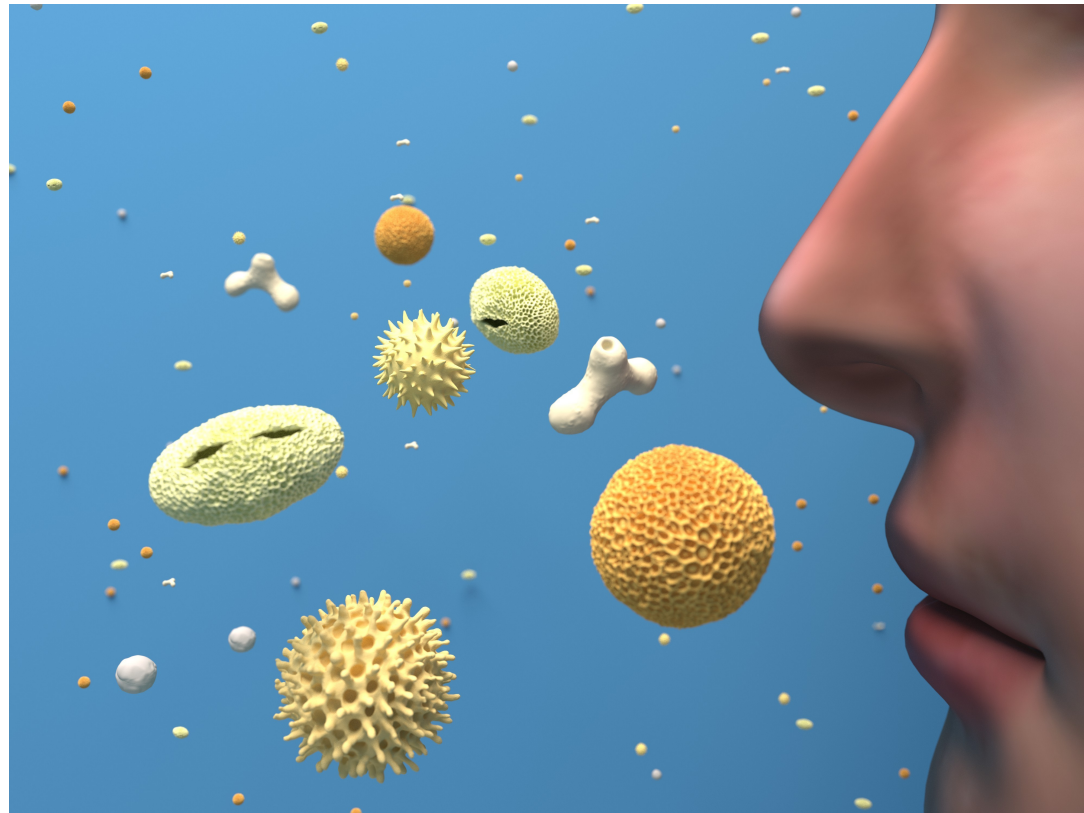


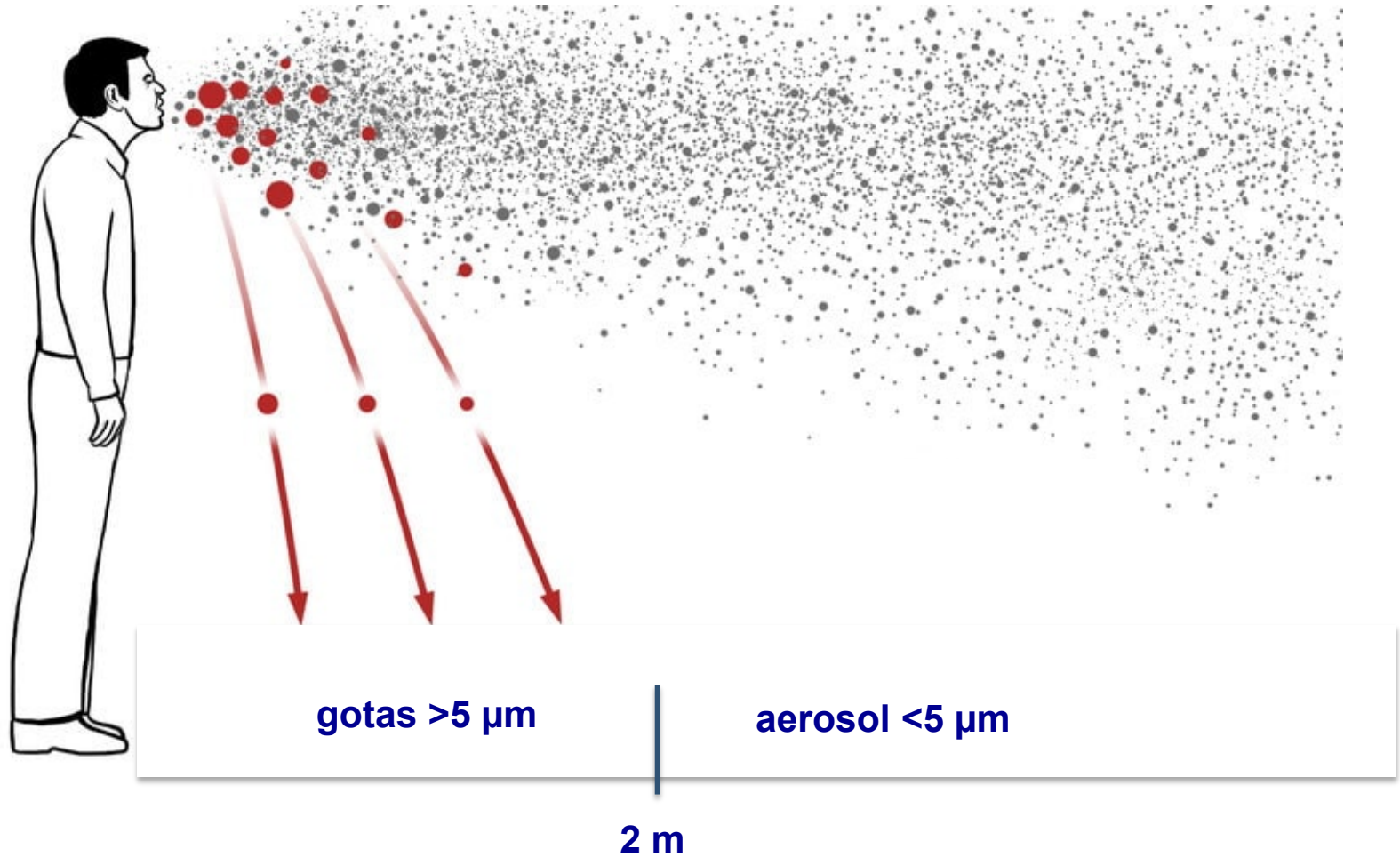
Detección de SARS-CoV2 en muestras de aire: papel en la vigilancia

Antonio Alcamí

Centro de Biología Molecular Severo Ochoa, CSIC-UAM



¿Es relevante la transmisión de SARS-CoV2 por aerosoles?

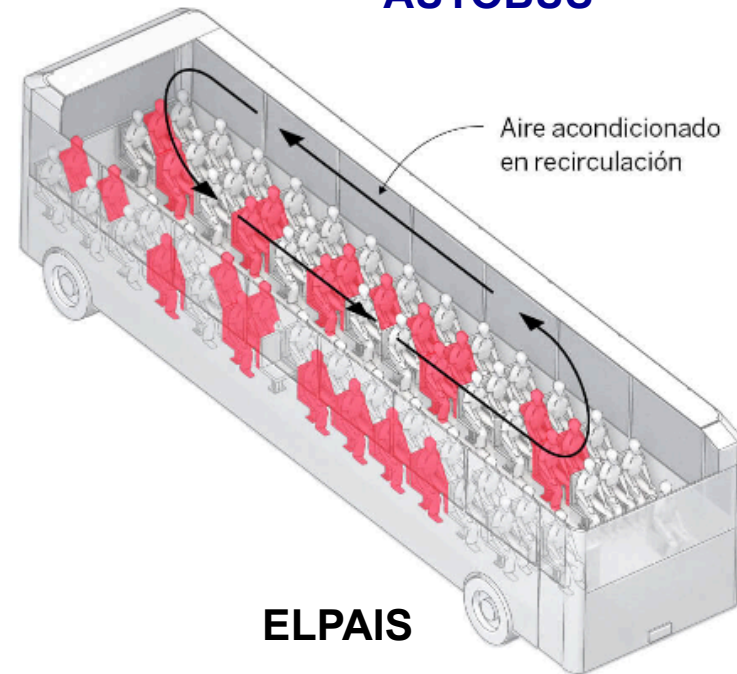


¿Es relevante la transmisión de SARS-CoV2 por aerosoles? Evidencias científicas

EVIDENCIAS EPIDEMIOLÓGICAS

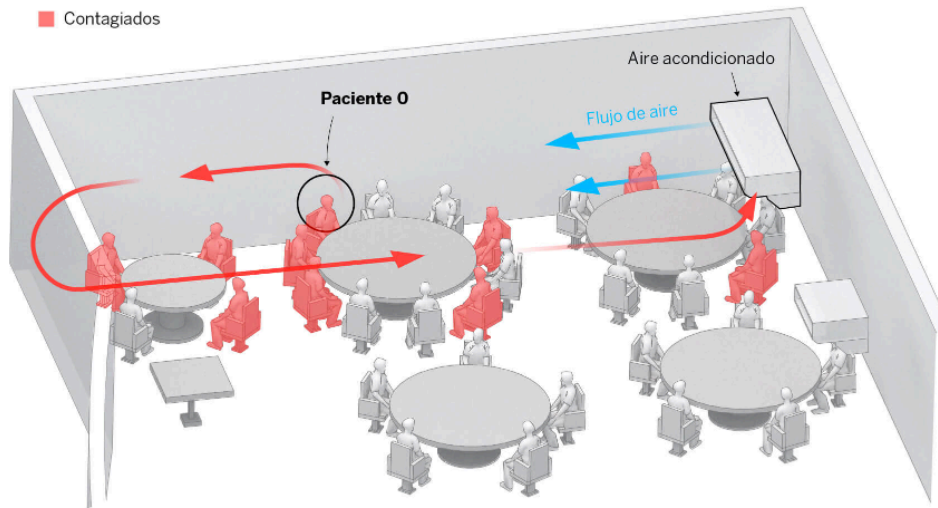
■ 23 contagiados por Covid 19 en el autobús

