



CENTRO DE BIOLOGÍA MOLECULAR SEVERO OCHOA

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TO WHOM IT MAY CONCERN

Counterfog Technology Bioaerosol Fast Sampler (BIAFTS)

Our laboratory at Centro de Biología Molecular Severo Ochoa (CBMSO), a biomedical research institute of the Spanish National Research Council (CSIC), has collaborated with Counterfog to test the efficacy of the Bioaerosol Fast Sampler (BIAFTS) to capture viruses present in aerosols.

Initial experiments done with aerosols containing the bacteriophage Phi29, a model virus, have demonstrated that BIAFTS efficiently captures Phi29 present in aerosols, with the same efficiency (100%) as the PFTE (Teflon) filters, previously demonstrated to capture virus-containing aerosols and used as a control. See Example 1.

In addition, the Counterfog technology BIAFTS was able to capture SARS-CoV-2 in the air of a hospital room with COVID-19 patients at Hospital Universitario Fundación Alcorcón, which was detected with a SARS-CoV-2 specific RT-qPCR carried out in our laboratory. See Example 2.

These results demonstrate the utility of the BIAFTS technology developed by Counterfog to capture viruses in aerosols. The technology is expected to capture other human and animal pathogens present in aerosols, and will have many applications in the control of airborne infectious diseases and future pandemics.

Antonio Alcamí, PhD
Research Professor CSIC

Centro de Biología Molecular Severo Ochoa (CSIC-UAM)
Nicolás Cabrera 1
Campus de Cantoblanco
28049 Madrid

Email aalcami@cbm.csic.es

C/Nicolás Cabrera 1
Cantoblanco (Campus UAM)
28049-Madrid .
Teléfono: +34-911964401
Fax: +34-911964420

EXAMPLE 1

Identification of bacteriophage Phi29 in the air of a train carriage

Method: Bacteriophage Phi19 was nebulized using the Counterfog system (CF) in a train carriage. Air was subsequently collected at different times by filtration through PTFE filters in three locations of the carriage (10 min, 30L/min), or with the Counterfog system (2 min, 4 m³/min). Filters were immersed in 0.1% bovine serum albumin in phosphate buffered saline and Counterfog samples collected in liquid media (0.1% bovine serum albumin in phosphate buffered saline) were concentrated with an Amicon 50kDa to 2 ml. Viable Phi19 (expressed as plaque forming units) was determined by plaque assay on *Bacillus Subtilis* lawns. The presence of infectious Phi29 per m³ of air at different times after generating the aerosol is represented.

Result: Phi29 was detected in all samples, taken at different times after aerosol generation. No virus was detected before the aerosol was generated. The amount of virus recovered with the Counterfog system was higher than that retained in PTFE filters.

	CONTROL			t 0_10 min			t 15_25 min					
	1	2	3 CF control	1	2	3 CF 5 min	1	2	3 CF 20 min			
Viral titer, pfu/ml	0	0	0	0	4,25E+05	3,80E+05	3,25E+05	4,15E+05	1,75E+04	1,90E+04	1,60E+04	2,75E+04
Viral titer per volume (2 ml filters, 160 ml CF): TOTAL PFU	0,00E+00	0,00E+00	0,00E+00	0,00E+00	8,50E+05	7,60E+05	6,50E+05	6,64E+07	3,50E+04	3,80E+04	3,20E+04	4,40E+06
Viral titer / m ³ : 0,3 m ³ PTFE filter and y 8,16 m ³ in CF	0,00E+00	0,00E+00	0,00E+00	0,00E+00	2,83E+06	2,53E+06	2,17E+06	8,14E+06	1,17E+05	1,27E+05	1,07E+05	5,39E+05
Virus in the train carriage	0,00E+00	0,00E+00	0,00E+00	0,00E+00	4,43E+08	3,96E+08	3,39E+08	1,27E+09	1,83E+07	1,98E+07	1,67E+07	8,44E+07

	t 30_40 min			t 50_60 min				
	1	2	3 CF 35 min	1	2	3 CF 55 min		
Viral titer, pfu/ml	6,40E+02	1,65E+03	1,25E+03	4,10E+03	1,55E+02	1,98E+02	1,05E+02	6,65E+03
Viral titer per volume (2 ml filters, 160 ml CF): TOTAL PFU	1,28E+03	3,30E+03	2,50E+03	6,56E+05	3,10E+02	3,95E+02	2,10E+02	1,06E+06
Viral titer / m ³ : 0,3 m ³ PTFE filter and y 8,16 m ³ in CF	4,27E+03	1,10E+04	8,33E+03	8,04E+04	1,03E+03	1,32E+03	7,00E+02	1,30E+05
Virus in the train carriage	6,68E+05	1,72E+06	1,30E+06	1,26E+07	1,62E+05	2,06E+05	1,10E+05	2,04E+07

