

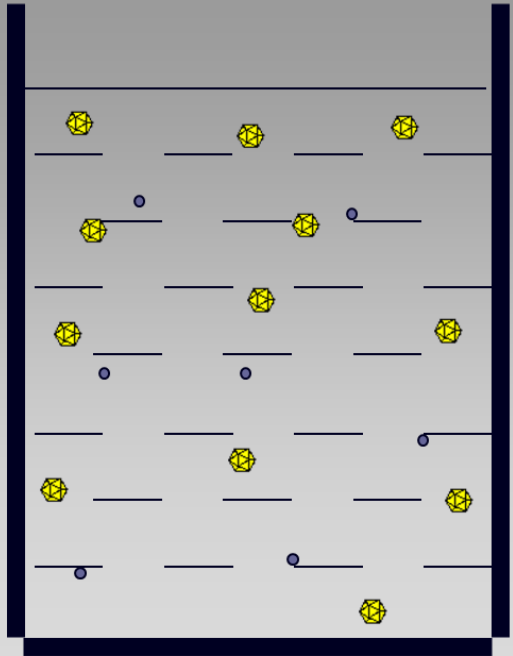


Desarrollo del proyecto.
Perspectiva de los laboratorios de investigación

Albert Bosch

Virus detection in the water environment:

A problem that requires concentration...

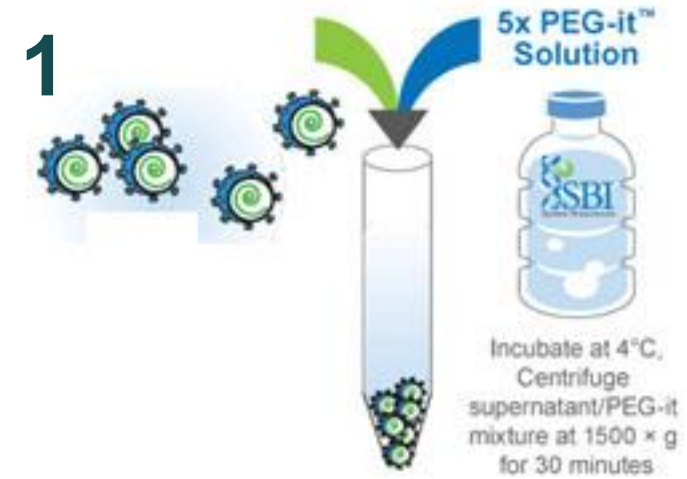


PEG Precipitation (1)

Aluminum Hydroxide Adsorption-Precipitation (2)

Centricon Filtration (3)

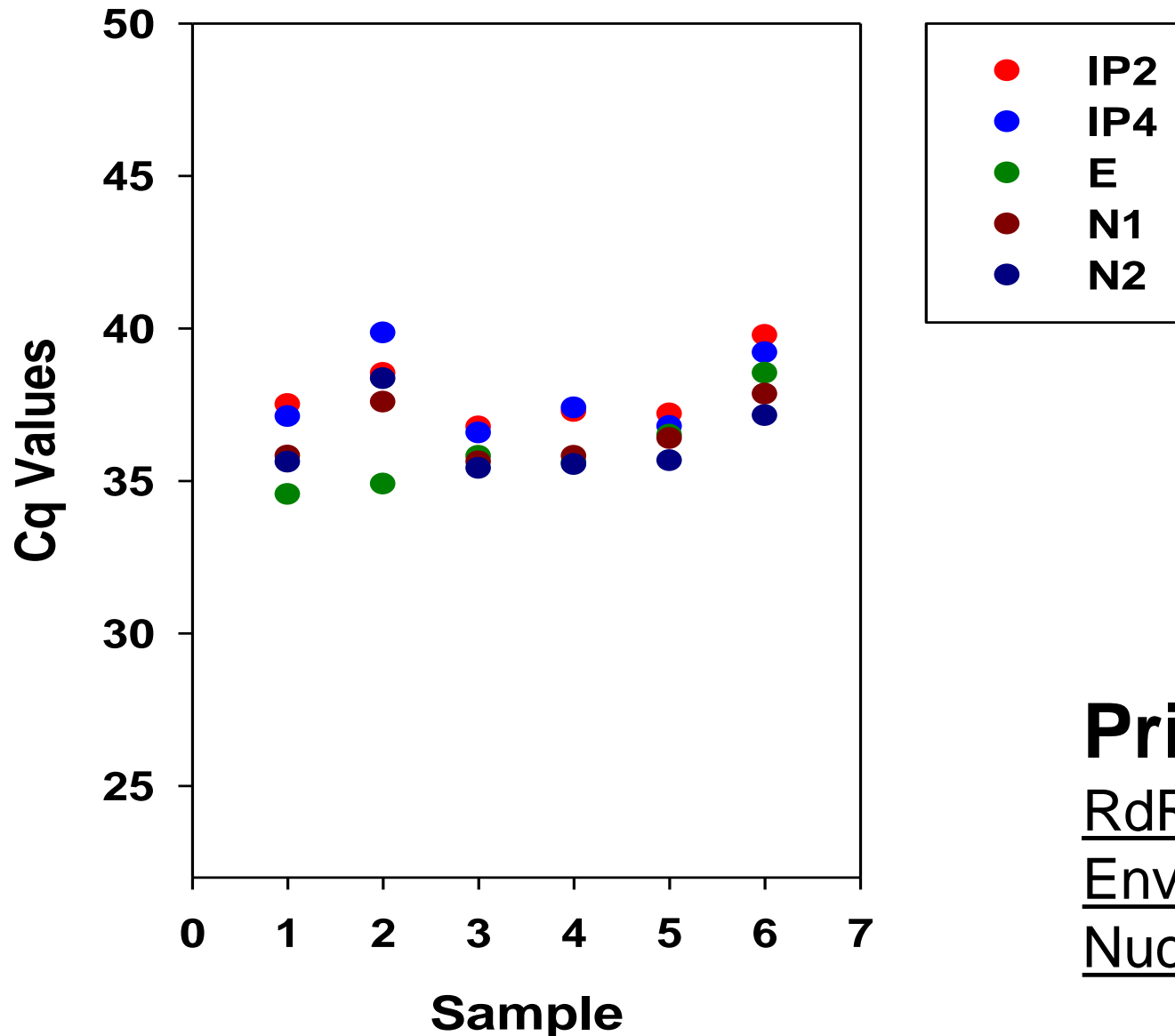
Innovaprep Concentrating Pipette (4)



Safely concentrate Virus
10- to 100-fold



Levels of SARS-CoV-2 genomes (Cq Values) in 6 sewage samples employing the five targets (IP2, IP4, E, N1 and N2)



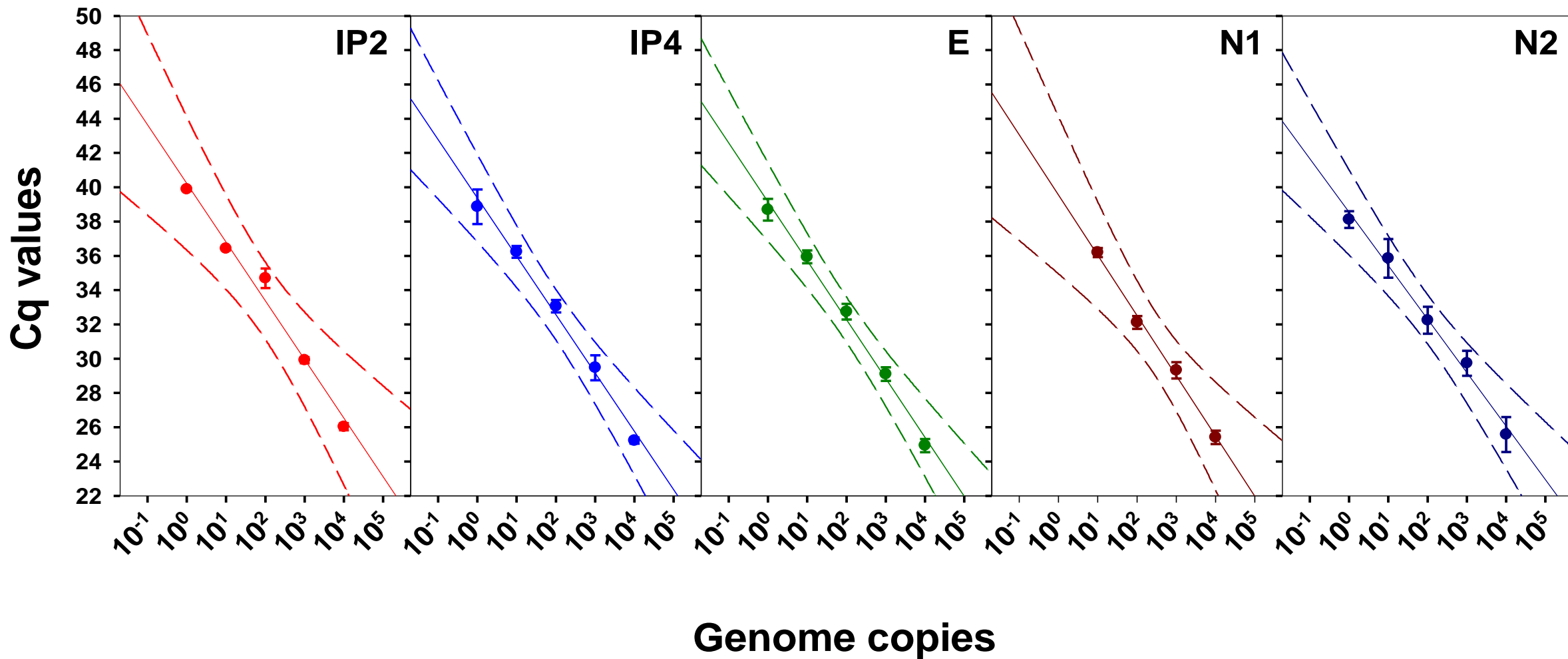
Primer regions:

