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Short paper

Measuring the effectiveness of gaseous virus disinfectants

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ABSTRACT

The efficacy of gaseous disinfection is critical for prevention and treatment of microbial contamination in biotechnological facilities. For an evaluation of gaseous disinfection efficacy, a down-scaled laboratory model was established, using currently available carrier tests and a custom-made dry fog box. A mixture of peroxyacetic acid and hydrogen peroxide (PAA/HP) was investigated as example, at concentrations between 0.4 and 2.9 mL/m^3 for up to 3 h for inactivation of a panel of lipid-enveloped and non-lipid-enveloped viruses. The influenza viruses were most sensitive to PAA/HP treatment and minute virus of mice was most resistant. Bovine viral diarrhea virus and reovirus III showed intermediate stability and similar inactivation kinetics. Use of the dry fog box circumvents dedicating an entire lab for the investigation, which renders the generation of data more cost-effective and allows for production of highly reproducible kinetic data.

1. Introduction

Gaseous disinfection is a procedure commonly used to inactivate microbes in biotechnological manufacturing facilities and laboratories. The disinfectants should be effective against all relevant microbial agents; however, information supplied by disinfectant manufacturers does not necessarily cover targeted microbes. This lack of information about relevant targets has prompted a variety of past investigations on disinfectant effectiveness, which were typically performed directly in the laboratory environment [1–5]. The resulting data was rather limited, as the experimental setup did not allow to obtain sequential samples at different times during the inactivation run (kinetic samples) for an assessment of virus inactivation, as requested by regulatory guidance [6,7]. Additionally, a complete laboratory shutdown was required, which is costly and time consuming. These constraints were circumvented by performing small-scale evaluations using biosafety class III cabinets that were connected to vapor generators [8,9]. Biosafety class III cabinets however, are only rarely available. As an alternative and more practical approach we established a robust, simple and cost-effective down-scaled investigation procedure, which simulates the practical conditions around biotechnology applications, i.e. in manufacturing facilities as well as laboratory units that allows for kinetic investigation of gaseous disinfectant procedures. Using a custom made Dry Fog box and currently available carrier tests [10], the virus inactivation capacity of the *Minncare*® Dry Fog decontamination system that uses a mixture of peroxyacetic acid and hydrogen peroxide (PAA/HP) was investigated with a panel of viruses, including the lipid-enveloped viruses bovine viral diarrhea virus (BVDV) and influenza A and B viruses, as well as the non-lipid-enveloped respiratory enteric orphan virus type III (Reo III) and the parvovirus minute virus of mice (MVM).

2. Materials and methods

2.1. Viruses, cells and infectivity assay

Bovine viral diarrhea virus (BVDV, strain Nadl; ATCC, Rockville, Maryland) was titrated on BT cells (ATCC). Influenza A H3N2 virus (H3N2; strain Victoria; WHO Collaborating Centre for Reference and Research on Influenza, Melbourne, Victoria, Australia) and influenza B virus (Flu B; strain Hubei-Wujiagang; NIBSC, Potters Bar, UK) were titrated on MDCK cells (ATCC).

Abbreviations: PAA/HP, peroxyacetic acid/hydrogen peroxide; BVDV, bovine viral diarrhea virus; Reo III, respiratory enteric orphan virus type III; MVM, minute virus of mice; H3N2, influenza A H3N2 virus; H5N1, influenza A H5N1 virus; Flu B, influenza B virus; $TCID_{50}$, median tissue culture infectious dose 50%; rH, relative humidity.

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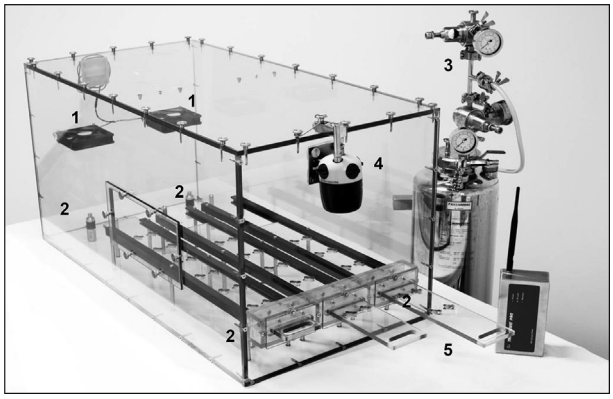

Fig. 1. Custom-made dry fog box. Numbers indicate the ventilators (1), the TS Pro2 X Logger –Units (2), the Dry Fog generating unit with its 3 manometers (3), the diffusion head of the Dry Fog unit (4), and the slipcase with sample holders (5).

Influenza A H5N1 virus (H5N1; strain Vietnam; CDC, Atlanta, Georgia) and respiratory enteric orphan virus type III (Reo III; strain Dearing; ATCC) were titrated on Vero cells (ATCC). Minute virus of mice (MVM; strain Prototype; ATCC) was titrated on A9 cells (ATCC).

Infectious virus titers were determined by median tissue culture infectious dose assay ($TCID_{50}$), using eightfold replicates of serial half-log sample dilutions of virus-containing samples that were titrated on the cell lines indicated above. The cells were incubated at 36 °C, before the cytopathic effect was evaluated by visual inspection under an inverted microscope (Nikon Eclipse TS100). $TCID_{50}$ titers were calculated according to the Poisson distribution and expressed as $\log_{10}$ [$TCID_{50}$/mL]. Virus reduction factors were calculated in accordance to the EU Committee for Proprietary Medicinal Products guidance [7], from at least two independent runs.

