
Test Report MPG 2021.06.03

Evaluation of virucidal activity of citric acid treatment on MS2 Bacteriophage

Client:

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A. Test method

MS2 bacteriophage is a non-enveloped virus that is used as a meaningful surrogate for enteric viruses such as norovirus but MS2 has been also proposed as a conservative surrogate for the SARS-CoV-2 virus, as non-enveloped viruses are more resistant to decontamination than enveloped viruses. MS2 is a virus belonging to the Leviviridae family. It typically attaches to a “male” F pilus on the surface of infects *Escherichia coli* this latter is therefore used for the detection of infective virions.

The modified ISO standard 10705-1:1995 has been used for the present analyses.

The principle is the following: The sample is mixed with a small volume of semisolid nutrient medium. A culture of host strain is added and plated on a solid nutrient medium. After this, incubation and reading of plates for visible plaques takes place.

For the essays, droplets of MS2 phage suspension are added, dried onto the surface of test and control samples and held at room temperature for a 2-hour contact time. Viral particles are collected then assayed for “viable” virus as described above.

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B. Test specimens and treatments

All experiments have been made on PlasmaSPOT equipment with 50% aqueous citric acid solution (CAS 77-92-9).

Each experiment has been made using coupons with a size of 10 x 10 cm.

Material type and Lot number: Deltrian Nanowave ES98PLUS

Sample preparation date: 12.03.2021

Aging protocol: Accelerated aging protocol at 55°C and 88% relative humidity. 37 days aging at accelerated conditions corresponds to 12 months real time aging at 22°C room temperature (according to norm ASTM F1980-16)

Sample ID	Real time aging	# of passes on SPOT	Treatment
Control for 462, 463			No treatment / Control
462	0 month	3	BeCitric Acid 50%
463	0 month	6	BeCitric Acid 50%
Control for 477, 478, 481, 482			No treatment / Control
477	1 month	3	BeCitric Acid 50%
478	1 month	6	BeCitric Acid 50%
481	2 months	6	BeCitric Acid 50%
482	2 months	6	BeCitric Acid 50%
Control for 491, 492			No treatment / Control
491	3 months	3	BeCitric Acid 50%
492	3 months	6	BeCitric Acid 50%
Control for 515, 516, 519, 520			No treatment / Control
515	6 months	3	BeCitric Acid 50%
516	6 months	6	BeCitric Acid 50%
519	9 months	3	BeCitric Acid 50%
520	9 months	6	BeCitric Acid 50%
Control for 526, 527			No treatment / Control
526	12 months	3	BeCitric Acid 50%
527	12 months	6	BeCitric Acid 50%

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C. Test conditions and results

In the next tables, "Samples" designate treated coupons and "Controls" designate non treated samples.

Sample ID	Test description			Results	
MPG MS2 bacteriophage	Quantity of test organism deposited	Number of viral particles spiked (PFU/sample)	Number of viral particles recovered after contact time (PFU/sample)	Viral Load Reduction (log value)	Viral load Reduction (%)
2 h contact time					
Controls (n=3)	20 droplets x 5 µl of MS2 bacteriophage	8.80E+05	5.41E+05	/	
462 (n=2)			6.63E+02	2.90	> 99.92
463 (n=2)			7.25E+02	2.84	> 99.91
Controls (n=3)		7.35E+05	5.16E+05	/	
477 (n=2)			5.10E+04	1.00	> 93.06
478 (n=2)			1.65E+04	1.48	> 97.75
481 (n=2)			3.94E+04	1.12	> 94.63
482 (n=2)			8.69E+03	1.77	> 98.81
Controls (n=3)		1.20E+06	4.64E+05	/	
491 (n=2)			1.10E+04	1.62	> 99.07
492 (n=2)			1.66E+04	1.44	> 98.60
Controls (n=3)		1.20E+06	4.24E+05	/	
515 (n=2)			9.95E+03	1.61	> 99.16
516 (n=2)			1.73E+04	1.39	> 98.55
519 (n=2)			4.32E+03	1.99	> 99.63

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520 (n=2)			7.80E+03	1.71	> 99.34
Controls (n=3)			5.57E+05		
526 (n=2)		1.23E+06	9.58E+03		
527 (n=2)			3.35E+04	1.21	> 97.26

D. Conclusions

Whatever the aging, the log reduction remains above 1 unit. The highest reduction of MS2 loads have been observed for samples without aging (2.8 – 2.9 log reduction).