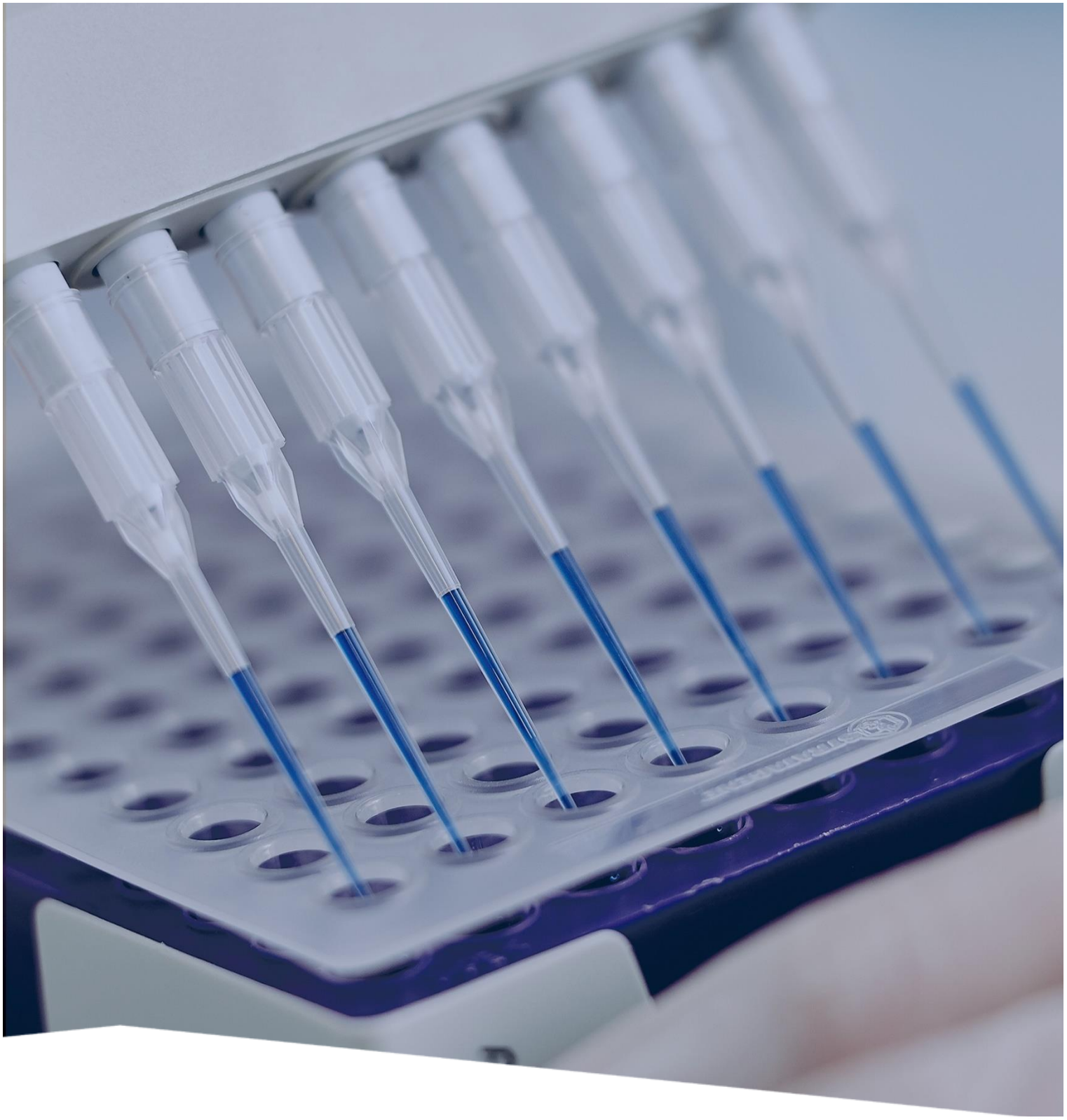

Report no. 966271_1

Inactivation of aerosolized viruses: MS2 bacteriophages

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Summary

The purpose of the test is to determine the efficacy of the air purifier to reduce the concentration of active aerosolized MS2 bacteriophages using a modified ISO 16000-36:2018 method. The tested air purifier is VBreathe Tasman by VBreathe, which utilizes HEPA filtration and vaporization of VAactive gel. The air purifier is tested at maximum fan speed.