AUTOBÚS



RESTAURANTE

■ Contagiados

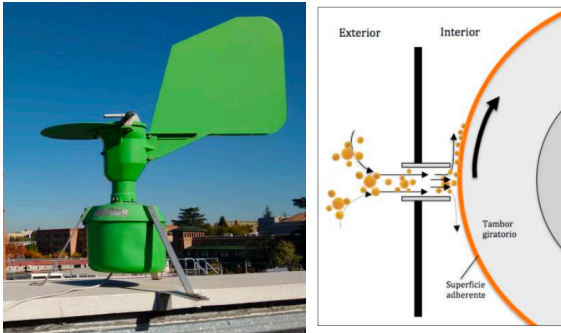


ELPAIS

ELPAIS

Métodos de captación de virus en el aire (I)

Captador Burkard (tipo Hirst): impacto (polen y esporas de hongos)



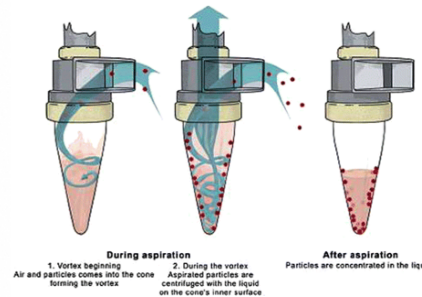
Captador Millipore M Air T: impacto (bacterias)



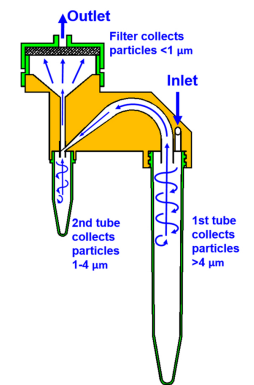
Biosampler: borboteo (polen, esporas hongos, bacterias v virus)



Coriolis biosampler: ciclónico (polen, esporas hongos, bacterias y virus)



NIOSH biosampler: ciclónico (polen, esporas hongos, bacterias y virus)

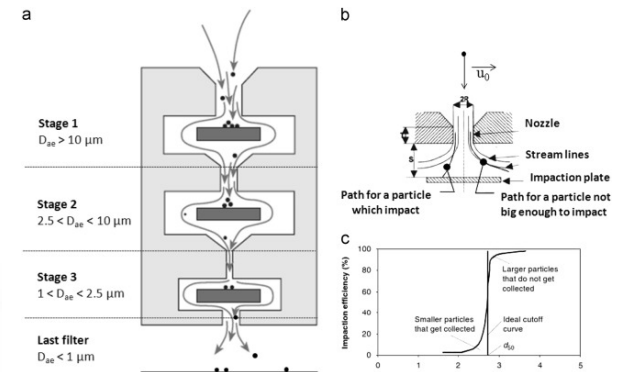


Métodos de captación de virus en el aire (II)

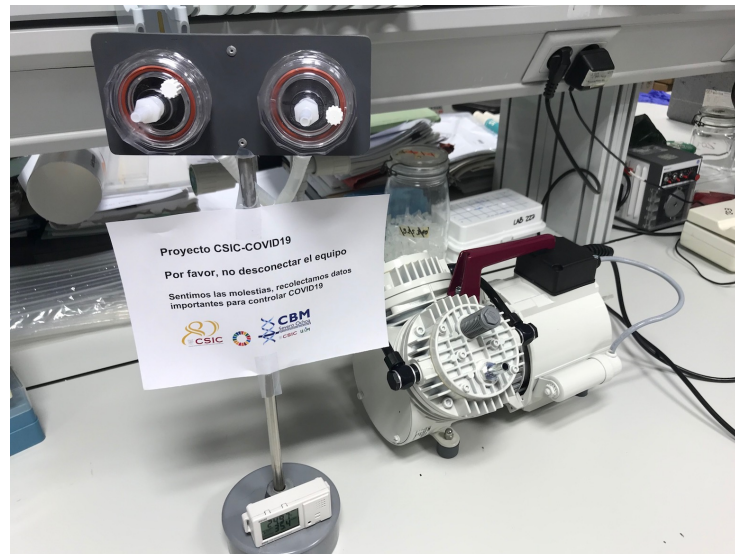
Sartorius filtros gelatina: filtración (polen, esporas hongos, bacterias y virus)



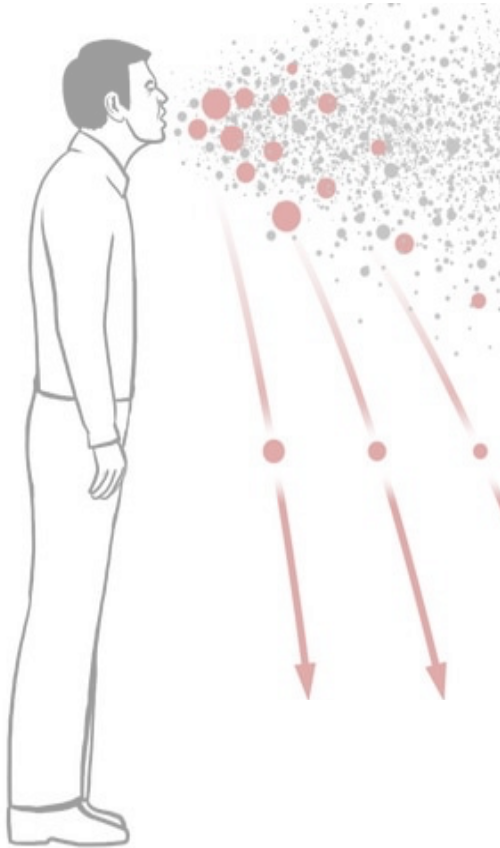
Impactador en cascada: impacto (polen, esporas hongos, bacterias y virus)



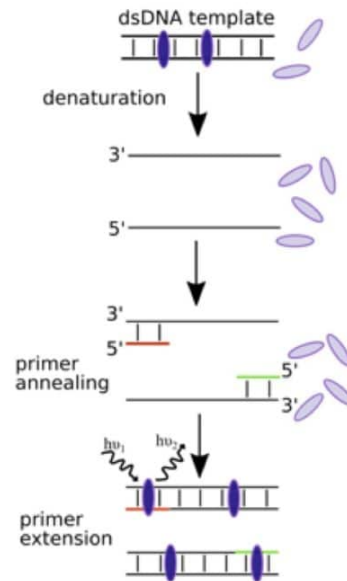
Sistemas filtración teflón (PTFE, polytetrafluorethylene): filtración (polen, esporas hongos, bacterias y virus)



Métodos de detección (qRT-PCR)

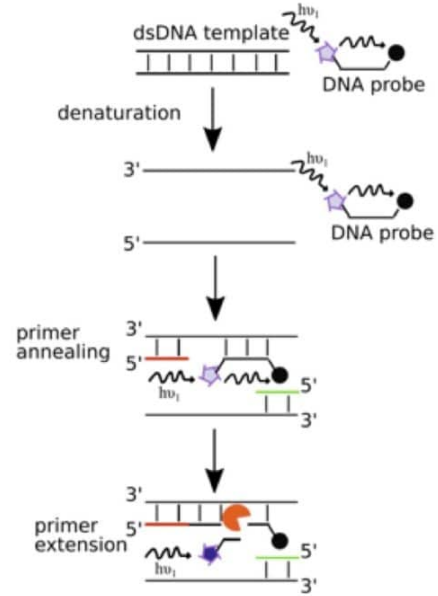


Fluorescent dye-based real-time PCR



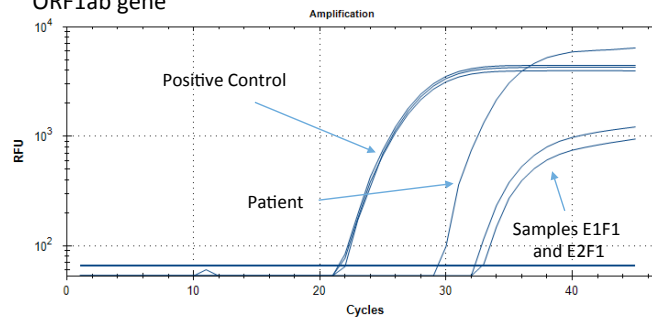
Key:
 ○ ground state fluorophore
 ● excited state fluorophore
 ● fluorescence quencher

