

FEB. 02-03, 2021

CONFERENCE ONLINE



NOVEL SARS-CoV-2 VIABILITY RT-qPCR ASSESSMENT ON COMPLEX MATRICES

Enric Cuevas-Ferrando, Walter Randazzo, Irene Falcó, Alba Pérez-Cataluña, Gloria Sánchez
Department of Preservation and Food Safety Technologies, Instituto de Agroquímica y Tecnología de Alimentos – Consejo Superior de Investigaciones Científicas (IATA-CSIC), Valencia, Spain

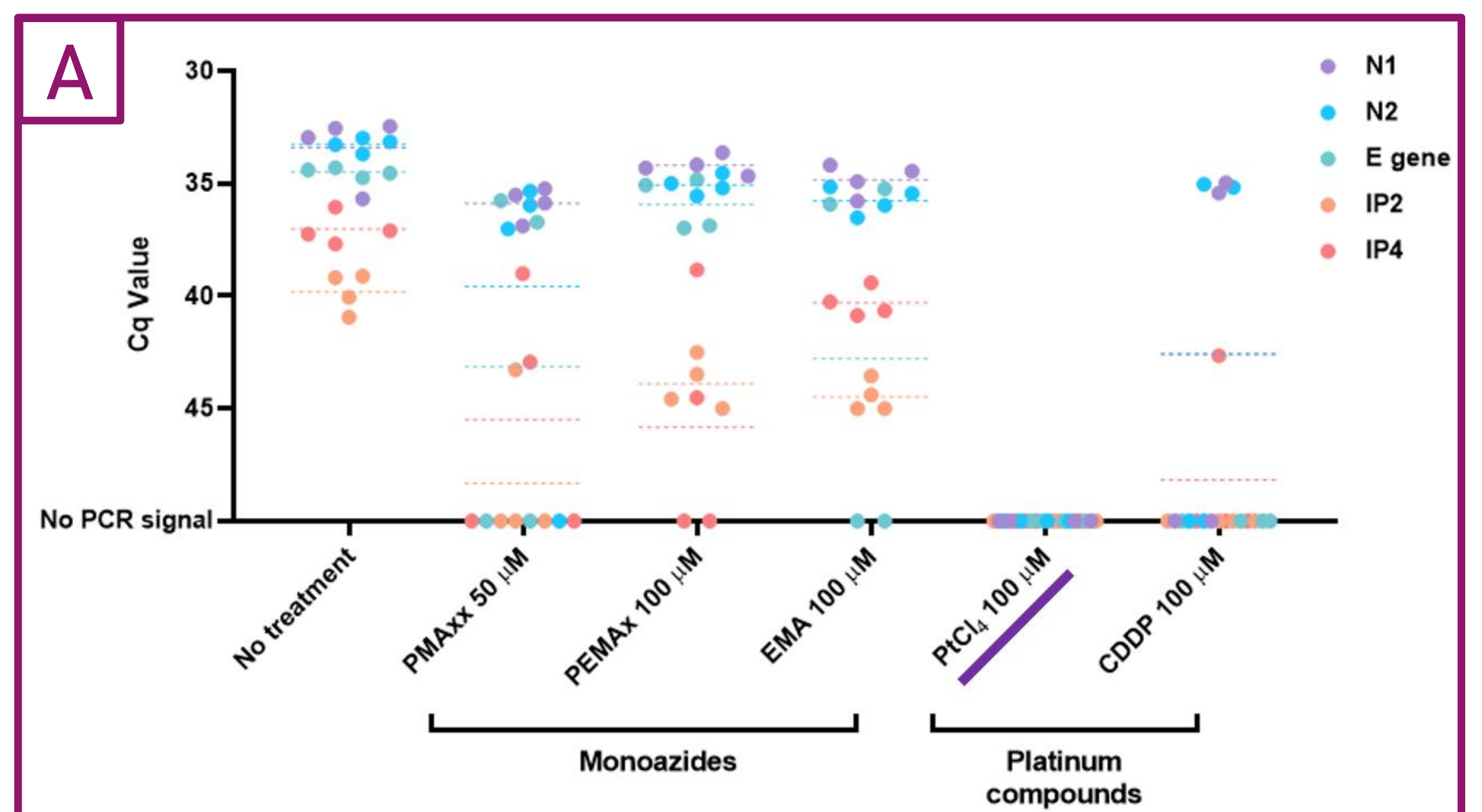
CSIC
CONSEJO SUPERIOR DE INVESTIGACIONES CIENTÍFICAS