RdRp: **IP2**, **IP4**, Institut Pasteur

Envelope protein: **E**, Charité Berlin

Nucleoprotein: **N1**, **N2**, CDC

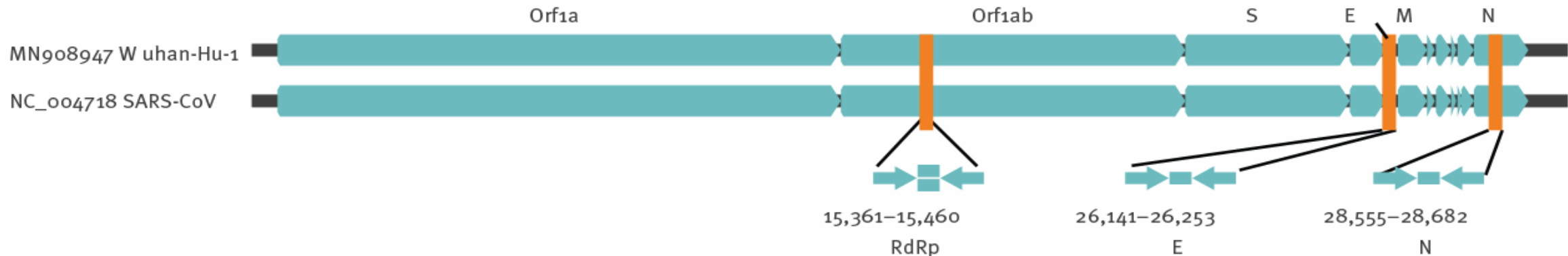
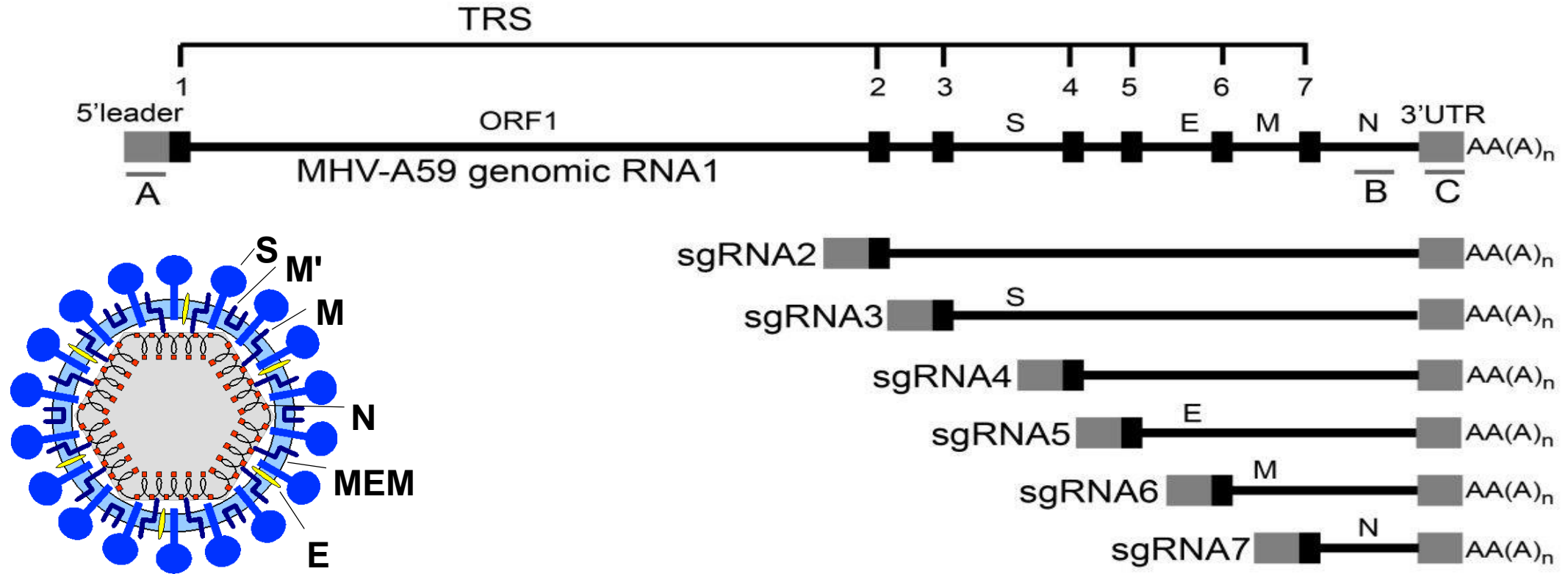
Average standard curves for each of the targets used in this study (IP2, IP4, E, N1 and N2). Regression line (solid lines) and their 99% confident (dashed lines).



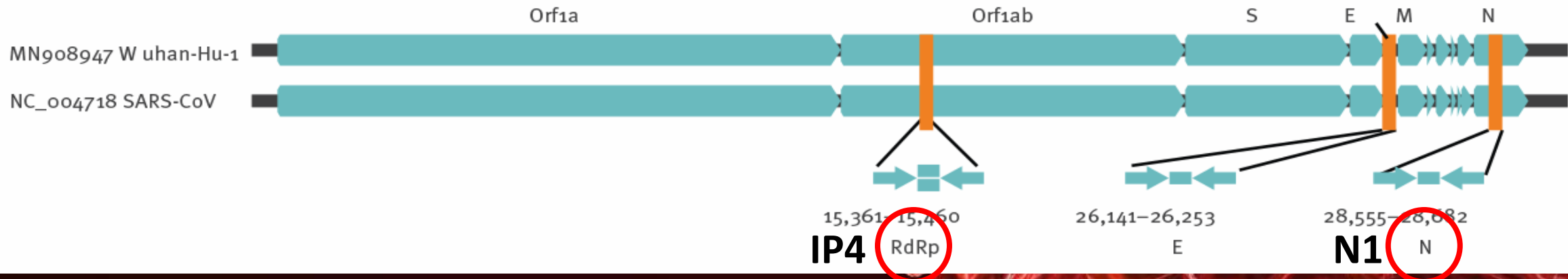
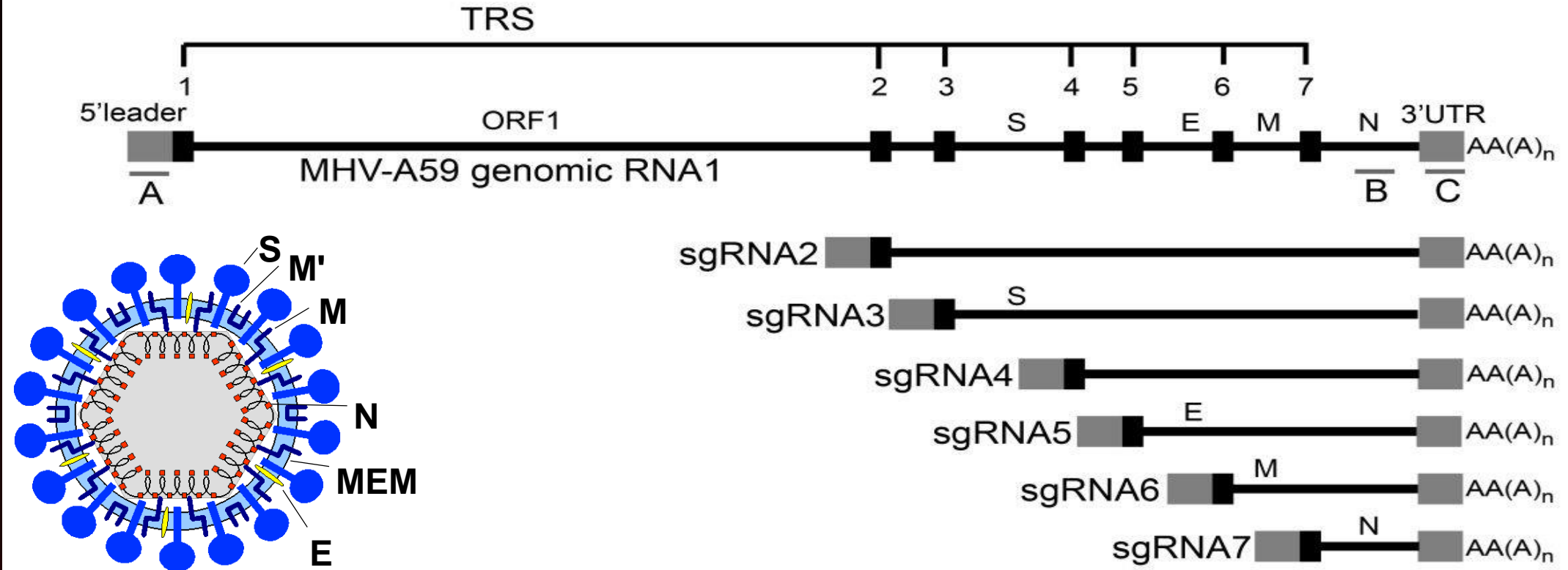
RT-qPCR comparison: Cq values of positive samples