DNA probe-based real-time PCR



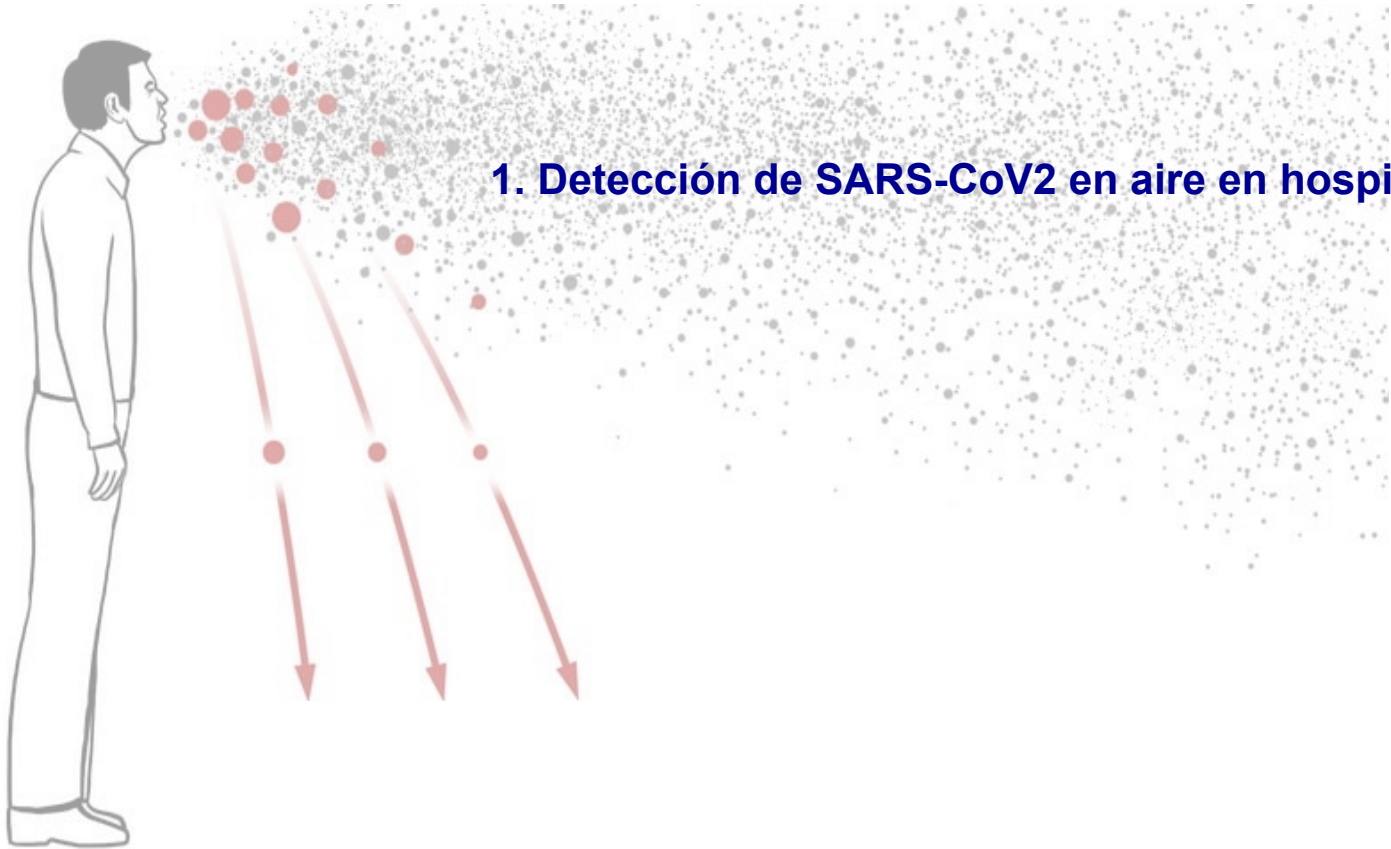
☆ quenched fluorophore
 ● excited state fluorophore
 ● Taq polymerase

ORF1ab gene



¿Es relevante la transmisión de SARS-CoV2 por aerosoles?

EVIDENCIAS CIENTÍFICAS



1. Detección de SARS-CoV2 en aire en hospitales

Detección de SARS-CoV2 en aire de hospitales

nature

Article

Aerodynamic analysis of SARS-CoV-2 in two Wuhan hospitals

<https://doi.org/10.1038/s41586-020-2271-3>

Received: 14 March 2020

Accepted: 20 April 2020

Yuan Liu^{1,6}, Zhi Ning^{2,6,8}, Yu Chen^{1,6,8}, Ming Guo^{1,6}, Yingle Liu¹, Nirmal Kumar Gali², Li Sun², Yusen Duan³, Jing Cai⁴, Dane Westerdahl², Xinjin Liu¹, Ke Xu¹, Kin-fai Ho^{9,8}, Haidong Kan^{4,8}, Qingyan Fu^{3,8} & Ke Lan^{1,8}

Table 1 | RNA concentration of airborne SARS-CoV-2 at different locations in Wuhan

Category	Sites	Sample type	Concentration (copies/m ³)
Patient areas			
Fangcang Hospital	Zone A workstation ^a	TSP ^b	1
		TSP ^c	9
	Zone B workstation	TSP	7
	Zone C workstation ^a	TSP ^b	5
		TSP ^c	0
Renmin Hospital	Patient mobile toilet room	TSP	19
	Intensive care unit	TSP	1
	Intensive care unit	Deposition	31 ^d
	Intensive care unit	Deposition	113 ^d
	Coronary care unit	TSP	0
Ward zone 16	TSP	0	
Medical staff areas			
Fangcang Hospital	PPAR of zone A ^a	TSP ^b	16
		TSP ^c	0
	PPAR of zone B	Size-segregated	0
	PPAR of zone C ^a	Size-segregated ^d	2
		TSP ^b	0
	Male staff change room	TSP	20
	Female staff change room	TSP	1
	Medical staff's office	Size-segregated	2
	Meeting room	TSP	18
	Warehouse ^a	TSP ^b	21
		TSP ^c	0
Renmin Hospital	Passageway for medical staff	TSP	6
	Dining room for medical staff	TSP	6

Workstation 1-9 copies/m³

Intensive care units 0 copies/m³

Protective removal rooms 16-42 copies/m³

Staff change room 11-20 copies/m³

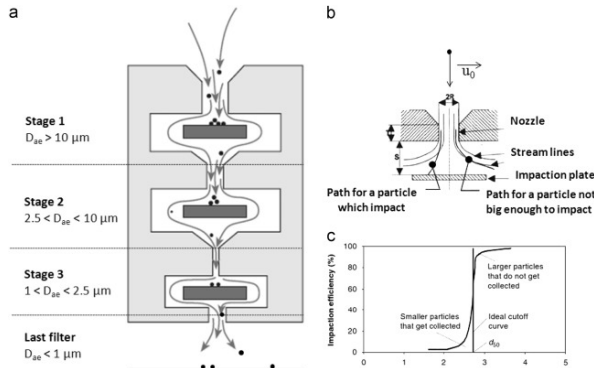
Medical staff room 18-20 copies/m³

Public areas 0-3 copies/m³

Public areas

Location	Sample type	Concentration (copies/m ³)
Fangcang Hospital pharmacy	TSP	3
Renmin Hospital doctor office	TSP	0
Renmin Hospital outpatient hall	TSP	0
Renmin Hospital outdoor	TSP	7
University office doorside	TSP	0
University hospital outpatient hall	TSP	0
Community checkpoint	TSP	0
Residential building	TSP	0
Supermarket	TSP	0
Department store 1	TSP	11
Department store 2	TSP	3
Blank control ^a	Field blank ^b	0
	Field blank ^c	0

Impactador en cascada + droplet digital PCR



Protective removal rooms
0.25-1 μm 10-40 copies/m³
(aerosols deposited on protective apparel)

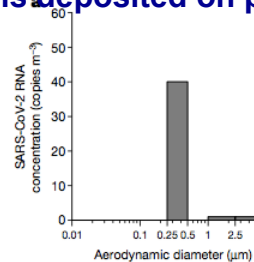
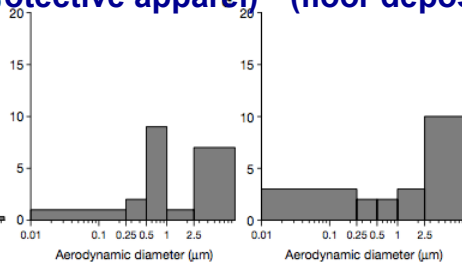


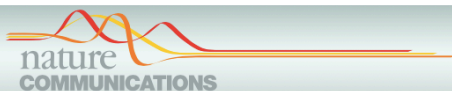
Fig. 1 | Concentration of airborne SARS-CoV-2 RNA in different aerosol size bins. a, Concentration of SARS-CoV-2 in a protective-apparel removal room in zone B of Fangcang Hospital. **b**, Concentration of SARS-CoV-2 in a protective-apparel removal room in zone C of Fangcang Hospital.

Medical staff room
>2.5 μm 10 copies/m³
(floor deposited virus)



c, Concentration of SARS-CoV-2 in the medical staff's office of Fangcang Hospital. The x axis represents the aerodynamic diameter on a logarithmic scale to cover the multiple magnitudes of measured aerosol diameters.

