and has previously been shown to effectively eliminate infectious microorganisms including mycobacteria, bacteria and viruses, from specimens.

One previous study commissioned by the manufacturer to measure the inactivation of SARS-CoV-2 in Σ–MM™, has shown it to be very effective, with a log reduction of 5.78 achieved. However, these studies are difficult to carry out with the target virus, enumeration of SARS-CoV-2 is carried out in cell culture, which takes several days, requiring specialised laboratory facilities and biosafety level 3 conditions.

BS EN 144761 provides a framework for evaluating chemical disinfectants for virucidal activity using described viruses that are considered resistant, however the stated enveloped virus is Vaccinia virus and is very different to the target coronaviruses.

Bacteriophages have been used as surrogates to measure virus survival and inactivation, for example the use of the non-enveloped MS2 bacteriophage as a surrogate for norovirus (Dawson et al.[3]). The bacteriophage Phi6 has been suggested to be a useful surrogate for coronavirus (Casanova & Weaver[4]). It is an enveloped RNA virus of similar size and shape to coronavirus.

The advantages of using Phi 6 are:

- Ease of propagation - consistent production of large titres of virus
- Reduced risk to handlers - biosafety level 1 (BSL-1) biohazard rating
- Ease of assay – ability to rapidly and accurately quantify virus infectivity via the use of plaque assay – allowing processing of large numbers of samples

The aim of this study was to use Phi 6 to demonstrate the effective inactivation of infectious virus in the Σ–MM™ inactivation medium.

Materials & Method:

Stocks of Phi 6 (DSM 21518, passage no. 5) were produced. 200µl of Phi 6 were added to 300µl of clean interfering substance (0.3% Bovine Albumin, an interfering substance is used to mimic "other" components that would be present in the sample, eg. saliva) to produce high and low-level inoculum containing 10^8-10^9 pfu/ml (plaque forming units/ml). Separate aliquots (1.5ml) of Σ–MM™ inactivation medium (MWMM, lot: 22A05M) stored at 20°C were inoculated with the inoculum, 500µl inoculum into 1.5ml test medium.

The samples were thoroughly mixed and stored in a shaking water bath at 20± 1°C for the contact time (1, 5 & 30 minutes). At the end of the contact time the samples were removed from the water bath mixed thoroughly and 1ml was transferred to a tube containing 8ml neutraliser (Inactivator BS as described in BS EN 1276) and 1ml of glass distilled water. This was then thoroughly mixed and enumerated for levels of Phi 6.

Several control tests were carried out alongside:

- Interference control (A) – to establish that the neutralised test substance does not act on the host bacteria in such a way as to inhibit the ability of the host bacteria to be infected by the virus
- Toxicity control (B) – to establish if the neutraliser is toxic to the bacteriophage.
- Neutralisation control (C) – to validate that the neutralisation method is effective.
- Cytotoxic control (D) – to establish if the neutraliser is toxic to the host bacteria

For the test to be valid the following conditions need to be met:

- In order to observe the required reduction, the initial suspension of the virus needs to contain a minimum of $1x10^8$ pfu/ml (plaque forming units/millilitre)
- Controls A-C must be within 0.5 logs of the comparator value
- Cytotoxic control (D) – if there is a detectable pfu/ml result of this control, the limit of detection of the assay will be affected

Inoculated test samples of product and controls were enumerated for levels of Phi 6 by use of a plaque assay with *Pseudomonas syringeae* (DSM 21482) as the host bacterium, summarised in Figure 1.