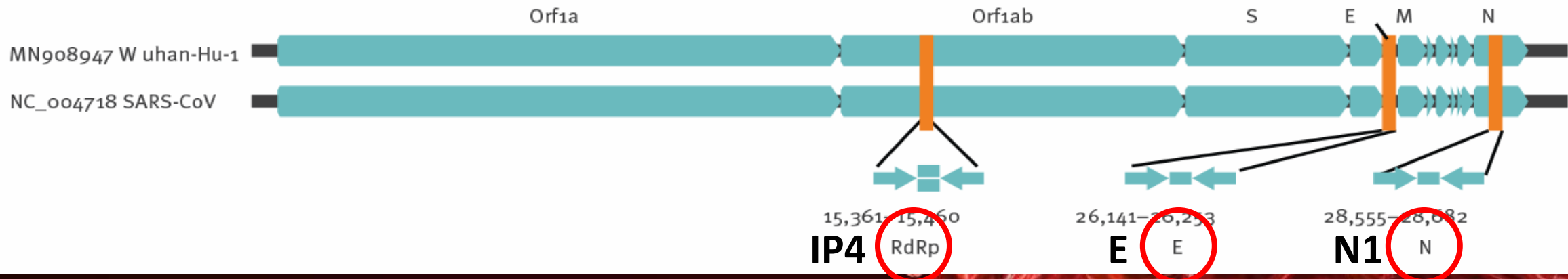
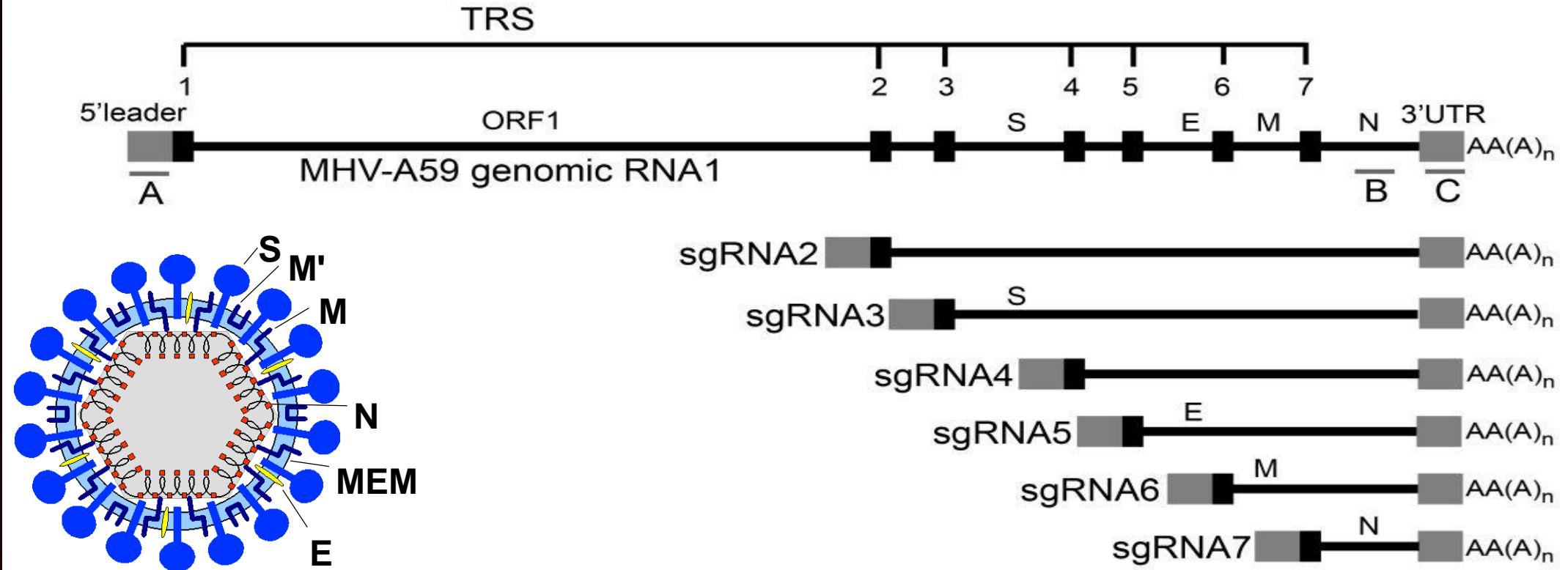
CoV Genome Organization and Expression



CoV Genome Organization and Expression

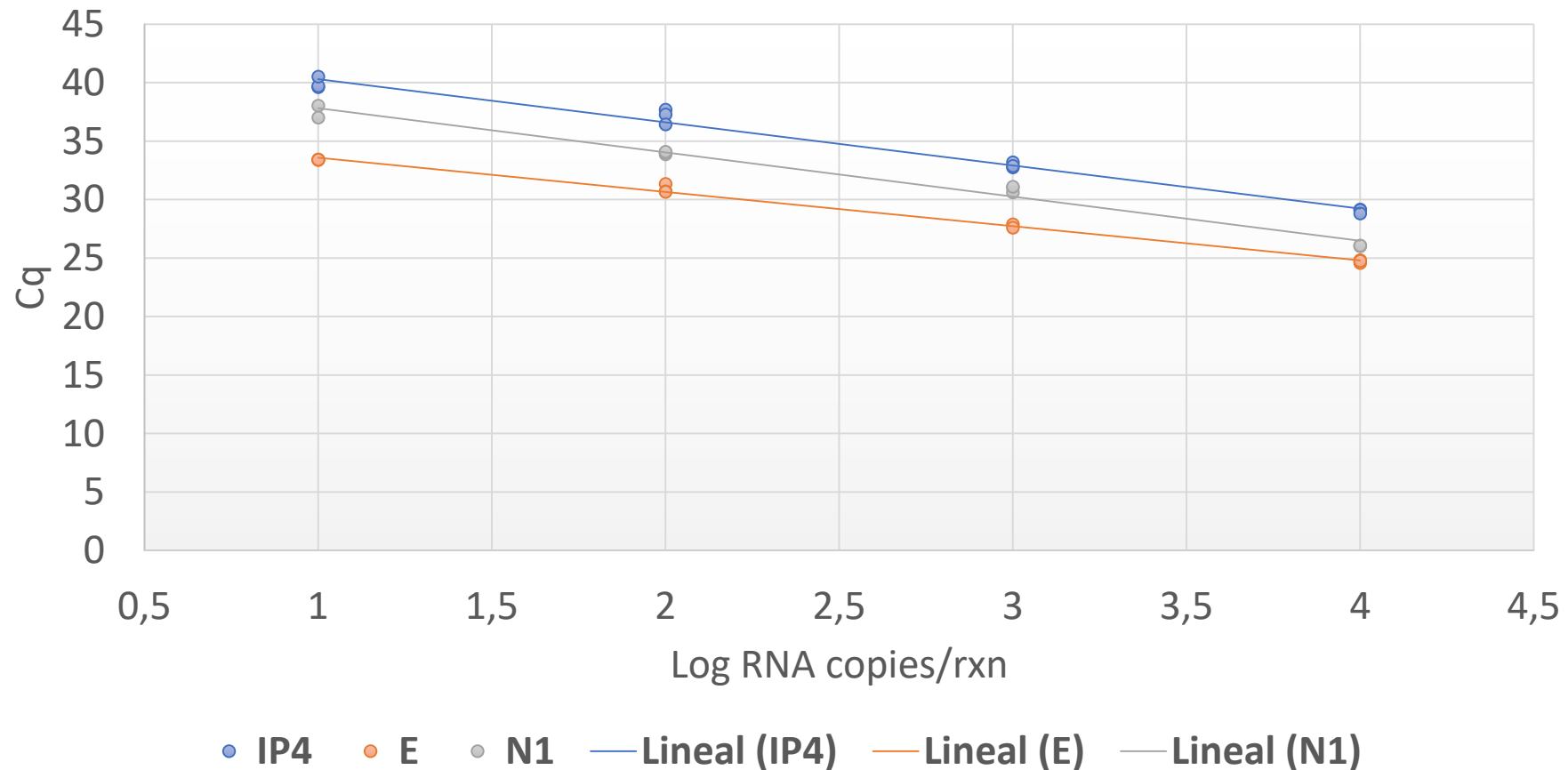


CoV Genome Organization and Expression



Standard Curves Parameters						
Target	Slope	Intercept	R2	Efficiency (%)	RT-qPCR Kit	qPCR Instrument
IP4-UB	-3,694	43,992	0,9863	86,52	Takara	Stratagene
E-UB	-2,922	36,189	0,9949	119,90	Takara	CFX96 BioRad
N1-UB	-3,779	41,59	0,9853	83,91	Takara	CFX96 BioRad

Twist Synthetic SARS-CoV-2 RNA Control 2 (MN908947.3) (Twist Bioscience)



Ejercicios de intercomparación

AI-3

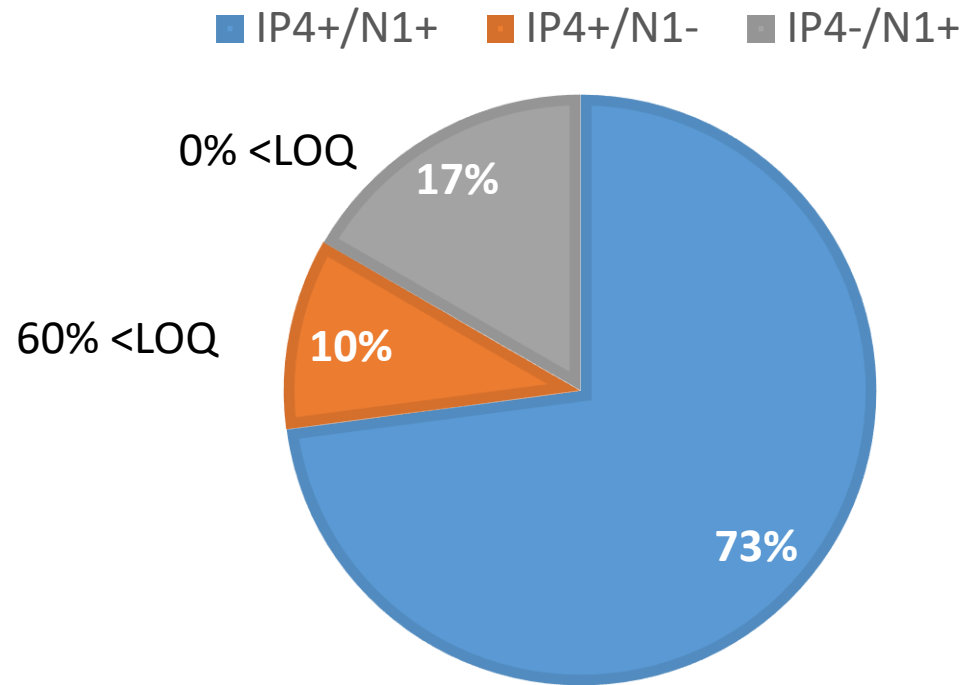
	UB	USC	CEBAS	IATA	Promedio	Desv Est
IP4	2.11E+04	1,36E+03	6.33E+03	7.42E+04	3.39E+04	3.37E+04
E	8.70E+04	8,94E+05	2.54E+06	6.17E+04	8.96E+05	1.25E+06
N1	1.98E+06	1,98E+06	1.89E+06	8.67E+05	1.58E+06	9.38E+05