Detección de SARS-CoV2 en aire de hospitales



ARTICLE

<https://doi.org/10.1038/s41467-020-16670-2>

OPEN



Detection of air and surface contamination by SARS-CoV-2 in hospital rooms of infected patients

Po Ying Chia^{1,2,3,11}, Kristen Kelli Coleman^{4,11}, Yian Kim Tan^{5,11}, Sean Wei Xiang Ong^{1,2,11}, Marcus Gum⁵, Sok Kiang Lau⁵, Xiao Fang Lim⁵, Ai Sim Lim⁵, Stephanie Sutjipto^{1,2}, Pei Hua Lee^{1,2}, Than The Son⁴, Barnaby Edward Young^{1,2,3}, Donald K. Milton⁶, Gregory C. Gray^{4,7,8}, Stephan Schuster⁹, Timothy Barkham^{2,10}, Partha Pratim De^{2,3}, Shawn Vasoo^{1,2,3}, Monica Chan^{1,2}, Brenda Sze Peng Ang^{1,2,3,10}, Boon Huan Tan⁵, Yee-Sin Leo^{1,2,3,10}, Oon-Tek Ng^{1,2,3,12}, Michelle Su Yen Wong^{5,12}, Kalisvar Marimuthu^{1,2,10,12} & for the Singapore 2019 Novel Coronavirus Outbreak Research Team*

NIOSH Sampler (ciclónico)

polytetrafluoroethylene (PTFE) filter

1 m from patient

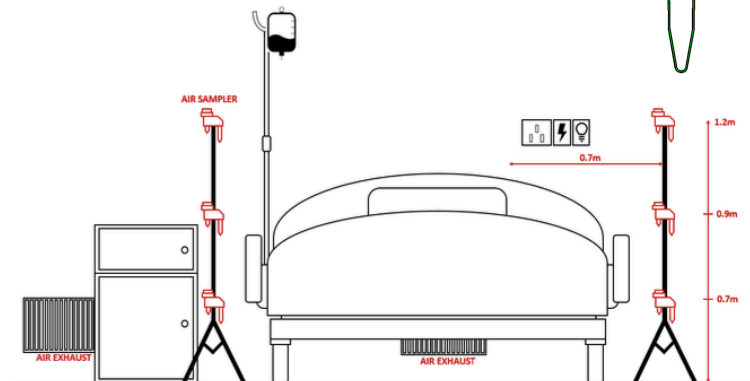
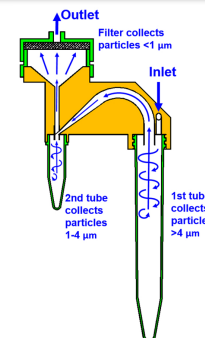


Table 1 Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) detections in the air of hospital rooms of infected patient.

Patient	Day of illness	Symptoms reported on day of air sampling	Clinical Ct value ^a	Airborne SARS-CoV-2 concentrations (RNA copies m^{-3} air)	Aerosol particle size	Samplers used
1	9	Cough, nausea, dyspnea	33.22	ND	$>4\ \mu\text{m}$	NIOSH
				ND	$1-4\ \mu\text{m}$	
				ND	$<1\ \mu\text{m}$	
				ND	-	
2	5	Cough, dyspnea	18.45	2,000	$>4\ \mu\text{m}$	SKC filters NIOSH
				1,384	$1-4\ \mu\text{m}$	
				ND	$<1\ \mu\text{m}$	
				ND	-	
3	5	Asymptomatic ^b	20.11	927	$>4\ \mu\text{m}$	NIOSH
				916	$1-4\ \mu\text{m}$	
				ND	$<1\ \mu\text{m}$	
				ND	-	

ND none detected.

^aPCR cycle threshold value from patient's clinical sample.

^bPatient reported fever, cough, and sore throat until the day before the sampling. Patient reported no symptoms on the day of sampling, however was observed to be coughing during sampling.

qRT-PCR

Quantification?

Deteción de SARS-CoV2 en aire de hospitales

Title: Investigating SARS-CoV-2 surface and air contamination in an acute healthcare setting during the peak of the COVID-19 pandemic in London

Authors: Jie Zhou,^{1*} Jonathan A. Otter,^{2,3*} James R. Price,^{2,3} Cristina Cimpeanu,³ Danel Meno Garcia,³ James Kinross,^{3,4} Piers R Boshier,^{3,4} Sam Mason,^{3,4} Frances Bolt,^{2,3} Alison H. Holmes,^{2,3} Wendy S. Barclay¹

Clinical Infectious Diseases 2020

Coriolis Sampler (ciclónico)

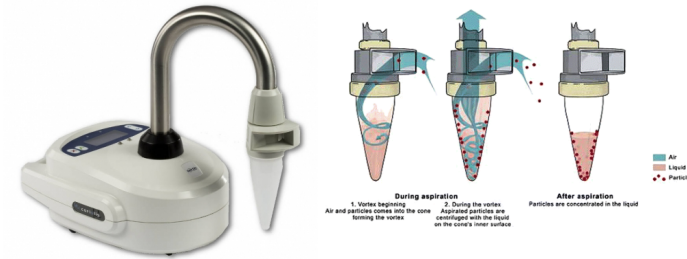


Table 1. PCR results from surface and air samples.

		AIR SAMPLES		
		Result	Concentration (copies/m ³)	Notes
Cohort ward A	Staff room	Negative		
	Nurse station	Negative		
	Toilet B (outside the patients' bay)	Negative		
	Cohort bay B	Positive	7048	
Cohort ward B	Staff room	Negative		
	Patients' toilet (in the ward)	Suspect	464	
	Male bay	Suspect	1335	
	Male bay (side room)	Suspect	163	
Adult acute admission unit	Ward managers office	Negative		
	Nurse station	Positive	404	
	Patient bay 2	Negative		
	Patient bay 1	Negative		
Adult emergency department	'Green' majors	Negative		
	Nurse station	Negative		
	Ambulatory waiting	Negative		
	Patient assessment cubicles			
	Male toilet (next to the nurse station)	Suspect	35	
	Resus bay (last patient > 2 hours)	Suspect	1574	

Area	Location	Result	Concentration	Notes
Hospital public areas	QEQM main entrance	Suspect	1574	
	Male toilet at QEQM main entrance	Suspect	1545	
	Lift area QEQM ground floor	Negative		
Temporary CPAP ward	Nurse station	Suspect	1922	
	CPAP unit	Suspect	31	< 1m from 2 patients
		Negative		> 2 m from patients
	PPE doffing area	Negative		
Adult ICU	Staff room	Suspect	249	
	Nurse station inside ICU	Negative		
	Bay area	Suspect	164	
	Side room bay area	Suspect	307	
Theatres	Theatres	Negative		Before tracheostomy
		Negative		During tracheostomy
		Suspect	1163	During tracheostomy
		Negative		During tracheostomy
Total		2/31 (6.4%) positive; 12/31 (38.7%) suspect		

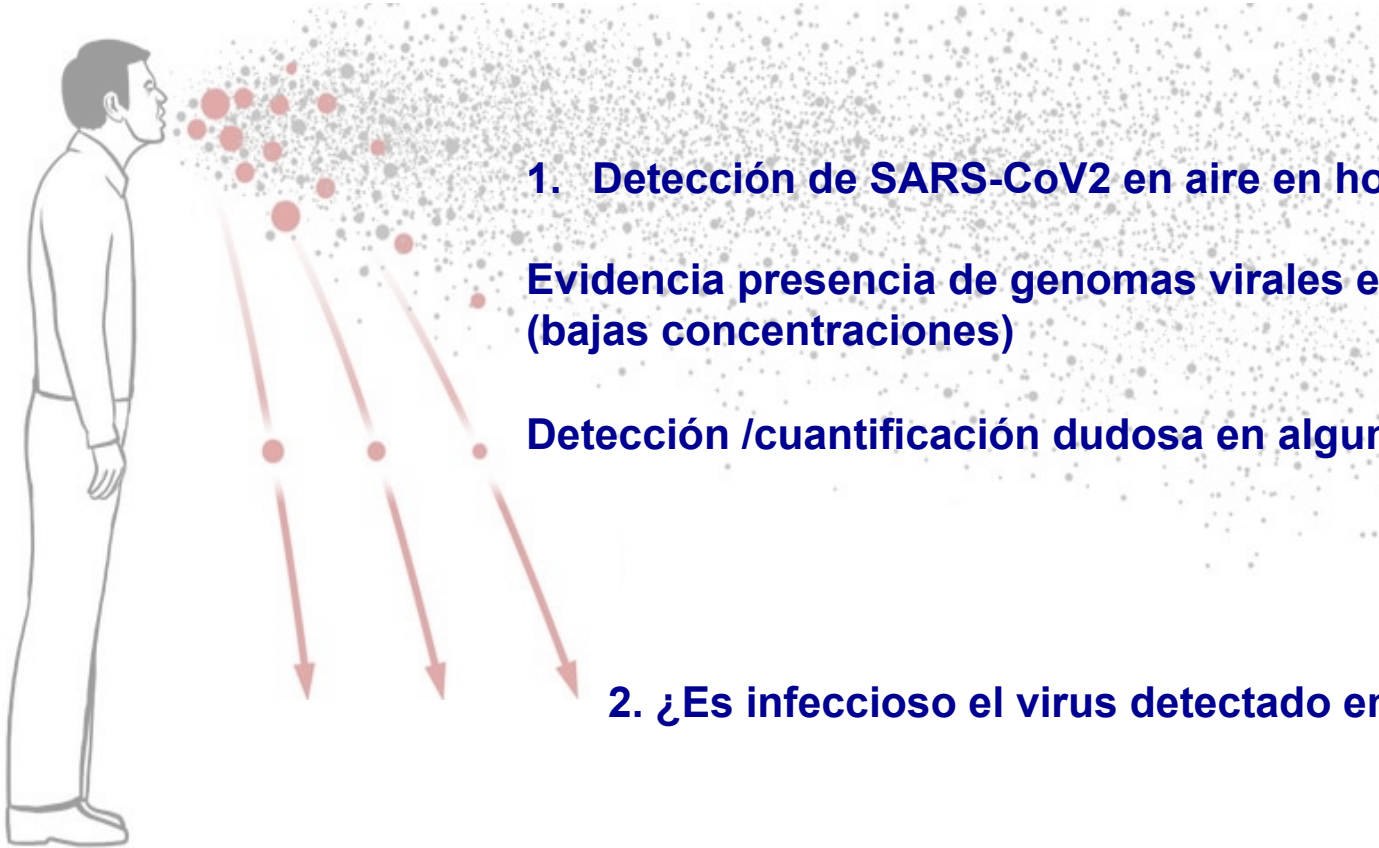
qRT-PCR

Viabilidad (NEGATIVO)

2 positive
 Cohort ward (7048 copies/m³)
 Admission unit (404 copies/m³)
 12 suspect (31-1922 copies/m³)
 17 negative

¿Es relevante la transmisión de SARS-CoV2 por aerosoles?