Method that looks for the infectious action of the virus

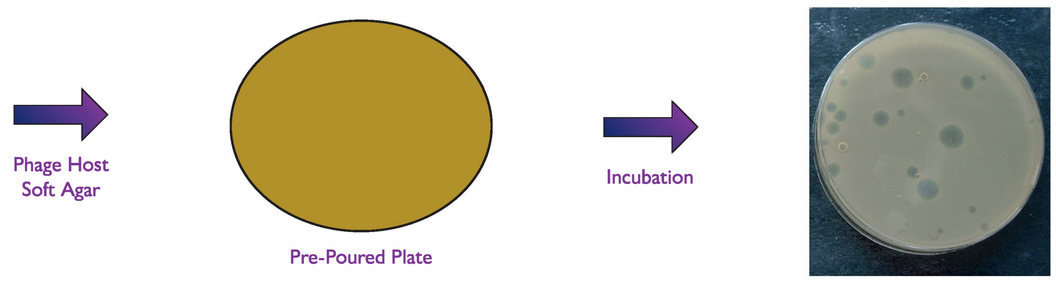

Figure 1 - Bacteriophage plaque assay - Phi6, sample containing phage is mixed with the host bacteria and then soft agar. This is then layered onto a pre-poured agar plate, allowed to set and incubated at 25°C for 18 - 24 hours. The resultant plaques in the bacterial growth is counted and reported as plaque forming units.

Results & Discussion:

The mean results of the three replicate tests are shown in Table 1 and the control verification is described in Table 2. Under all conditions tested, the Σ–MM™ inactivation medium, reduced the levels of Phi 6 to below the limit of detection, from a starting concentration of 10^8 pfu/ml, producing log^{10} reductions of 6.5 - 7.0.

This is similar to the results of the previous study in which SARS-CoV-2 were shown to be reduced to below the limit of detection, when inoculated into the Σ–MM™ providing log_{10} reductions of up to 5.89. BS EN 14476 requires that for a solution to be considered virucidal then a titre reduction of more than 4 log_{10} needs to be demonstrated, both of these trials exceeded this expectation for Σ–MM™.

The use of bacteriophage for this type of test allows for a greater reduction to be observed.

Due to the ease of use with a bacteriophage surrogate, large numbers of samples can be processed, allowing for more extensive trials to be carried out, for example with a range of recipes during development, or under a various storage conditions or inoculation levels, than those that would be possible using a virus that required testing under biosafety level 3 conditions or with use of tissue culture assays.

Table 1 Mean levels of Phi 6 in control & test suspensions and mean log reduction

Innoculum Level	Number of Replicates	Analysis	Level of Phi 6			
			Control	Test *(various application times)*		
				1 minute	5 minute	30 minute
Low	3	Mean Log pfu/ml	8.2	<1.7	<1.7	<1.7
		Standard Deviation	0.15	0	0	0
		Mean Log Reduction	-	6.5	6.5	6.5
High	3	Mean Log pfu/ml	8.7	<1.7	<1.7	<1.7
		Standard Deviation	0.04	0	0	0
		Mean Log Reduction	-	7.0	7.0	7.0

Key – pfu/ml = plaque forming units per millilitre

Table 2. Verification of controls

	Control				Comparator	Cytotoxic Control (D)
	Interference Control (A)	Toxicity Control (B)	Neutralisation Control (C)			
			0 minutes	5 minutes		
Count (Log pfu/ml)	6.1	5.9	6.5	5.9	6.4	<2
Control - Comparator	-0.3	-0.5	+0.1	-0.5	-	N/A
Verified	Yes	Yes	Yes	Yes	-	Yes

Conclusion:

The product, ∑-MM™ (Batch code 22A05M), possessed viricidal activity against Phi 6, an enveloped virus, used as a surrogate for coronavirus i.e. showed greater than a 4-log reduction, when tested by a method based on BS EN 1276:2019 and BS EN 14476:2013 standard methods.

References: 1. *BS EN 14476:2013 Chemical disinfectants and antiseptics. Quantitative suspension test for the evaluation of virucidal activity in the medical area. Test method and requirements (Phase 2/Step 1). BSI. 2. BS EN 1276:2019 Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic and institutional areas - Test method and requirements (phase 2, step 1) 3. Dawson, D.J. et al. (2004) Survival of Viruses on Fresh Produce using MS2 as a Surrogate for Norovirus. Journal of Applied Microbiology, 98, 203-209. 4. Casanova LM, Weaver SR. 2015. Evaluation of eluents for the recovery of an enveloped virus from hands by whole-hand sampling. J Appl Microbiol 118:1210–1216.*