PEG

	UB	USC	CEBAS	IATA	Promedio	Desv Est
IP4	4.18E+04			7.12E+04	5.65E+04	2.08E+04
E	4.05E+02			2.04E+05	1.02E+05	1.44E+05
N1	3.89E+03			1.09E+05	5.64E+04	7.43E+04

Diana	Analizadas	% positividad	% positivos <LOQ
IP4	55	73%	15%
E	45	53%	42%
N1	55	80%	0%

POSITIVIDAD IP4/N1



Mandatory quality controls for reference methods: ISO 15216-1 & 2

CONTROLS

ISO 15216-1 & 2

ANALYSES

REPRODUCIBLE & REPEATABLE

COMPLEX METHOD

Several controls for each step

EXTRACTION EFFICACY

VIRUS PROCESS CONTROL [MENGOVIRUS Vmc0]

RT-PCR EFFICIENCY

Internal positive control (RNA molecules)

QUANTIFICATION

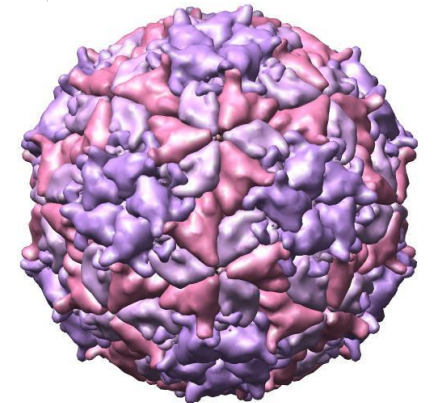
PLASMIDS, dsDNA molecules

RT-PCR

CONTROLS [VIRAL RNA AND PLASMIDS]

**NEGATIVE
CONTROLS**

PRETREATMENT & RT-PCR



Mandatory quality controls for reference methods: ISO 15216-1 & 2

CONTROLS

ISO 15216-1 & 2

ANALYSES

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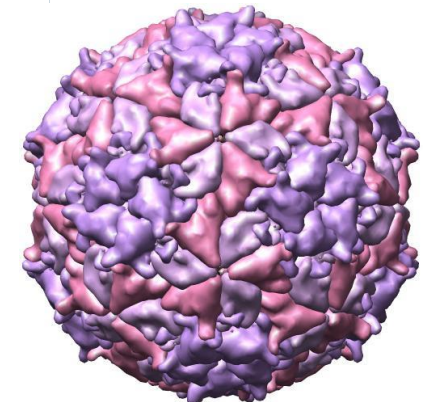
PLASMIDS, dsDNA molecules

RT-PCR

CONTROLS [VIRAL RNA AND PLASMIDS]

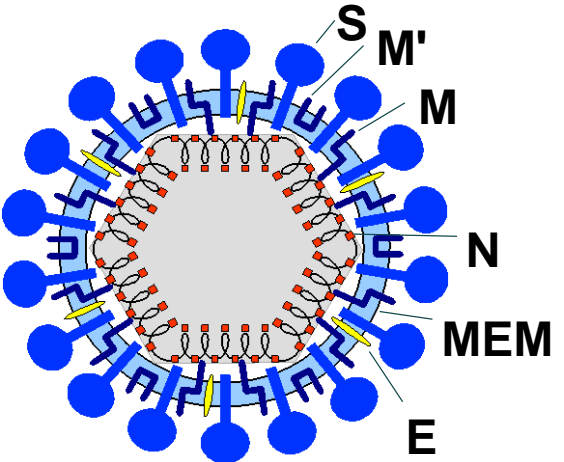
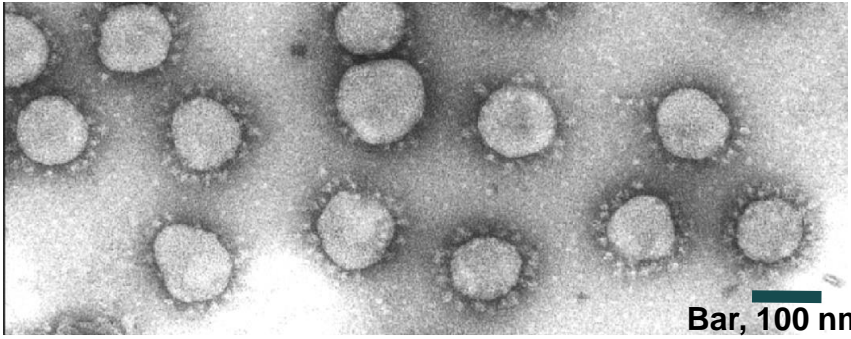
NEGATIVE
CONTROLS

PRETREATMENT & RT-PCR

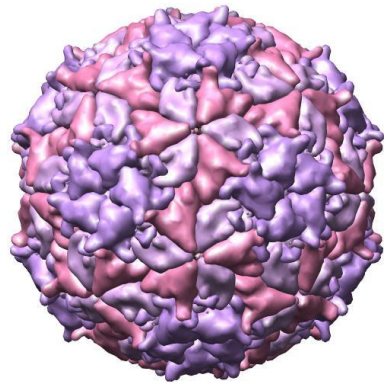
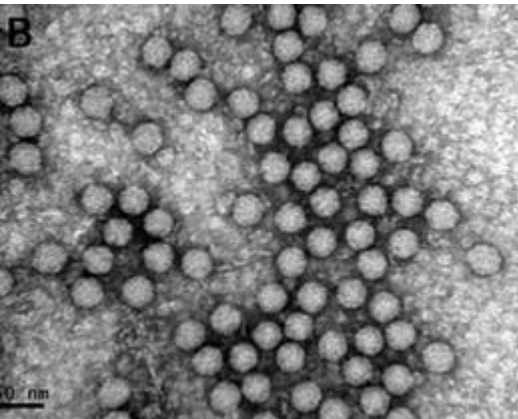


Process control virus for SARS-CoV-2 detection in wastewater

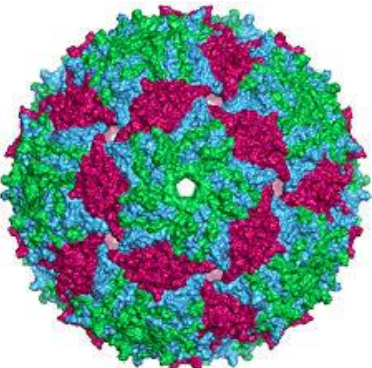
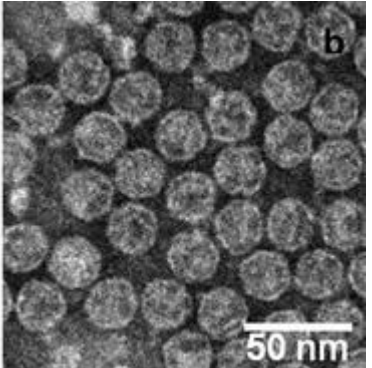
CoV



MV

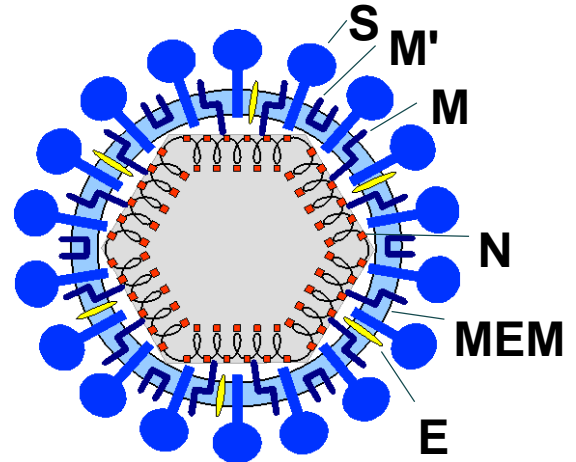
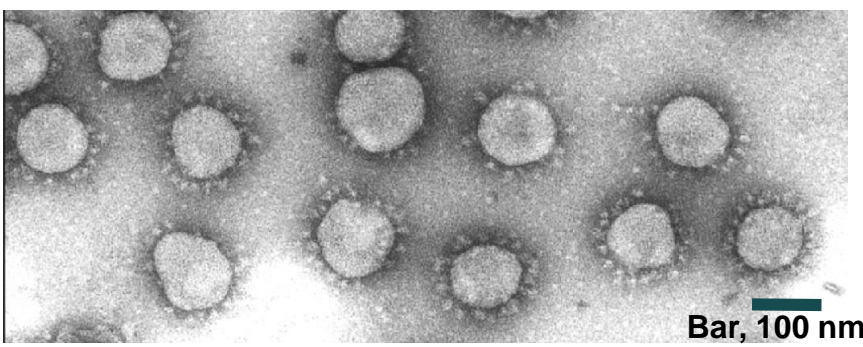


MS2



Process control virus for SARS-CoV-2 detection in wastewater

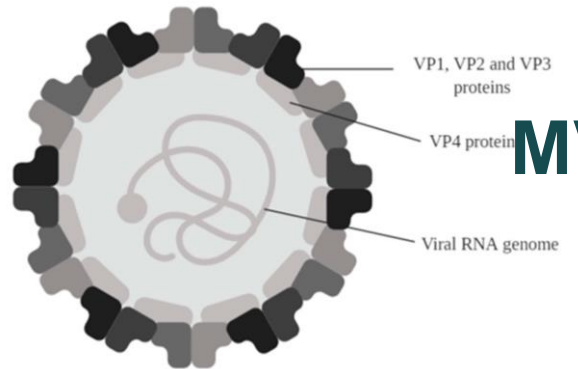
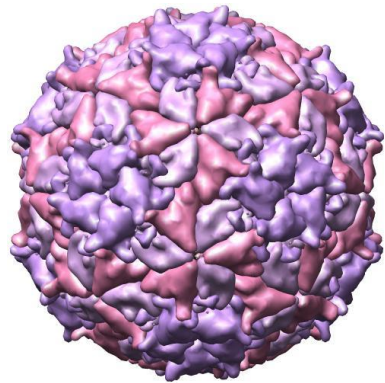
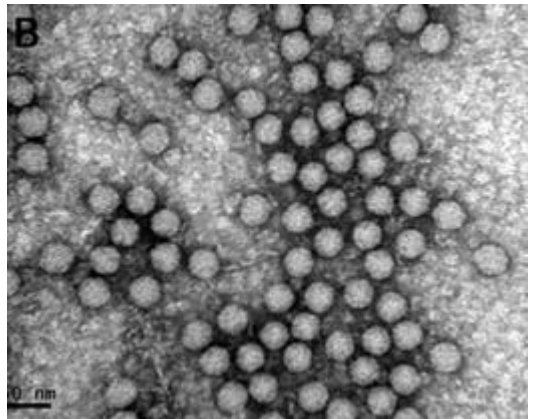
CoV