EVIDENCIAS CIENTÍFICAS



1. Detección de SARS-CoV2 en aire en hospitales

**Evidencia presencia de genomas virales en el aire
(bajas concentraciones)**

Detección /cuantificación dudosa en algunas publicaciones

2. ¿Es infeccioso el virus detectado en el aire?

Viabilidad de SARS-CoV2 en aire de condiciones experimentales

The NEW ENGLAND JOURNAL of MEDICINE

CORRESPONDENCE



Aerosol and Surface Stability of SARS-CoV-2 as Compared with SARS-CoV-1

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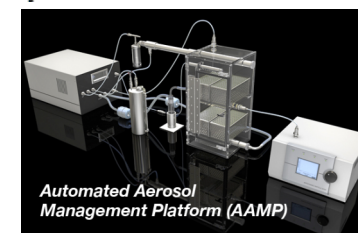
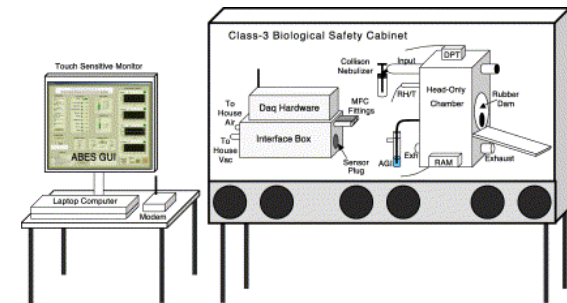
Comparative dynamic aerosol efficiencies of three emergent coronaviruses and the unusual persistence of SARS-CoV-2 in aerosol suspensions

Fears, A.C.¹, Klimstra, W.B.², Duprex, P.², Hartman, A., Weaver, S.C.³, Plante, K.C.³, Mirchandani, D.³, Plante, J.A.³, Aguilar, P.V.³, Fernández, D.³, Nalca A.⁴, Totura, A.⁴, Dyer, D.⁴, Kearney, B.⁴, Lackemeyer, M.⁵, Bohannon, J.K.⁵, Johnson, R.⁵, Garry, R.F.¹, Reed, D.S.², Roy C.J.¹

Infectividad < 3 h



Infectividad < 16 h

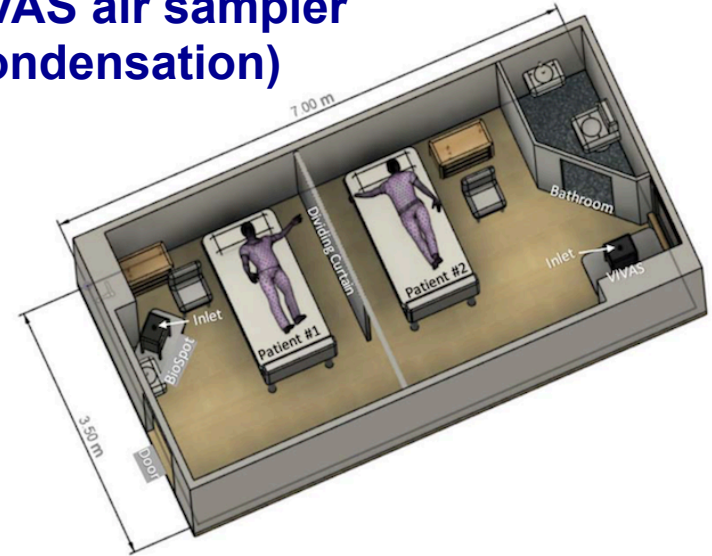


Automated Aerosol Management Platform (AAMP)

Viabilidad virus en aerosoles

medRxiv preprint doi: <https://doi.org/10.1101/2020.08.03.20167395>; this version posted August 4, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted medRxiv a license to display the preprint in perpetuity. It is made available under a [CC-BY-ND 4.0 International license](https://creativecommons.org/licenses/by-nd/4.0/).

VIVAS air sampler (condensation)



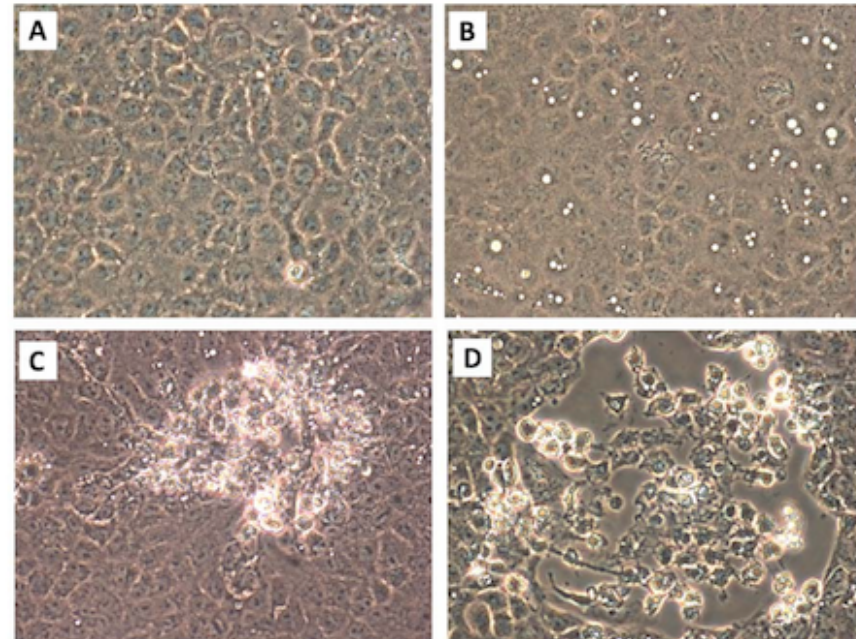
- 1 **Viabile SARS-CoV-2 in the air of a hospital room with COVID-19 patients**
- 2
- 3 John A. Lednicky, PhD^{1,2*}, Michael Lauzardo, MD^{2,3}, Z. Hugh Fan, PhD^{4,5}, Antarpreet Jutla, PhD,⁶
- 4 Trevor B. Tilly, PhD,⁶ Mayank Gangwar⁶, Moiz Usmani⁶, Sripriya Nannu Shankar⁶, Karim Mohamed⁵,
- 5 Arantza Eiguren-Fernandez, PhD⁷, Caroline J. Stephenson^{1,2}, Md. Mahbulul Alam^{1,2}, Maha A. Elbadry,
- 6 PhD^{1,2}, Julia C. Loeb^{1,2}, Kuttinchantran Subramaniam, PhD^{2,8}, Thomas B. Waltzek, PhD^{2,8}, Kartikeya
- 7 Cherabuddi, MD³, J. Glenn Morris, Jr., MD^{2,3}, and Chang-Yu Wu, PhD⁶
- 8

Table 3. Estimate of viable virus counts based on TCID₅₀ tests.

Sample ID	Virus genome equivalents/L of air ^a	TCID ₅₀ /100 μl	Viable virus count/L air
1-1 BioSpot	94	2.68E+04	74
1-2 BioSpot + HEPA	-	0	0
1-3 BioSpot	30	6.31E+03	18
2-1 VIVAS	44	1.00E+04	27
2-2 VIVA S+ HEPA	-	0	0
2-3 VIVAS	16	2.15E+03	6

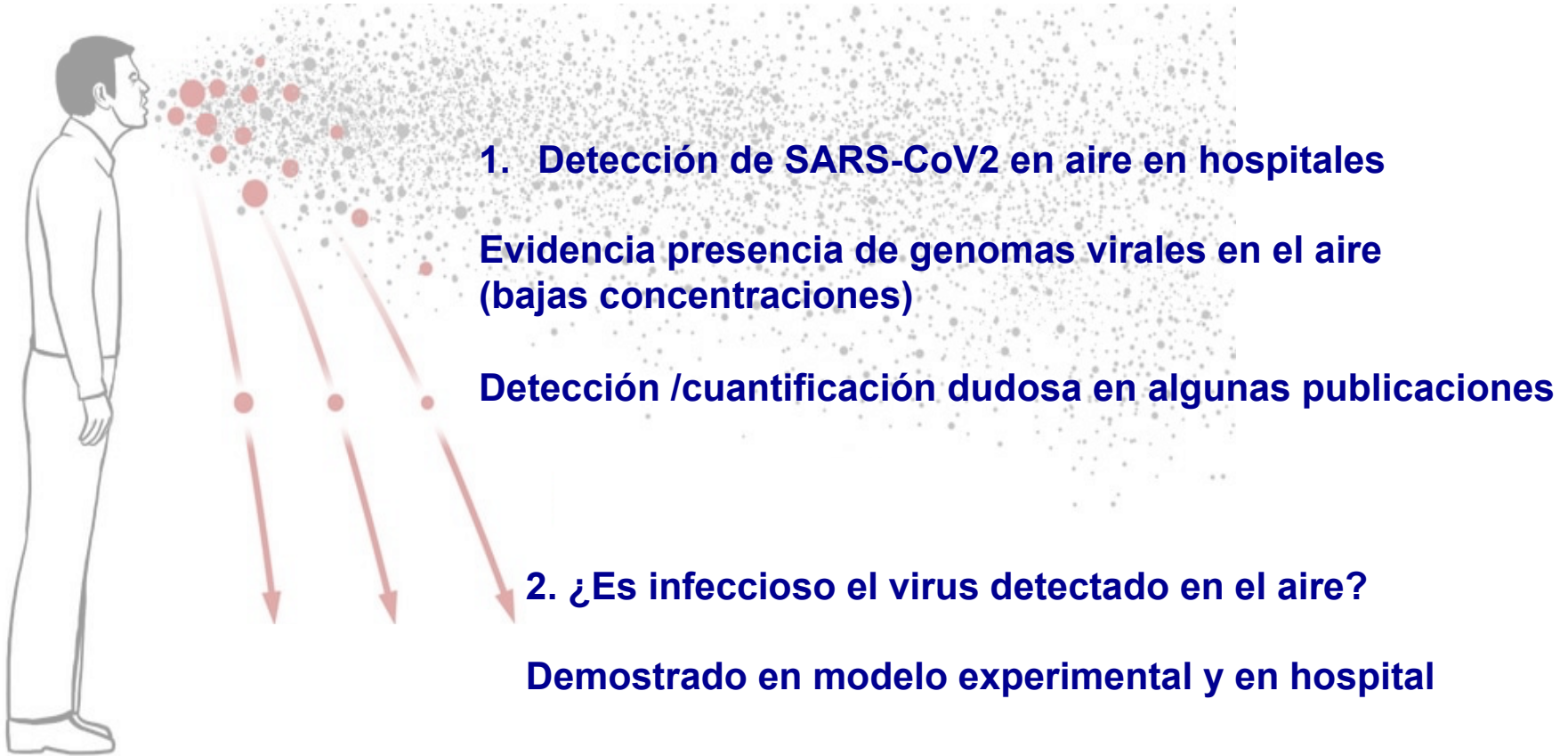
^aFrom Table 2.