MS2 bacteriophages is chosen as virus surrogate as this is a recognized RNA and non-enveloped model virus, that furthermore is robust enough to survive aerosolization and air sampling.

The reduction rate at 30 minutes is 65 % and 47 % at 60 and 120 minutes. The reduction rates are calculated as described in ISO 16000-36:2018 section 8.3.

The measured decay over time of the concentration of active MS2 during the tests is attributed to a natural decay of the aerosol and an attribution of the air purifier. The test only shows a small attribution of the air purifier to the decay of active MS2 in addition to the natural decay over time. The log10-reduction attributed to the air purifier is 0.08. However, the quantification of this attribution is limited by the sensitivity of the test, as the measured log10-reduction of the product test is less than 10% higher than the reference test and thus highly uncertain. The effect of the air purifier is most significant during the first 30 minutes of the test. However, the inactivation rate of MS2 after 30 minutes is similar between the product and reference test.

Method and Materials

The purpose of this test is to determine the air purifier's ability to remove MS2 bacteriophages aerosolized in a test chamber. The natural decay rate of the concentration of active aerosolized MS2 is determined by sampling the air in the chamber over a 2-hour period and the enhanced decay rate due to the air purifier is determined in a similar manner.

The volume of the used chamber is 20 m^3 and it has an inert FEP lining for chemical resistance and easy cleaning. The room is airtight, and a fan is in the room to mix the air and secure a homogenous concentration of aerosols. The aerosol is generated within the room using a nebulizer (Palas AGK 2000) and the air purifier is placed on a table in the middle of the room. See the setup in Figure 1.

The room is cleaned thoroughly and heavily ventilated using clean air prior to the test.

The relative humidity in the test chamber during testing was 25 ± 2 %RH and temperature was 20.5 °C ± 0.5 °C

Figure 1: Image of setup in Test chamber.

The sampling of the air is done through a 6mm stainless steel tube in the sidewall of the room using GilAir plus pump at 4.0 L/min. A total of 20 L is extracted per sample into an impinger with 60mL SM-buffer. The timing of sampling is: 0, 30, 60 and 120 minutes after finishing the aerosolization. The exact time for the sampling is defined by the end of the sampling time of about 5 minutes. The start of the first sample (t = 0 minutes) is less than a minute after the nebulizer is stopped.

In the air purifier test the air purifier is started 24 hours prior to aerosolization of MS2 to allow for a buildup of the active gel by vaporization.

The procedure is the following:

1. The air purifier is switched on 24 hours prior to the aerosolization.
2. A suspension of MS2 in SM-buffer is prepared and the MS2 concentration is determined.
3. A background sample is taken before the test and injection of aerosol.
4. The product test is carried out *with* the air purifier turned on. The nebulizer is working at 2 bar pressure for a total time of 15 minutes.
5. The sampling is carried out according to the timing plan.
6. After the 2-hour test with the air purifier *on*, the room is flushed with clean air for 60 minutes and thoroughly rinsed using water.
7. The same procedure is followed for the reference test to measure the natural decay *without* the air purifier.
8. The sampling is carried out according to the timing plan.
9. The concentration of active MS2 is evaluated for each sample by mixing dilutions series with a fresh culture of the host bacteria, cultivation, and enumeration of PFU following incubation.

The test is performed 8. – 17. February 2021.

Experimental conditions for air cleaning

Test organism:	MS-2 bacteriophage, ATCC 15597-B1
Host organism for MS2:	*Escherichia coli*, ATCC 15597
Growth conditions for enumeration of pfu:	Coliform agar at 37±2°C for 18-24 h
Growth conditions for host organism:	First on TSA plates and then in TSB at 250 rpm. at 37±2°C for 20-24 h.
Sampling and dilution solution:	SM-buffer
Sample volume (SM-buffer):	60 mL per bottle
Test suspension for aerosolization:	SM buffer with $1.5 \cdot 10^{10}$ pfu/mL

Results

The vaporized amount of the VActive gel during the full 24 hours of the test was 7.6 g.

The concentration of active MS2 expressed as PFU/m^3 is shown in Table 1 and in graph in Figure 2. The room background is measured before the first injection of aerosols.