INTRODUCTION

- COVID-19 pandemic situation and its ongoing epidemiological changes highlight the need for reliable, rapid and easy-to-use techniques for SARS-CoV-2 detection and quantification.
- Molecular techniques alone cannot discriminate between infectious and non-infectious viral particles.
- Several compounds have been used as viability markers for different viruses, most of them based on capsid integrity discrimination [1][2][3].
- In this study, we develop a viability RT-qPCR approach for SARS-CoV-2 based on platinum chloride (IV) (PtCl₄) viability marker.
- Platinum chloride (IV) is an accessible low-cost compound, not harmful for mammals, and not sensible to visible light as other common viability markers (monoazide dyes).
- Satisfactory results have been obtained in SARS-CoV-2 genomic RNA, inactivated SARS-CoV-2 viral suspensions, and in artificially and naturally contaminated complex matrices (i.e. faeces, urine and wastewater).

METHODS AND RESULTS

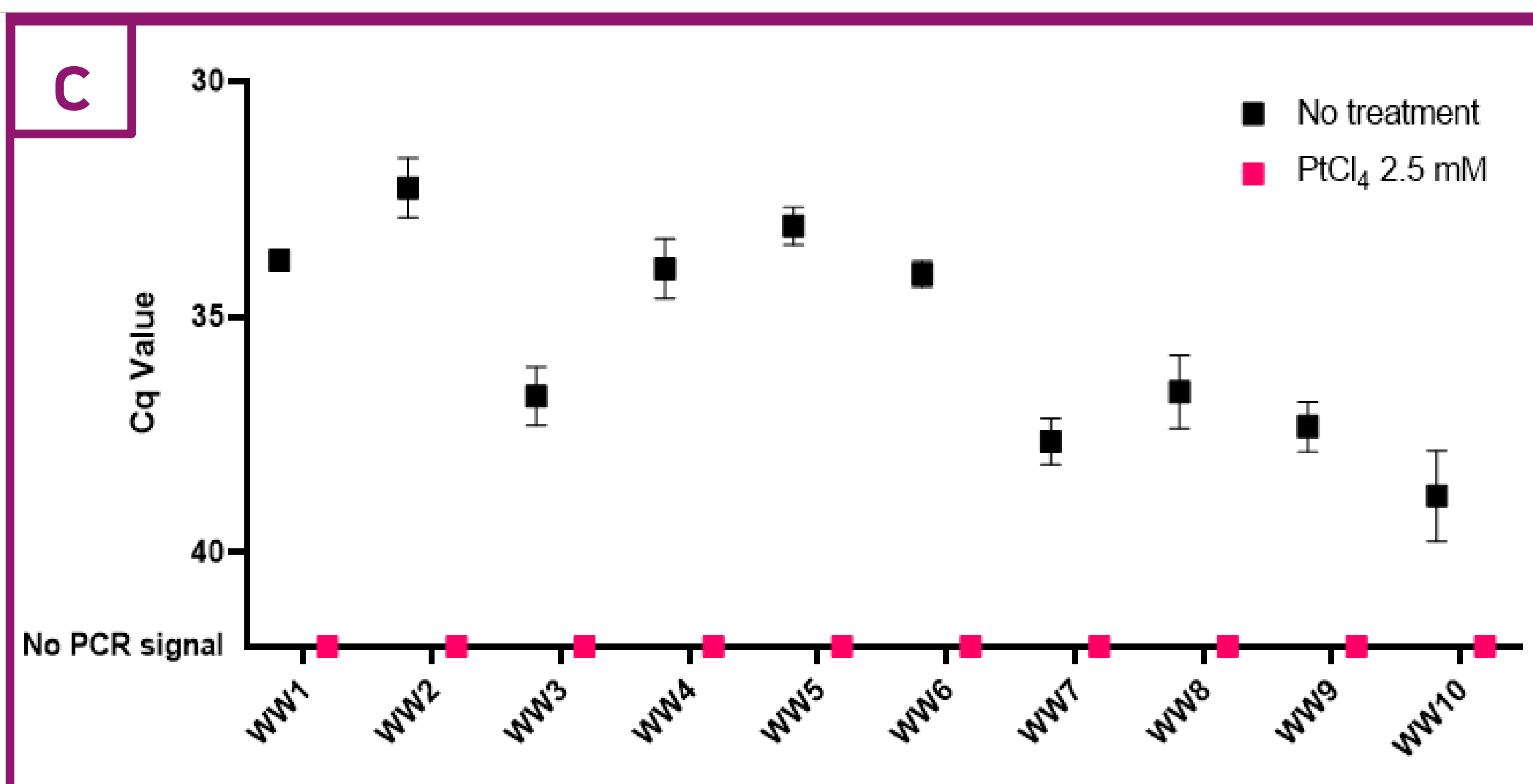
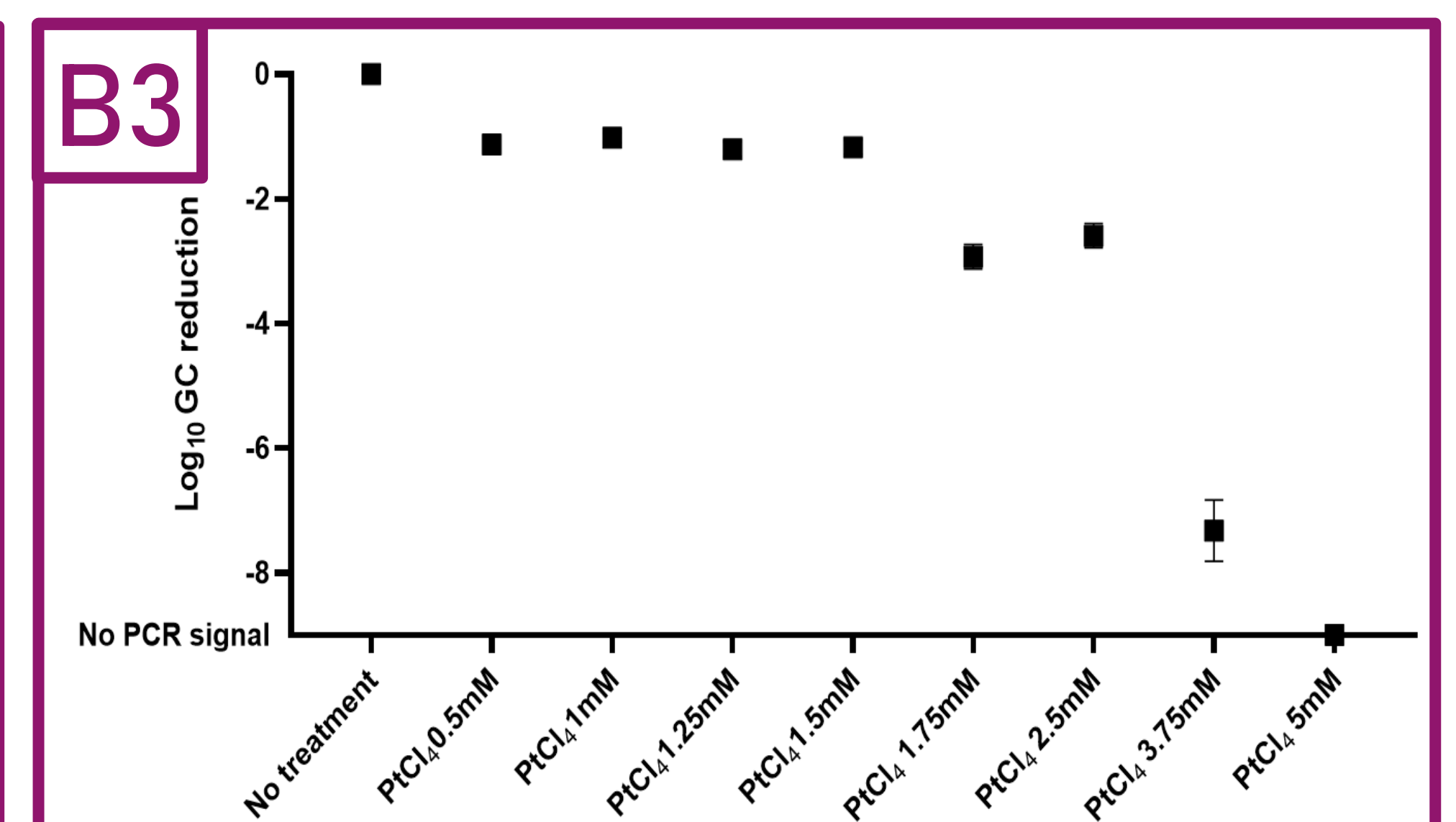
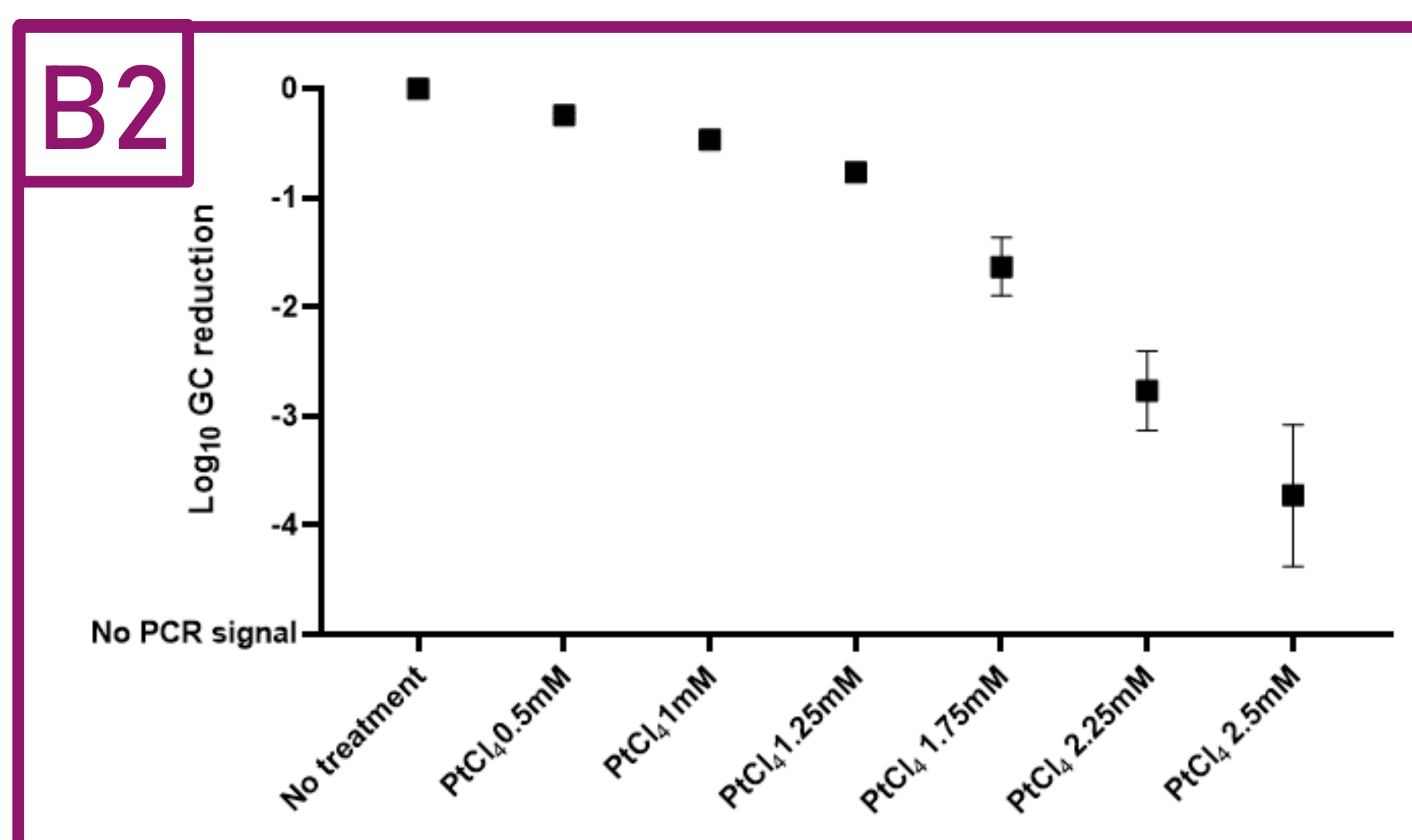
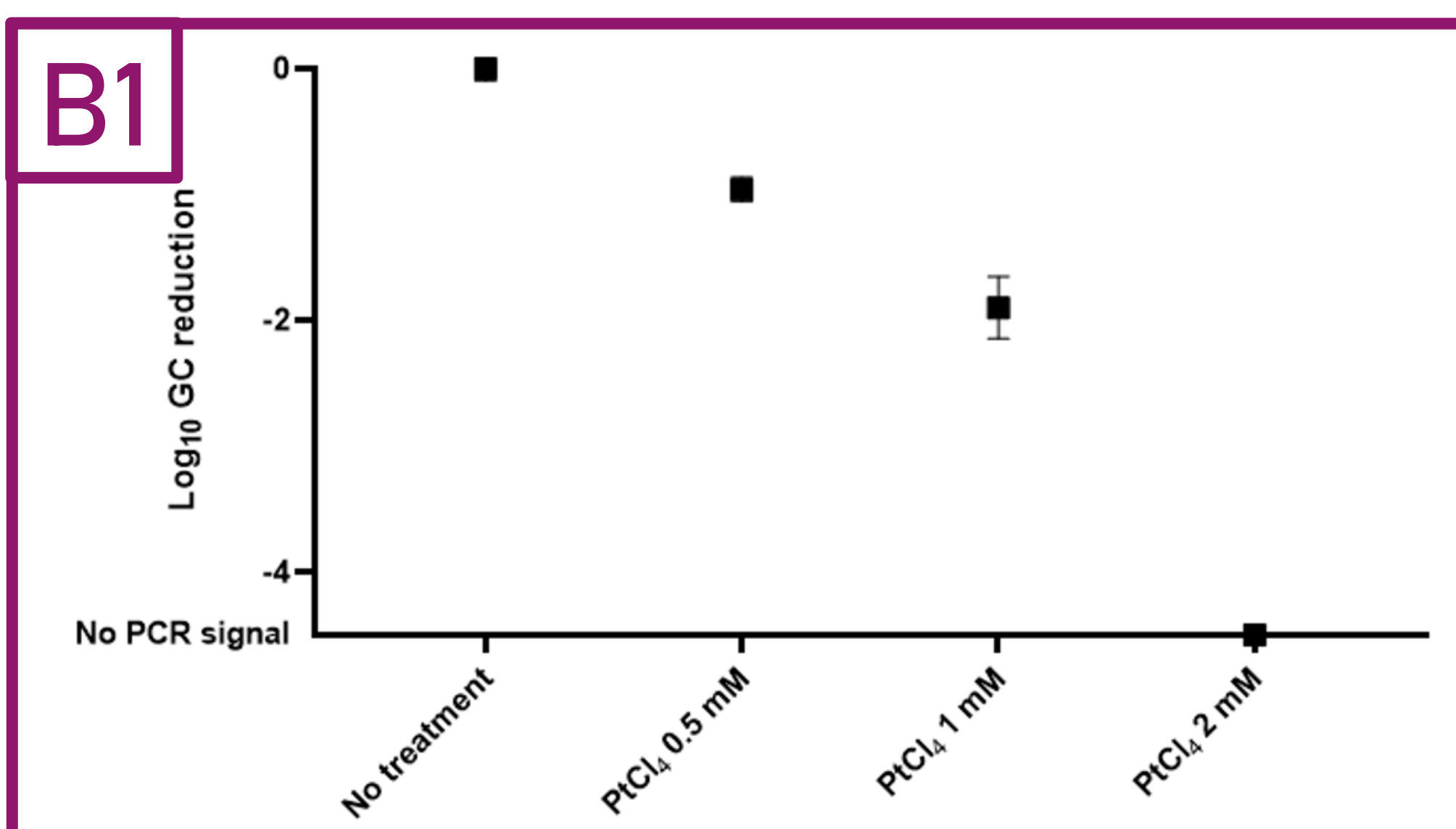
(A) Different viability markers (two monoazide dyes and two platinum compounds) were firstly evaluated on a genomic SARS-CoV-2 RNA targeting five different regions of SARS-CoV-2.