2.2. Dry fog box and data acquisition

The in-house custom made Dry Fog box consists of an acrylic glass box (Fig. 1), which is designed to fit into a common laminar flow bench class II (outer dimensions: 1 m × 0.5 m × 0.5 m). The box has a lateral manipulation opening and three rubber-sealed slots at the bottom front side including three movable carrier slides with twelve sample holders each. Two ventilators inside the box ensure the homogenous distribution of the disinfectant which was confirmed by measurements of relative humidity (rH) that did not vary by more than 3% between the different recorders. Temperature and relative humidity was recorded by four TS Pro2 X logger units and a Tracksense Pro Sky Access Point module. Data monitoring was done with the validated software ValSuitePro (Ellab A/S; Hilleroed, Denmark).

2.3. Gaseous disinfection

For all runs, 50 μL of virus stock suspension, as described in ASTM standard E2197-11 [10], was dried two to 4 h on the center point of e-polished stainless steel carriers (Ø 2 cm; AISI type 316L; Baumgartner & Co GmbH, Vienna, Austria) and transferred into the Dry Fog box. A Minntech® Dry Fog unit (Cantel Medical Corp./Mar Cor, Little Falls, NJ), was connected to the diffusion head via a magnetic valve for regulation of 10% aqueous *Minncare*® Cold Sterilant, containing peroxyacetic acid (PAA, 4.5%) and hydrogen peroxide (HP, 22.0%), that was released into the box. PAA/HP solution was applied in four different run designs (I–IV), at increasing concentrations ranging from 0.4 mL/m^3 (run design I) to 2.9 mL/m^3 (run design IV). PAA/HP solution concentrations were calculated according to the Excel spreadsheet "Minncare DRY FOG Calculations" provided by Minncare®. During the first 59 min of run design II (0.8 mL PAA/HP solution/m^3) re-fogging was performed as soon as the rH decreased by 5%. Kinetic samples were drawn after 5 min, 10 min, 30 min, 59 min by pulling the sample holder out of the Dry

Table 1
Virus inactivation by PAA/HP treatment: Mean virus titers and 95% C.I. values are given in $\log_{10}$ ($TCID_{50}$/mL). "Virus Control" is the recovered virus titer obtained after the drying phase. (a) Titers at this sampling stage were used for calculation of the log10 reduction factor. (b) For the influenza viruses H5N1, H3N2 and FLU B the virus titer calculated from successive negative samples is given where no viral infectivity was detected in successive kinetic samples up until the final sample, the volume of all successive negative samples were taken into account for calculation of the assay detection limit. A) lipid-enveloped viruses, B) non-enveloped viruses. n = number of sample series; n.a. = not applicable.

A

Sampling stage	Virus	H5N1	H3N2	Flu B	BVDV	
	Run design (*PAA/HP* [mL/m^3]; n)	I (0.4; 4)	I (0.4; 4)	I (0.4; 4)	I (0.4; 4)	II (0.8; 2)
Virus control (a)		4.3 ± 0.3	3.7 ± 0.3	3.7 ± 0.3	5.5 ± 0.3	5.8 ± 0.3
5 min		<1.1 + 0.6	1.1 ± 0.9	<1.1 + 0.6	5.2 ± 0.3	5.3 ± 0.3
10 min		0.6 ± 0.9	1.1 ± 0.9	<1.1 + 0.6	4.8 ± 0.3	5.1 ± 0.3
30 min		<0.6 + 0.6	1.2 ± 0.9	<1.1 + 0.6	4.0 ± 0.3	4.1 ± 0.3
59 min		<0.6 + 0.6	<1.1 + 0.6	<1.1 + 0.6	3.9 ± 0.3(a)	3.1 ± 0.3(a)
Virus titer calculated from the cumulative volume of successive negative samples (a) (b)		<0.2 + 0.6	<0.8 + 0.6	<0.5 + 0.6	n.a.	n.a.
Virus reduction factor (95% C.I./SD)		>4.1 (−0.6/0.0)	>2.9 (−0.6/0.0)	>3.2 (−0.7/0.0)	1.6 (±0.4/0.2)	2.6 (±0.4/0.1)

B

Sampling stage	Virus	Reo III		MVM			
	Run design (*PAA/HP* [mL/m^3]; n)	I (0.4; 4)	II (0.8; 2)	I (0.4; 4)	II (0.8; 2)	III (1.4; 2)	IV (2.9; 2)
Virus control (a)		5.9 ± 0.3	5.8 ± 0.3	6.7 ± 0.3	6.1 ± 0.3	6.1 ± 0.3	6.1 ± 0.2
5 min		4.6 ± 0.3	4.6 ± 0.3	6.7 ± 0.3	5.7 ± 0.3	5.6 ± 0.3	5.3 ± 0.3
10 min		4.2 ± 0.3	4.2 ± 0.3	6.4 ± 0.2	5.7 ± 0.3	5.2 ± 0.3	4.8 ± 0.3
30 min		3.9 ± 0.3	4.0 ± 0.3	6.3 ± 0.3	5.0 ± 0.3	4.1 ± 0.3	3.7 ± 0.3
59 min		4.0 ± 0.3	4.2 ± 0.3	6.0 ± 0.3	4.7 ± 0.3	4.0 ± 0.3	2.5 ± 0.4
114 min		3.8 ± 0.3	3.9 ± 0.3	5.8 ± 0.3	4.7 ± 0.2	3.1 ± 0.3	1.8 ± 0.6
174 min (a)		3.6 ± 0.3	3.7 ± 0.3	5.7 ± 0.3	4.5 ± 0.3	3.1 ± 0.3	<1.6 + 0.6
Virus reduction factor (95% C.I./SD)		2.3 (±0.4/0.2)	2.1 (±0.4/0.2)	1.1 (±0.4/0.1)	1.6 (±0.4/0.1)	3.0 (±0.4/0.1)	>4.6 (−0.7/0.0)