6-74 viable virus/l air



¿Es relevante la transmisión de SARS-CoV2 por aerosoles?

EVIDENCIAS CIENTÍFICAS



¿Cuál es la dosis de virus necesaria para iniciar infección?

Monitorizar SARS-CoV2 en el aire en hospitales



Captura de virus en filtros, detección por RT-qPCR y secuenciación de genomas

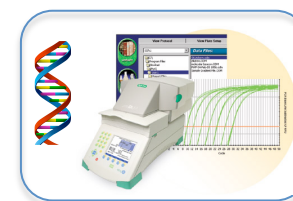
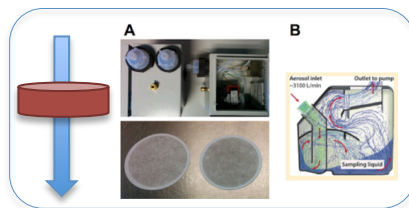
A. Alcamí, I. Casas, C. Calvo, M. L. García

Lab A. Alcamí: A. Rastrojo, B. Hernaez, R. Martín

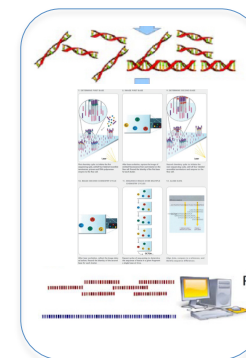
Servicio Genómica: L. Tabera, F. Carrasco, B. Aguado



Captura microbiota aire (filtro/impinger)



Amplificación directa qPCR / digital PCR (SARS-CoV2 genome)

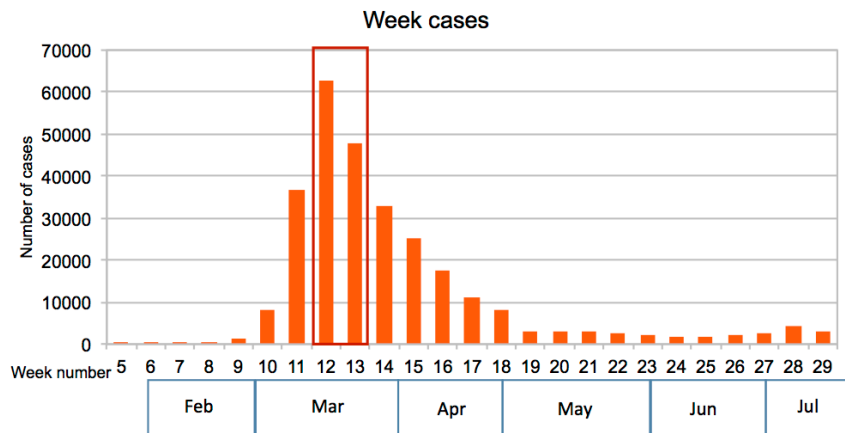


Metagenómica Secuenciación Illumina/MinION Bioinformática



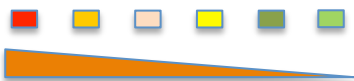
Muestreo en el Hospital La Paz, Madrid

Cristina Calvo
20/03/2020 – 15/05/2020



RT-qPCR (cuantitativa)

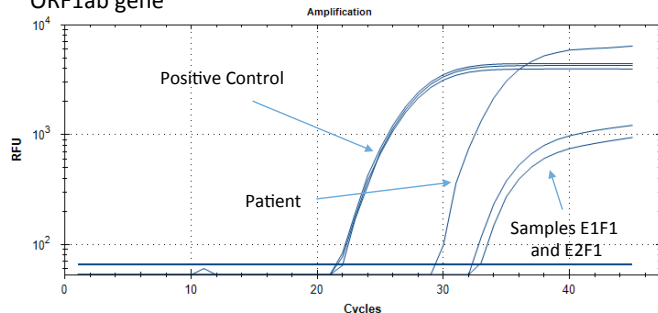
Control positivo Ct 20-25
Paciente Covid+ Ct 30
Negativo > Ct 40



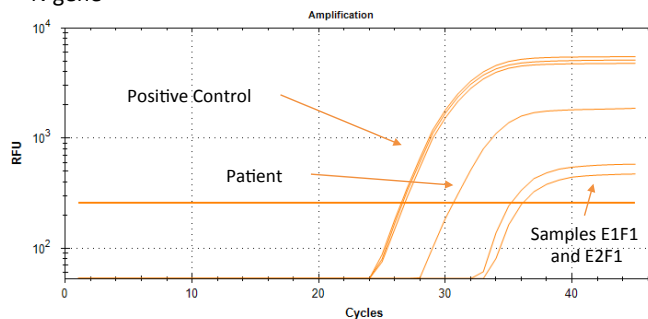
Device	Filter	Week 1	
		ORF-1ab	N gene
Neonatos	1	-	38,9
	2	-	-
Personal Sanitario	1	35,3	37,0
	2	33,8	35,7
Cuidados Intensivos 1	1	-	-
	2	36,1	-
Urgencias Pediatría	1	-	-
	2	-	-
Cuidados Intensivos 2	1	-	-
	2	35,0	36,9
Positive Control	-	22,88	26,29

Sample	Filter	Week 2	
		ORF1ab	N
Neonatos	1	-	-
	2	32,7	34,8
Personal Sanitario	1	-	36,3
	2	32,0	34,1
Cuidado Intensivos 2	1	38,4	38,5
	2	40,7	40,1
Urgencias Pediatría	1	32,8	36,1
	2	34,9	35,3
Cuidados Intensivos 2	1	33,5	34,9
	2	-	37,3
Positive Control	-	21,7	25,8