EXAMPLE 2

Identification of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in air samples from hospitals

Method: Air samples from a coronavirus disease 19 (COVID-19) patient room or patient bathroom in Hospital Universitario Fundación Alcorcón (Madrid, Spain) were collected with Polytetrafluorethylene (PTFE) filters (100 min, 30 L/min) or the Counterfog system (1.5-3 min, 4 m³/min). Counterfog samples collected in liquid media (0.1% bovine serum albumin in phosphate buffered saline) were concentrated with an Amicon 50kDa to 2 ml. Viral RNA retained in filters and Counterfog liquid sample were extracted with the Maxwell® RSC Viral Total Nucleic Acid Purification Kit (Promega) in a Maxwell® RSC 48 Instrument (Promega). Purified RNA was analyzed in triplicate by RT-qPCR targeting the N gene (N1 and N2 amplicon) from SARS-CoV-2, and positive samples were considered only when 2/3 replicates were positive. Synthetic SARS-CoV-2 RNA control (Twists Bioscience) was used to build a standard curve for quantification of RNA copies. The Ct value in the RT-qPCR and quantification of SARS-CoV-2 RNA copies per m³ are indicated.

Result: SARS-CoV-2 genomes can be detected in the air of patient rooms by filtration through PTFE filters. Viral RNA can also be detected with the Counterfog sampler. The variability in the amounts of viral genomes detected may reflect the presence of SARS-CoV-2-containing aerosols for short periods of time in the room and the time of sampling. PTFE samples are collected during longer periods (approx. 100 min) whereas the Counterfog sampler detects the presence of virus-containing aerosols during a short time period (1-5-3 min).

AIR SAMPLE	Date	Sampler	Cq_viral	Sample time (min)	Air volume (m3)	viral RNA genomes/m ³
PTFE Filter 1	14/01/2022	PTFE Filter	33,37	103	3,09	258
PTFE Filter 2	14/01/2022	PTFE Filter	33,83	104	3,12	456
Patient Room 1	14/01/2022	Counterfog	0	3	12,24	12
Patient Room 2	14/01/2022	Counterfog	0	3	12,24	16
Patient Room 3	14/01/2022	Counterfog	0	2	8,16	0
Patient Room 4	14/01/2022	Counterfog	33,49	2	8,16	128
Patient Room 5	14/01/2022	Counterfog	0	1,5	6,12	0
Patient Room 6	14/01/2022	Counterfog	0	1,5	6,12	0
PTFE Filter 1	20/01/2022	PTFE Filter	34,67	117	3,51	234
PTFE Filter 2	20/01/2022	PTFE Filter	32,62	109	3,27	650
Patient Room 1	20/01/2022	Counterfog	29,55	2	8,16	1557
Patient Room 2	20/01/2022	Counterfog	36,53	2	8,16	30
Patient Room 3	20/01/2022	Counterfog	37,37	3	12,24	3
Patient Room 4	20/01/2022	Counterfog	36,69	3	12,24	53
Patient Room 5	20/01/2022	Counterfog	38,12	2	8,16	0
Patient Room 6	20/01/2022	Counterfog	0	2	8,16	0
PTFE Filter 1	27/01/2022	PTFE Filter	34,1	112	3,36	157,00
Patient Room 1	27/01/2022	Counterfog	36,45	3	12,24	12
Patient Bathroom 1	27/01/2022	Counterfog	38,32	2	8,16	5
Patient Bathroom 2	27/01/2022	Counterfog	38,22	2	8,16	5
Patient Bathroom 3	27/01/2022	Counterfog	38,96	2	8,16	2
Patient Room 2	27/01/2022	Counterfog	37,36	2	8,16	10
Patient Room 3	27/01/2022	Counterfog	37,56	2	8,16	8
Patient Room 4	27/01/2022	Counterfog	37,64	2	8,16	11
Patient Room 5	27/01/2022	Counterfog	38,39	2	8,16	0