TGEV
PEDV
MHV
229E



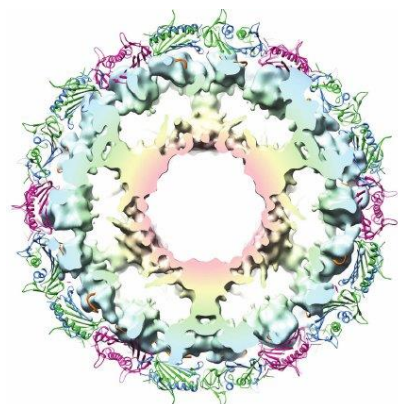
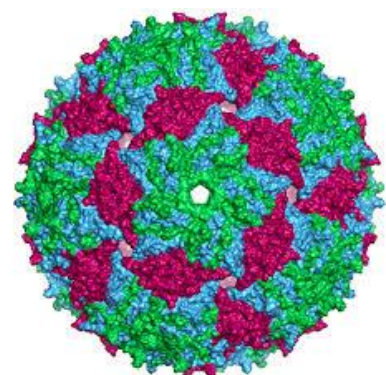
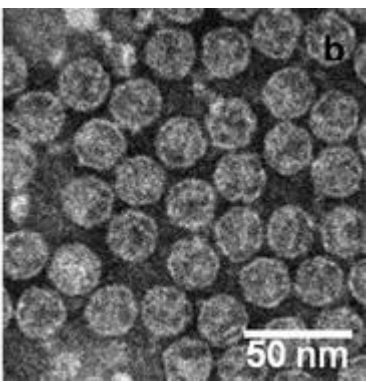
MV



MV



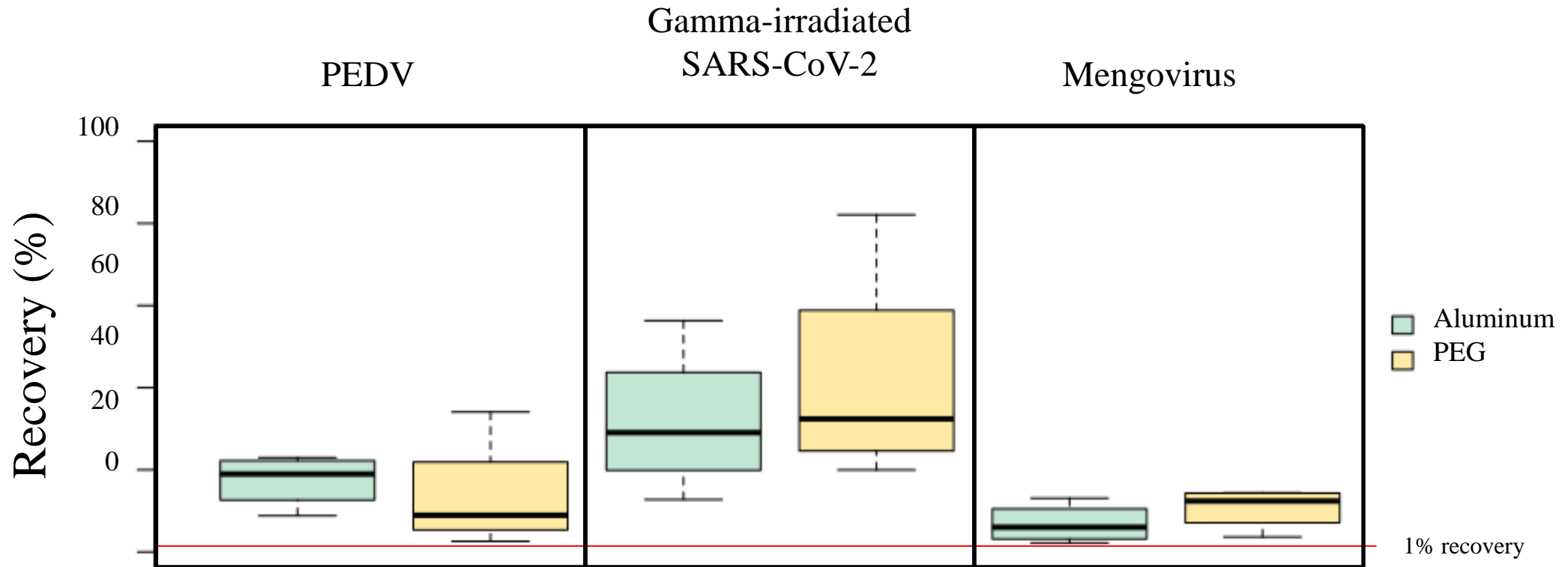
MS2



MS2



Concentration method comparison



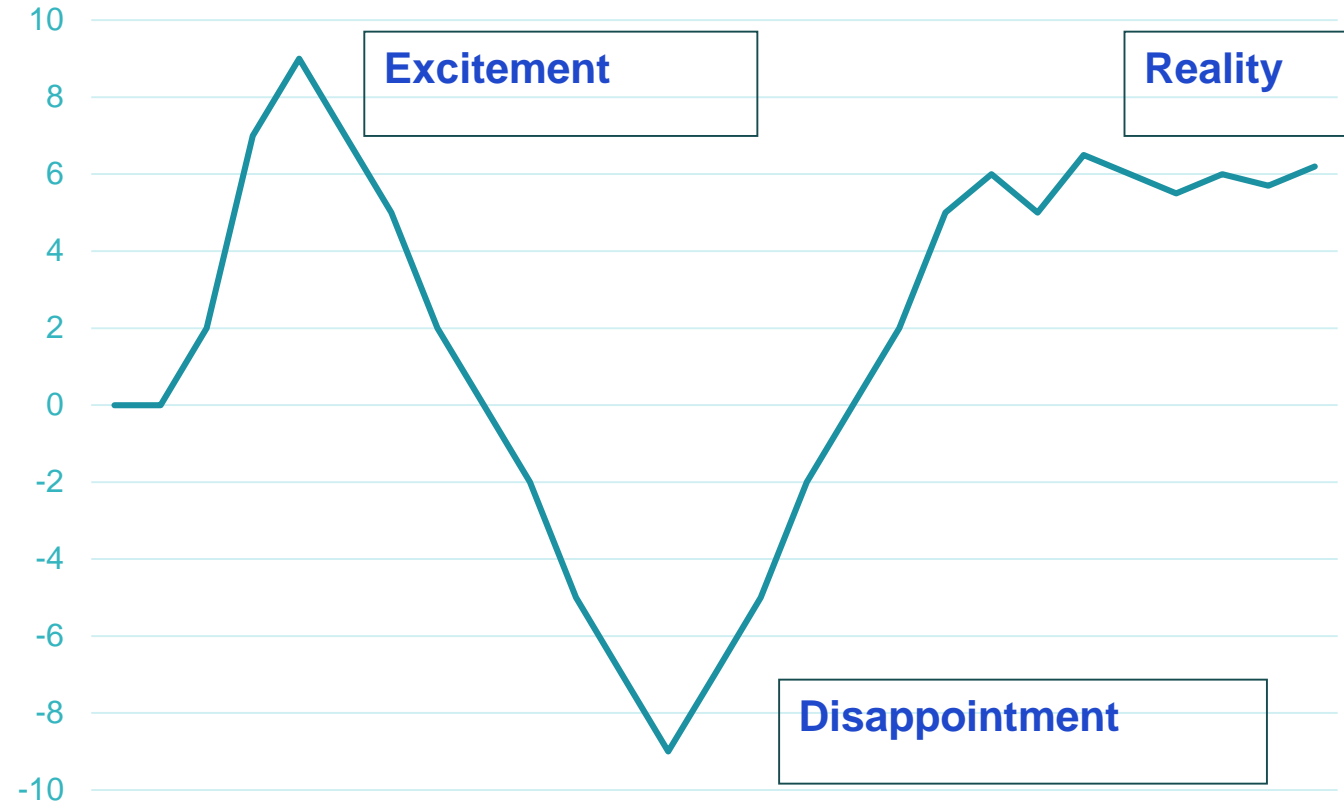
Limit of detection



Target	Levels of inoculated gamma SARS-CoV-2 (gc/mL)				LoD _{95%} (gc/L)
	1700	170	17	1.7	
N1	6/6	6/6	5/6	0/6	3.59E+04
N2	6/6	6/6	4/6	0/6	5.46E+04
E	6/6	6/6	3/6	0/6	8.19E+04
IP2	6/6	6/6	4/6	0/6	5.46E+04
IP4	6/6	6/6	1/6	0/6	1.65E+05

The ideal method for virus recovery from water

- Efficient
- Fast
- Cheap
- Adequate for different types of viruses
- Compatible with molecular detection assays
- No need for sophisticated equipment