ORF1ab gene



N gene



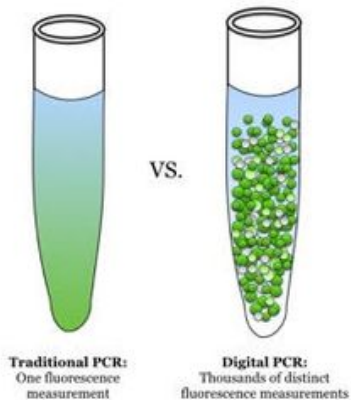


Muestreo en el Hospital La Paz, Madrid

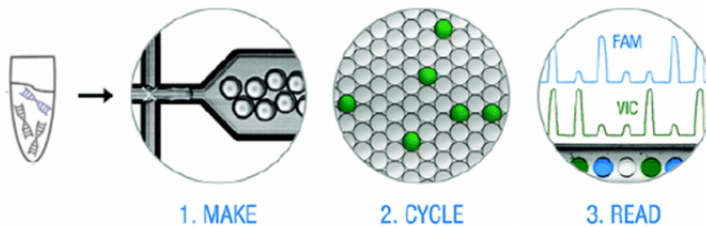
airCOVID19

GENGS GENOMICS & NGS

Cuantificación: Droplet digital PCR



Droplet digital PCR



Bio-Rad QX100

1. MAKE
Sample is partitioned into 20,000 droplets

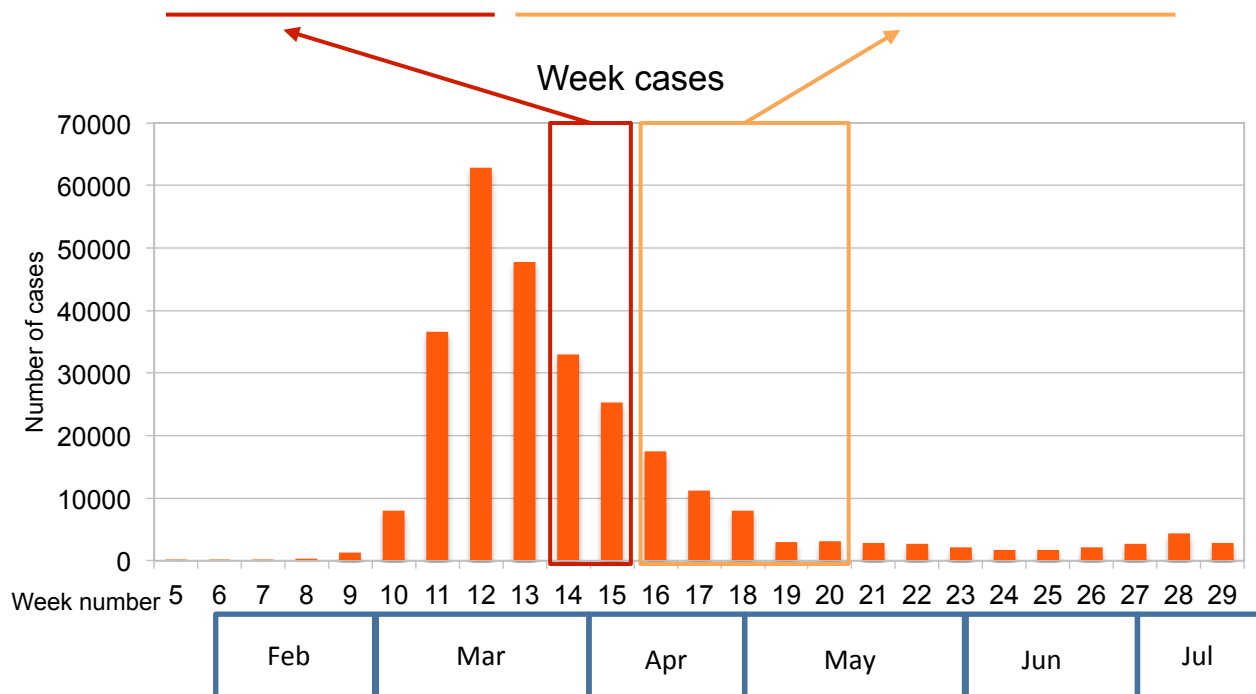
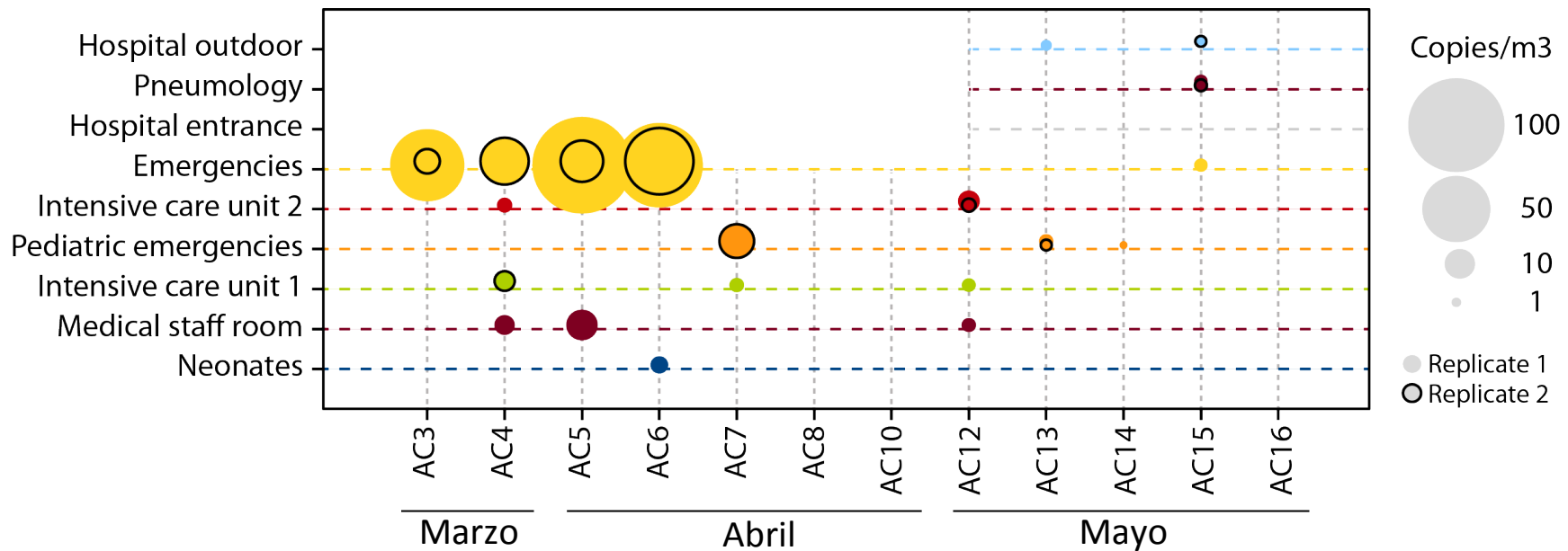
2. CYCLE
Run PCR cycles in all droplets simultaneously

3. READ
Measure fluorescence intensity in each droplet

Calculate concentration from number of positive droplets

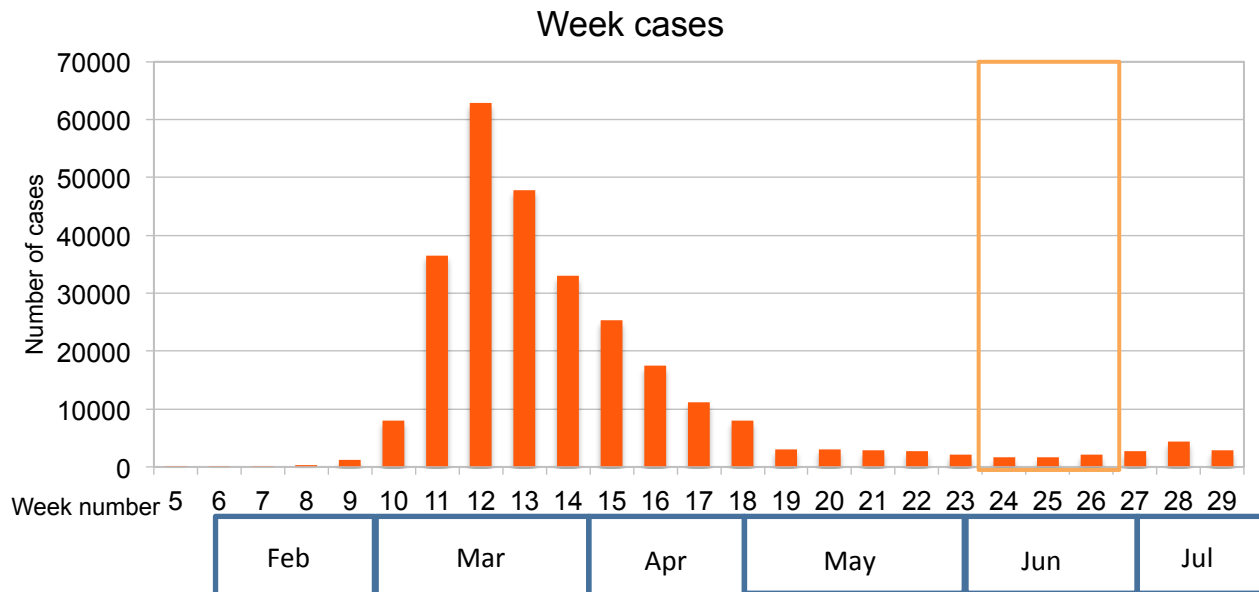
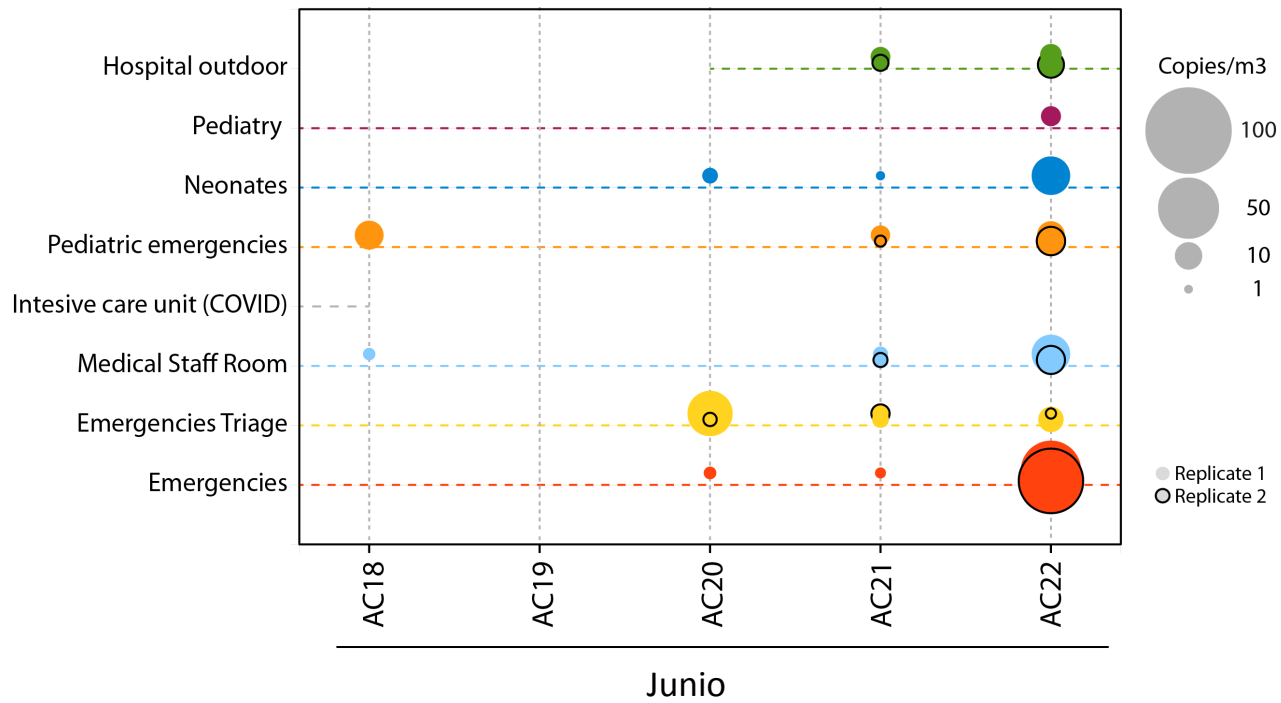
Donaciones Covid19