The results concerning the preparation of the test suspension and the enumeration of active virus in the samples, which were performed in an accredited microbiology laboratory, is furthermore presented in a separate report (Report number 965011)

Table 1: The concentration of active MS2 for the Natural decay and the Product test.

Time (min)	Natural decay (PFU/sample)	Product test (PFU/sample)
Background	0	
0	4.60E+05	9.30E+05
30	4.00E+05	2.80E+05
60	4.40E+04	4.70E+04
120	1.40E+04	1.50E+04

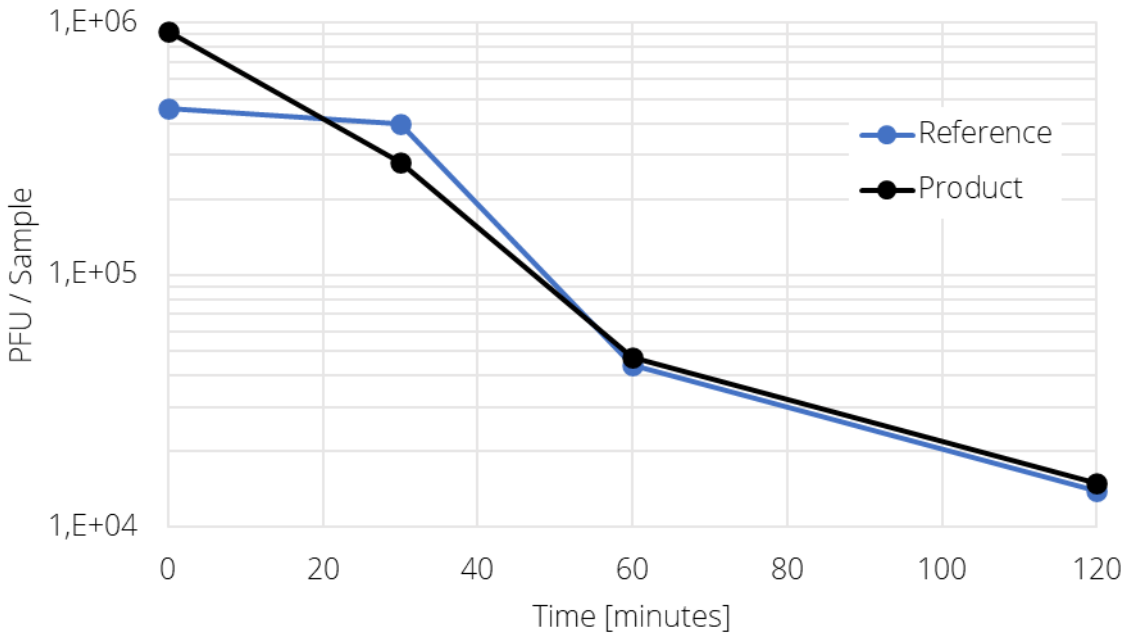

Figure 2: Concentration of active MS2 over time for the product test and the reference experiment.

The air purifier's capacity to reduce active MS2 is calculated based on the difference in absolute concentration at individual sampling points as specified in EN16000-36:2018.

The percentage-wise reduction is calculated as the relative difference in MS2 concentration over time for the product test and the reference experiment (natural decay). The MS2 concentration at t=0 for each run is defined as index 100%.

Table 2: Calculated reduction in MS2-concentration

	Relative Concentration (%)		Reduction in MS2-concentration (%)
Time, minutes	Natural Decay	Product Test	Product Attribution
0	100	100	0.00
30	87.0	30.1	65.4
60	9.6	5.1	47.1
120	3.0	1.6	47.0

In addition to the reduction rate calculated for individual sampling points as above, the log10-reduction over the full 2-hour period is calculated.

The air purifier's attribution to the overall decay of concentration of MS2 is calculated by the difference in decay constant (k) from the exponential fit to both the Natural decay and the Product test decay:

$$Active\ MS2\ [PFU/sample] = a \cdot \exp[-k \cdot time]$$

The decay constants are shown in Table 3. The Product attribution is calculated by subtracting the decay constant of the Product test and the Natural decay.

Table 3: Decay constant and corresponding log reduction.

	Decay constant, [min^{-1}]	Log10-reduction per hour
Natural decay	0.0318	0.83
Product test	0.0348	0.91
Product attribution	0.0030	0.08

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