(B) Different matrices were treated with various concentrations of PtCl₄ prior to RNA extraction and amplification by RT-qPCR:

- (B1) Gamma-inactivated SARS-CoV-2 suspension in PBS.
- (B2) 1% vol. faecal suspension spiked with inactivated SARS-CoV-2.
- (B3) 10% vol. urine suspension spiked with inactivated SARS-CoV-2.

(C) Optimized viability RT-qPCR was applied on SARS-CoV-2 naturally contaminated influent wastewater samples.



CONCLUSIONS

- In general, RT-PCR signal is efficiently removed or reduced at PtCl₄ concentrations ranging from 1 to 2.5 mM depending on matrix complexity.
- This study sheds light on developing and optimizing molecular protocols for detecting and quantifying potentially infectious SARS-CoV-2 particles.

CONTACT PERSON

Enric Cuevas Ferrando
enric.cuevas@iata.csic.es
Gloria Sánchez Moragas
gloriasanchez@iata.csic.es



REFERENCES

- [1] Randazzo et al., Viability RT-qPCR to Distinguish Between HEV and HAV With Intact and Altered Capsids., *Front Microbiol.* 2018 Aug 24;9:1973
- [2] Fraisse et al., Discrimination of infectious and heat-treated norovirus by combining platinum compounds and real-time RT-PCR., *Int J Food Microbiol.* 2018 Mar 23;269:64-74
- [3] Puente H et al., Rapid Selective Detection of Potentially Infectious Porcine Epidemic Diarrhea Coronavirus Exposed to Heat Treatments Using Viability RT-qPCR., *Front Microbiol.* 2020 Aug 21;11:1911



FEB. 02-03, 2021 CONFERENCE ONLINE