CSIC
CONSEJO SUPERIOR DE INVESTIGACIONES CIENTÍFICAS



Muestreo en el Hospital Severo Ochoa, Leganés María Luz García

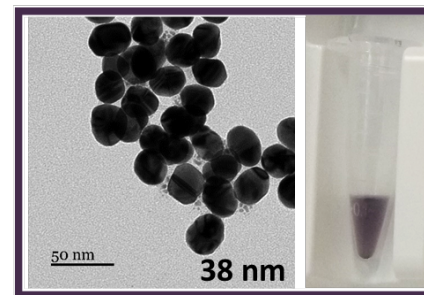
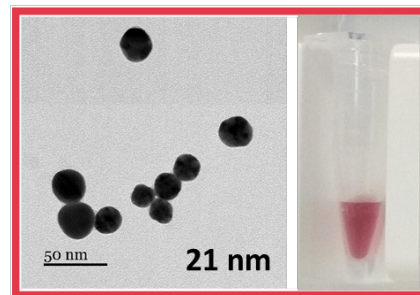
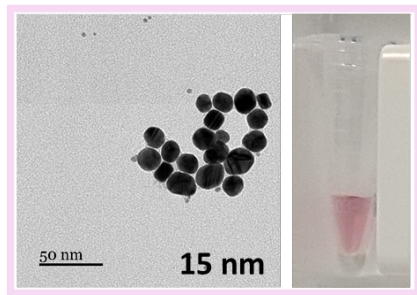
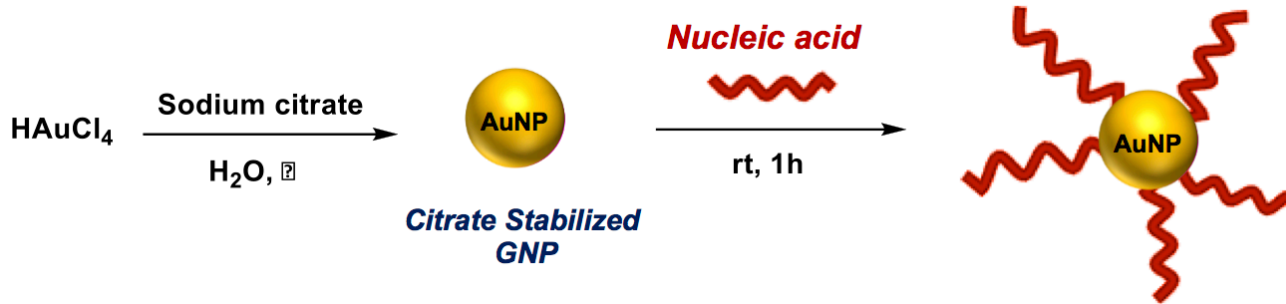




Detección rápida de virus mediante nanopartículas

Optimizar la detección colorimétrica de RNA y proteínas virales

A. Somoza, A. Alcamí

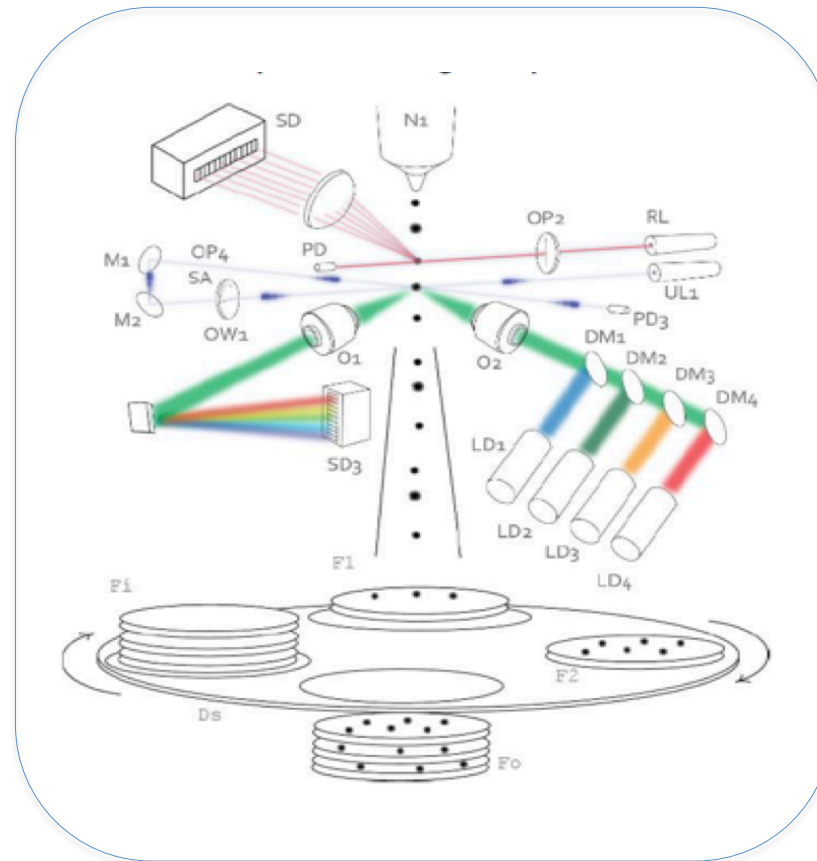


* Colaboración Luis Blanco (CBMSO) y Felipe Cortés (CNIO)
Métodos de amplificación isotérmica (Polimerasa Phi29)
Proyecto Covid19 ISCIII

Optimizar la tecnología LIF (laser-induced fluorescence)

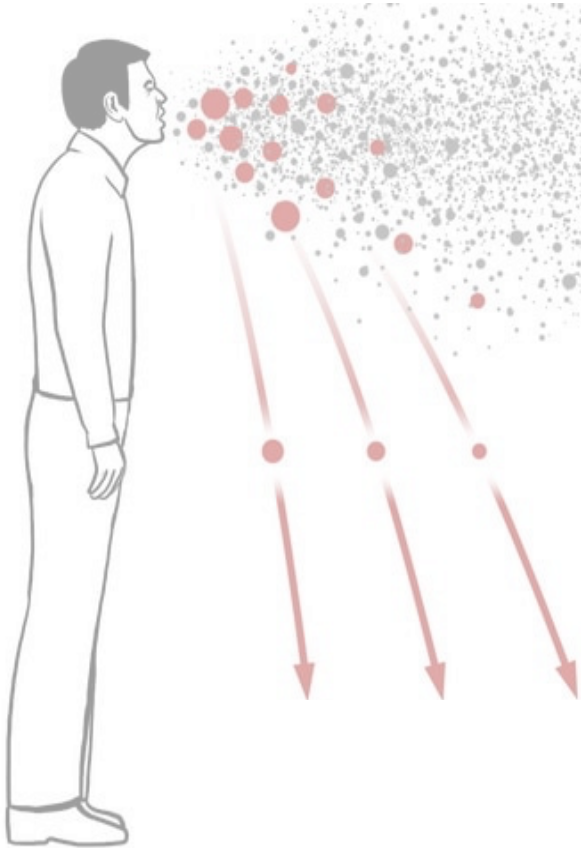
Detección de partículas virales en el aire en tiempo real

X. Rodó, A. Alcamí



Detección de SARS-CoV2 en muestras de aire: papel en la vigilancia

Antonio Alcamí, Centro de Biología Molecular Severo Ochoa, CSIC



Evidencias de transmisión aérea de SARS-CoV2

Evidencias epidemiológicas

Gemonas virales en aerosoles han sido detectados

- CAPTACIÓN: diversidad de métodos con eficacia variable
- DETECCIÓN PCR: cuantificación no es precisa en ocasiones

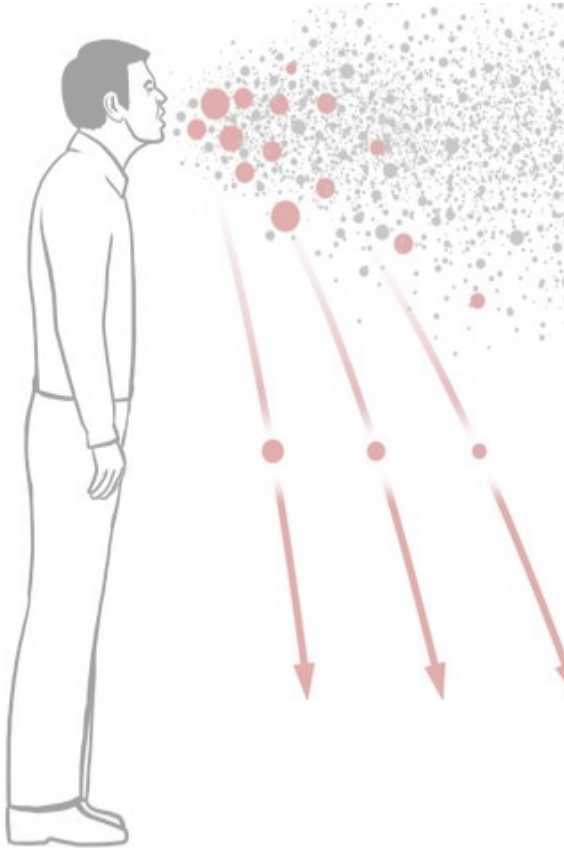
Viabilidad de la infectividad viral en aerosoles

- Demostrada en sistema experimental (hasta 3h o 16h)
- Una publicación reciente lo demuestra en ámbito hospitalario

Método válido para monitorizar presencia/transmisión del virus (hospitales La Paz y Severo Ochoa)

Detección de SARS-CoV2 en muestras de aire: papel en la vigilancia

Antonio Alcamí, Centro de Biología Molecular Severo Ochoa, CSIC



Temas a resolver en el futuro

Mejorar sistemas de detección en aire

- CAPTACIÓN: métodos mas eficaces que preservan infectividad
- MÉTODOS RÁPIDOS DE DETECCIÓN PCR: nanotecnología

Utilización de sistemas de detección de virus en aire en:
hospitales, transporte, espacios públicos

Viabilidad de la infectividad viral en aerosoles

¿Cuál es la dosis de virus necesaria para iniciar infección?

Necesario el desarrollo de tecnologías eficientes de inactivación
de SARS-CoV2 en el aire