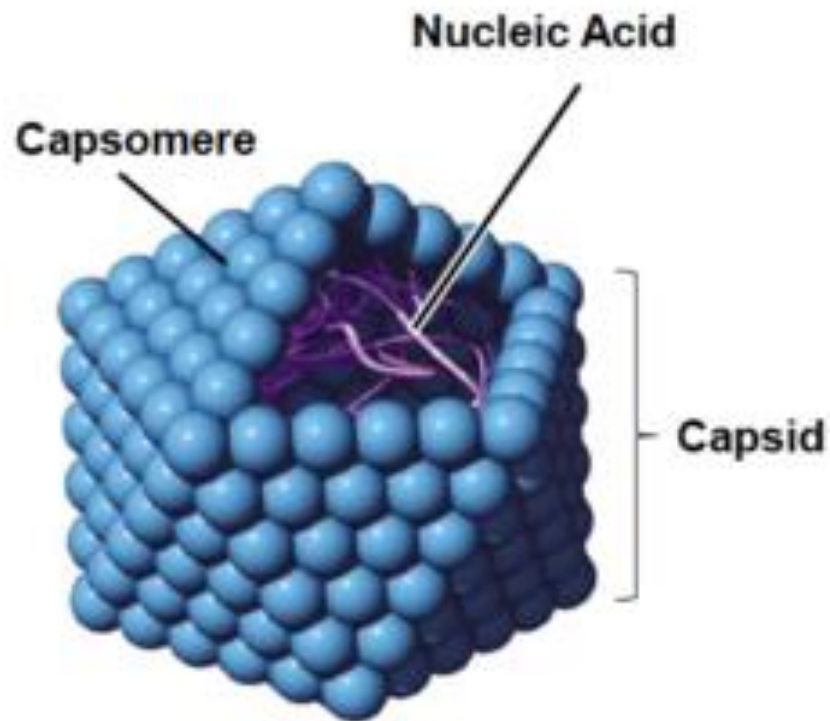
Life-cycle of a new assay

Vigilancia para Alerta Temprana en aguas residuales de CCOVID-19



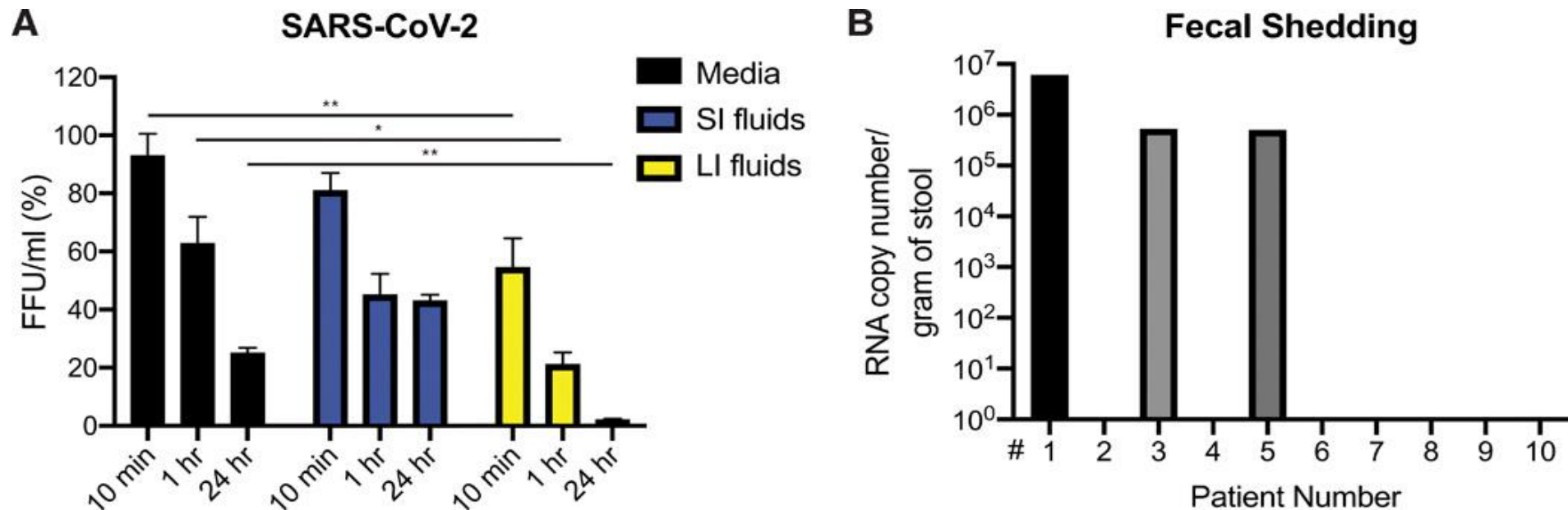
- IATA, CSIC, Valencia
- CEBAS, CSIC, Murcia
- Universidad de Santiago de Compostela

However,



- *What do genome copies imply in terms of public health?*
- *Genome copies do not necessarily correlate with infectious units*

CORONAVIRUS

TMPRSS2 and TMPRSS4 promote SARS-CoV-2 infection of human small intestinal enterocytesRuo Chen Zang^{1,2,*}, Maria Florencia Gomez Castro^{1*}, Broc T. McCune³, Qiru Zeng¹, Paul W. Rothlauf^{4,5}, Naomi M. Sonnek⁵, Zhuoming Liu¹, Kevin F. Brulois^{6,7}, Xin Wang², Harry B. Greenberg^{7,8}, Michael S. Diamond^{1,3,9}, Matthew A. Ciorba⁵, Sean P. J. Whelan¹, Siyuan Ding^{1†}Science Immunology
AAAS**SARS-CoV-2 rapidly lose infectivity in the human GI tract.**

Christian Drosten (La Charité, Berlin): *The proportion of infectious SARS-CoV-2 in respiratory secretions is very low (around 1 infectious unit in 10⁷ physical particles) and even less in feces*

Determinación de la presencia de genoma del SARS-CoV-2 en zonas de aguas de baño



CÓDIGO MUESTRA UB	Resultado SARS-CoV2*	EC (CFU/100mL)	CT (CFU/100mL)	SOMCPH (PFU/100mL)
ZB01_200622	Negativo	2	66	<1
ZB04_200623	Negativo	3	102	<1
ZB06_200623	Negativo	18	880	21
ZB07_200624	Negativo	50	760	<1
ZB10_200624	Negativo	66	2400	18
ZB02_200628	Negativo	5	21000	<1
ZB05_200629	Negativo	242	2317	58
ZB08_200630	Negativo	325	552381	45
ZB09_200701	Presunto Positivo	8250	>100000	670
ZB01_200705	Negativo	42	9650	NT
ZB04_200705	Negativo	41	272727	<1
ZB06_200706	Negativo	550	5300	38
ZB10_200706	Negativo	117	8200	164
ZB07_200707	Negativo	183	3800	<1
ZB01_200708	NT	19	5400	<1
ZB03_200708	Negativo	NT	NT	NT
ZB02_200712	Negativo	1	31650	<1
ZB05_200713	Negativo	8	5700	<1
ZB08_200714	Negativo	750	40500	15
ZB09_200715	Negativo	NT	NT	NT
ZB01_200719	Negativo	2	5150	<1
ZB04_200719	Negativo	35	8850	<1
ZB06_200720	Negativo	375	6591	74
ZB10_200720	Negativo	117	2545	11
ZB07_200721	Negativo	217	1400	<1
ZB03_200722	Negativo	1	50	3
ZB02_200726	Negativo	30	3200	<1
ZB05_200728	Negativo	13	2950	2
ZB08_200728	Negativo	6200	13400	26

EC: *Escherichia coli*; CT: coliformes totales; SOMCPH: colifagos somáticos

NT: "Not Tested", debido a que no llegó muestra para el análisis o llegó el envase roto para el análisis de indicadores microbiológicos.



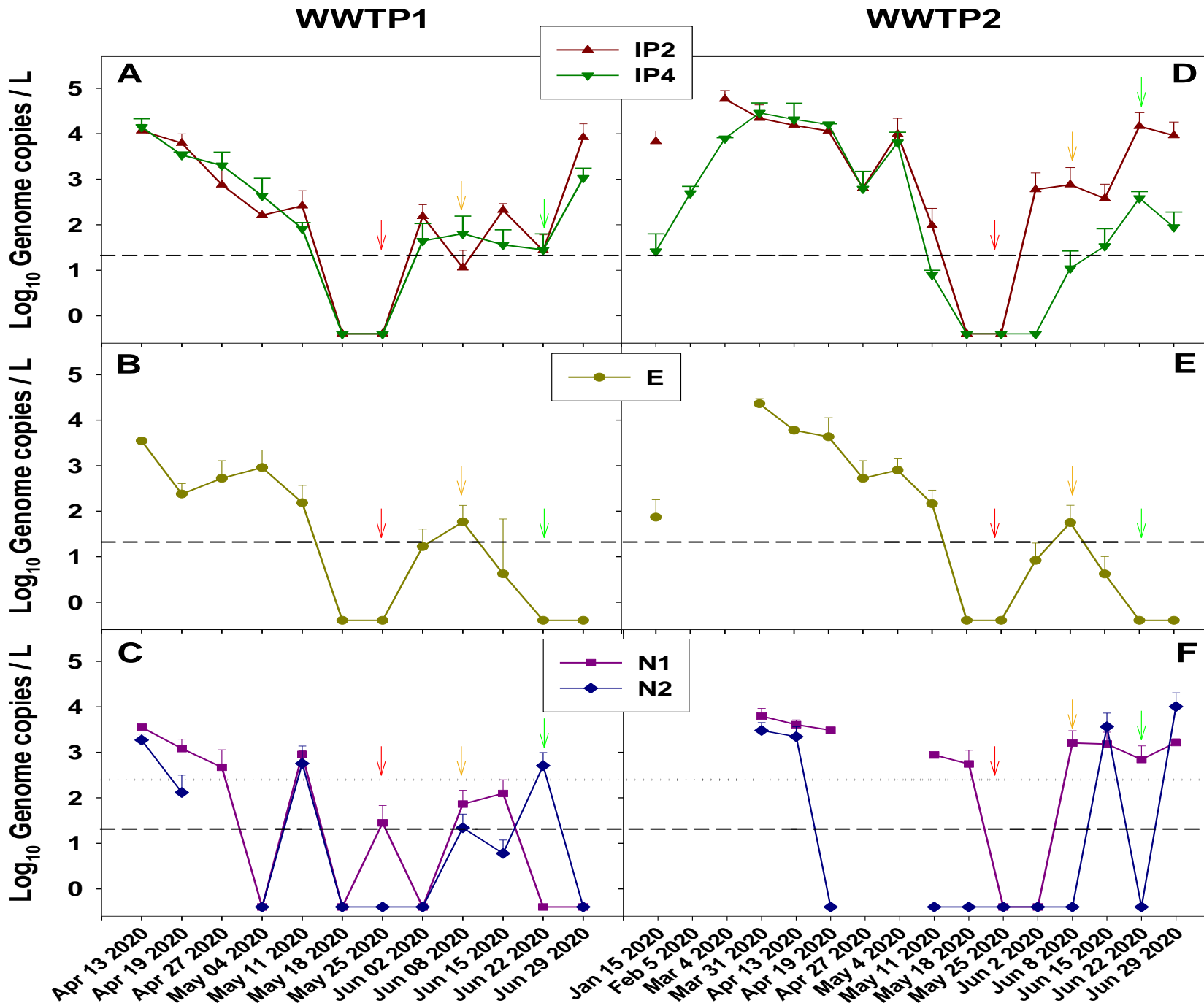
Detección de SARS-CoV-2 en muestras de agua residual tomadas con intervalos de una hora

CÓDIGO MUESTRA	HORA MUESTREO	EC (CFU/100mL)	CT (CFU/100mL)	SOMCPH (PFU/100mL)	IP4 (CG/L)	N1 (CG/L)	Resultado SARS-CoV2
200908-EDAR_27-E-1	08:00	1,76E+06	4,67E+07	1,08E+06	1,28E+05	6,87E+03	Positivo
200908-EDAR_27-E-2	09:00	5,71E+06	4,38E+07	2,57E+06	1,27E+05	3,22E+04	Positivo
200908-EDAR_27-E-3	10:00	1,30E+07	9,67E+07	4,67E+06	3,19E+04	2,58E+04	Positivo
200908-EDAR_27-E-4	11:00	1,50E+07	6,00E+07	7,95E+06	8,99E+04	5,44E+04	Positivo

EC: *Escherichia coli*

CT: coliformes totales

SOMCPH: colifagos somáticos



SARS-CoV-2 genome copy levels in two WWTP, detected with targets IP2 and IP4 (A, D), E (B, E) and N1 and N2 (C, F).

Recientemente, muchos laboratorios hemos tenido problemas con la diana E

JCM Accepted Manuscript Posted Online 20 July 2020

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1

1 A recurrent mutation at position 26,340 of SARS-CoV-2 is associated with failure of
2 the E-gene qRT-PCR utilized in a commercial dual-target diagnostic assay

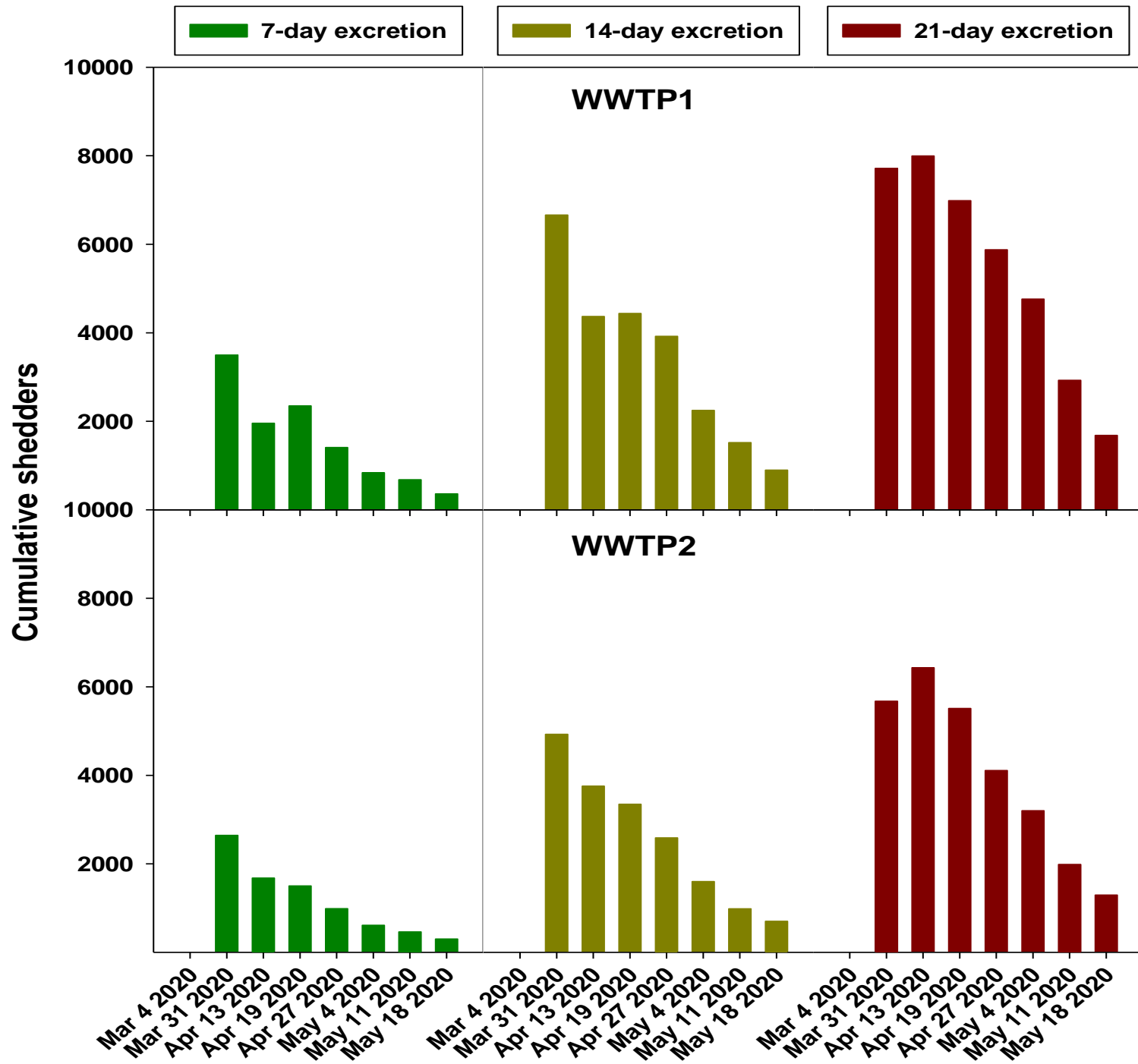
3

4 Maria Artesi^{1,#}, Sébastien Bontems^{2,#}, Paul Göbbels³, Marc Franckh³, Piet Maes⁴, Raphaël

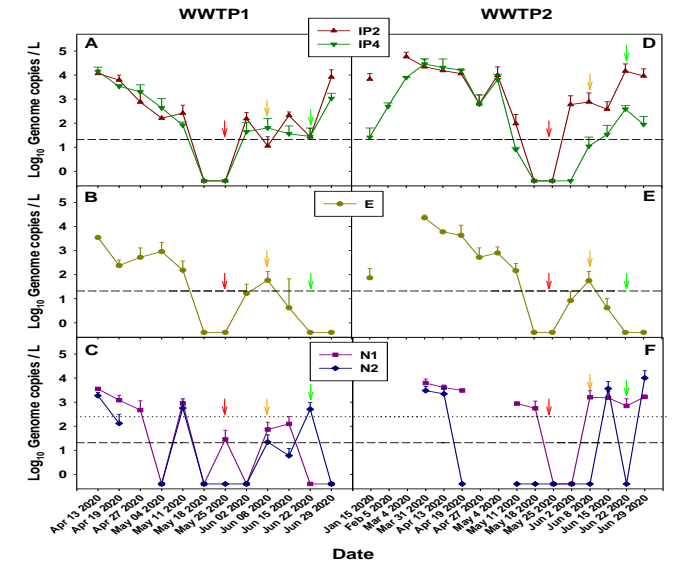
5 Boreux², Cécile Meex², Pierrette Melin², Marie-Pierre Hayette^{2,§}, Vincent Bours^{1,5,§}, Keith

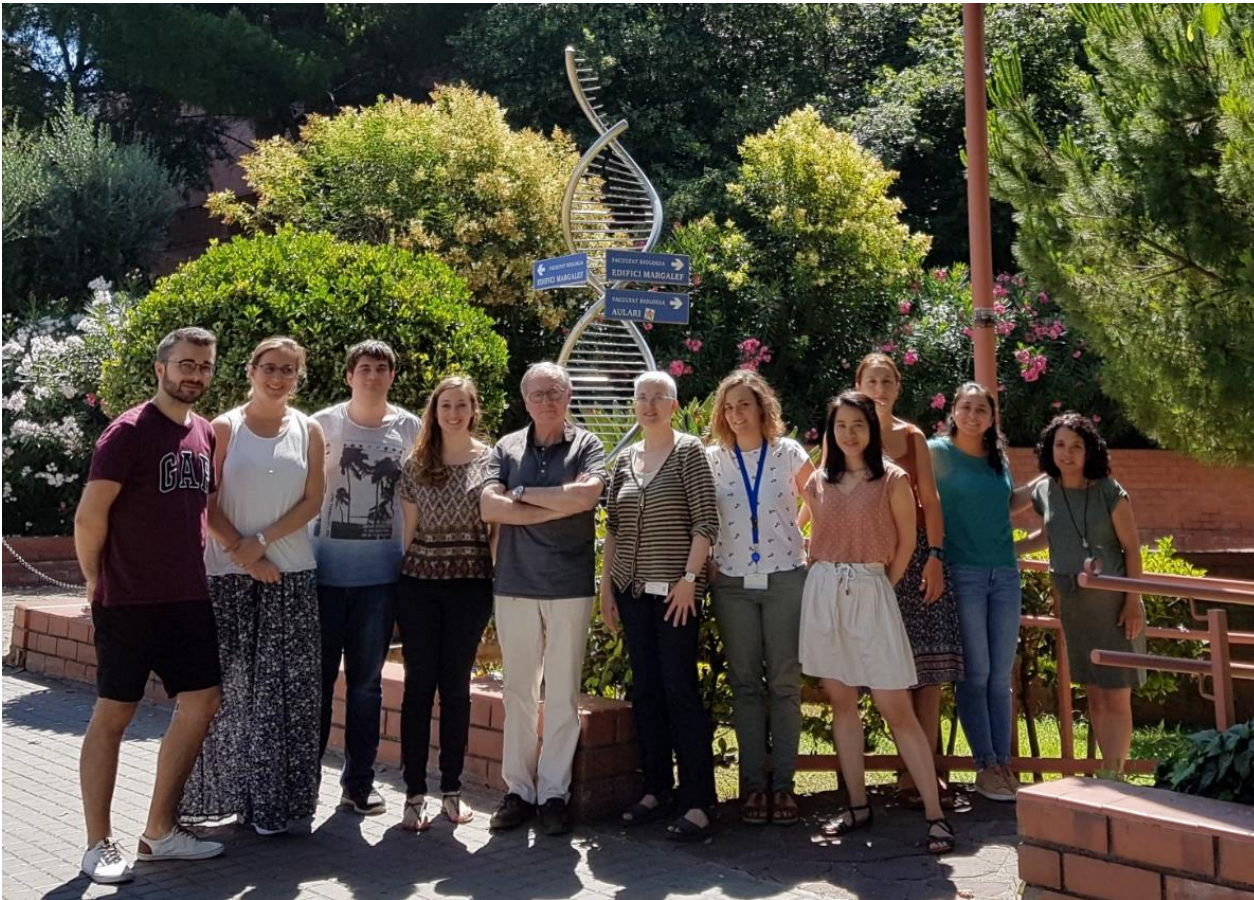
6 Durkin^{1,§}





Cumulated SARS-CoV-2 symptomatic shedders figured estimating fecal excretion periods of 7 days, 14 days and 21 days prior to case reporting



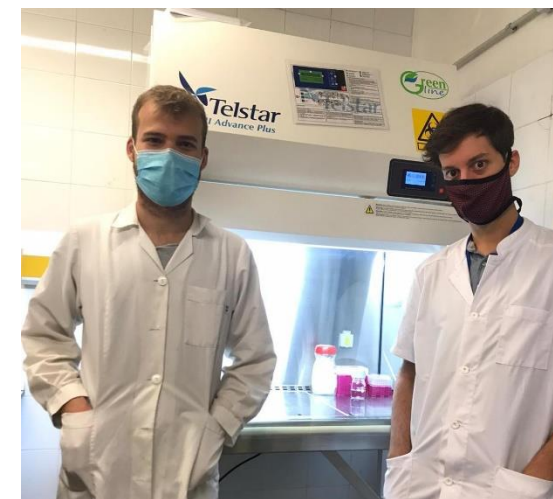


**Rosa M. Pintó
Susana Guix
Albert Bosch**

 VIRUS ENTÈRICS

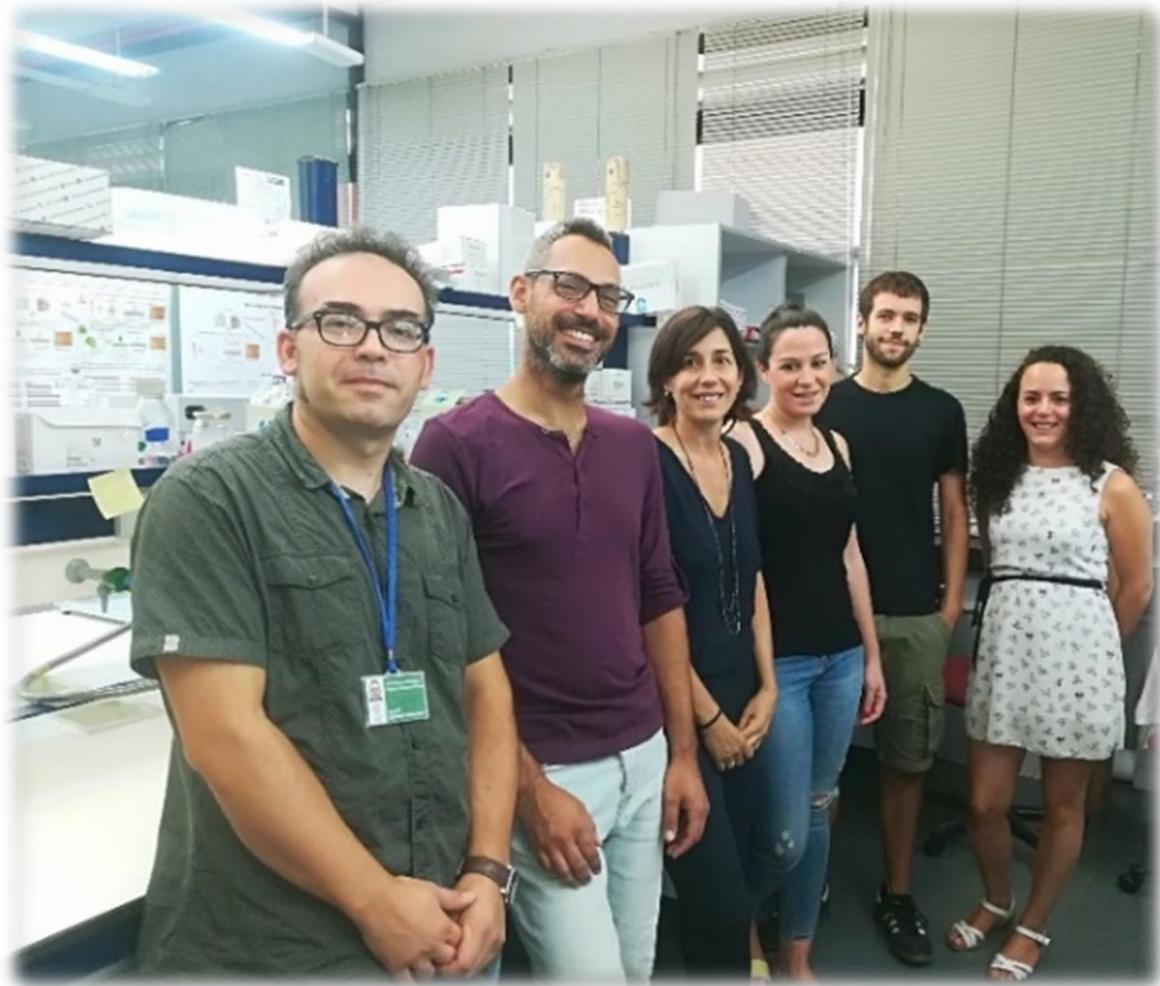


**Eduard Anfruns Estrada
Gemma Chavarria Miró
Cristina Fuentes
Adán Martínez
Mario Vázquez**





Jesús López Romalde
David Polo
Marta Lois
Alberto Lema



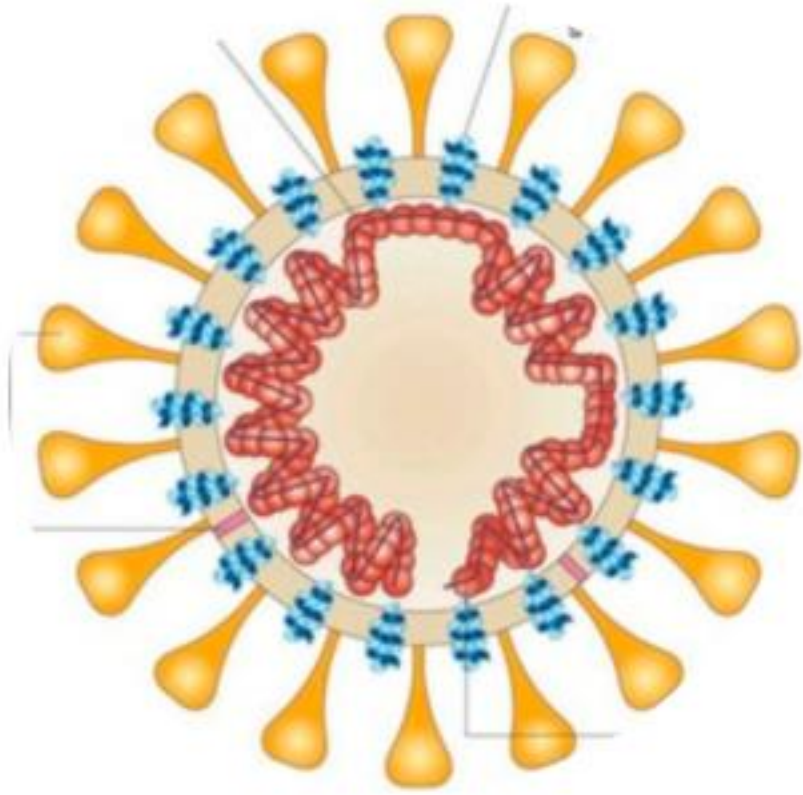
Gloria Sánchez
Walter Randazzo
Alba Pérez
Enric Cuevas
Azahara Diaz
Agustín Garrido
Andrea López de Mota



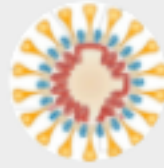
Instituto de Agroquímica
y Tecnología de Alimentos



Ana Allende
Pilar Truchado
Jennifer Cascales
Macarena Moreno
Ginés Abellán



MITERD



MITERD

Ana, Glòria, Jesús, Rosa, Susana, Tu

Buenas. os va bien tener un skype hoy jueves 13 a las 16h? Si no es así, proponed otra hora

13:05 ✓✓

Glòria Sánchez

Por mi bien

13:08

Susana Guix



13:09

Ana Allende



13:09

Jesús L Romalde

Yo tendría que hacerlo desde el teléfono, pero supongo que no tendré problema

13:09





¿Cual ha de ser nuestra respuesta frente a patógenos emergentes?



¿Cual ha de ser nuestra respuesta frente a patógenos emergentes?

Es necesario desarrollar y estudiar:

- **Métodos de detección / diagnóstico**
- **Controles . Sistemas de alerta temprana**
- **Estabilidad del patógeno en condiciones naturales y de desinfección**
- **Ecología, reservorios, vías de transmisión, ciclos, etc...**
- **Patogenicidad, hospedadores, dosis infecciosas, modelos animales, etc...**
- **Desarrollo de vacunas / tratamientos específicos**



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Wuhan, Hubei, China – Febrero 2020



Barcelona, Abril 2020



GRACIAS !!!

