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BIOSENSORS FOR PANDEMICS

Production and characterization of pseudotyped particles to develop an immunosensor for SARS CoV 2

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Introduction

Studying SARS-CoV-2 requires biosafety level 3 (BSL-3) facilities due to its virulence and transmission risk by aerosols. We have produced viral-like particles that express the spike glycoprotein of this emergent coronavirus by taking advantage of the pseudotyped particles tools to circumvent this issue. This simple, but highly beneficial tool, significantly reduces biological risk in the first steps of the immunosensor development requiring only a BSL-2 facility.

Methods and Results

Co-transfection

The pseudovirions were obtained by co-transfection of three plasmids as described by Millet et al [1]. Briefly, 800 ng of SARS-CoV-2 S plasmid, 600 ng of MLV-gag-pol plasmid and 600 ng Luciferase plasmid were co transfected in HEK-293 cells using linear Polyethylenimine. The supernatant of transfected cells was harvested 72 hours post-transfection.

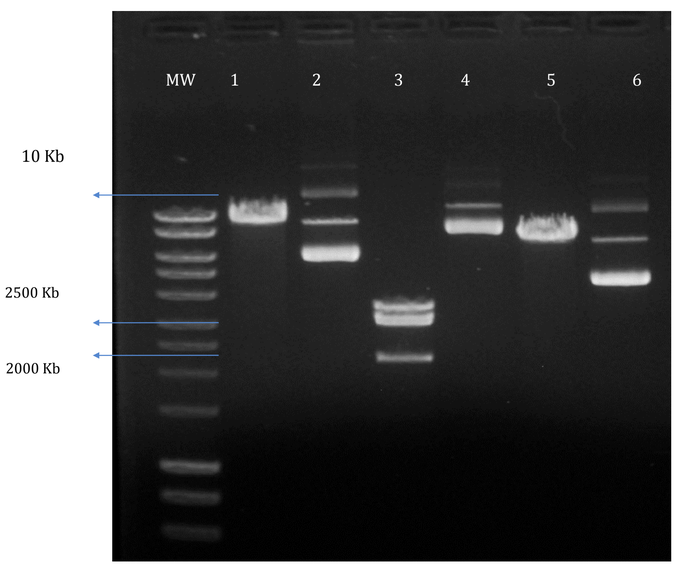


Figure 1. Plasmid rectriction digestion 1. SARS-CoV2 S plasmid after restriction using *Xba*I. **2.** SARS-CoV2 S plasmid **3.** MLV gag-pol plasmid after restriction using *EcoR*I **4.** MLV gag-pol plasmid **5.** Firefly luciferase gene/ MLV Ψ RNA / 5'/3' MLV LTR plasmid after restriction using *EcoR*I **6.** Firefly luciferase gene/ MLV Ψ RNA / 5'/3' MLV LTR.

Flow cytometry detection

The pseudotyped particles were characterized by flow cytometry, and indirect immunofluorescence, with a 13-color, 3-laser cytoFLEX S NVBR RVO flow cytometer (Beckman counter), operated with CytExpert v1.2 software. (Fig 2).

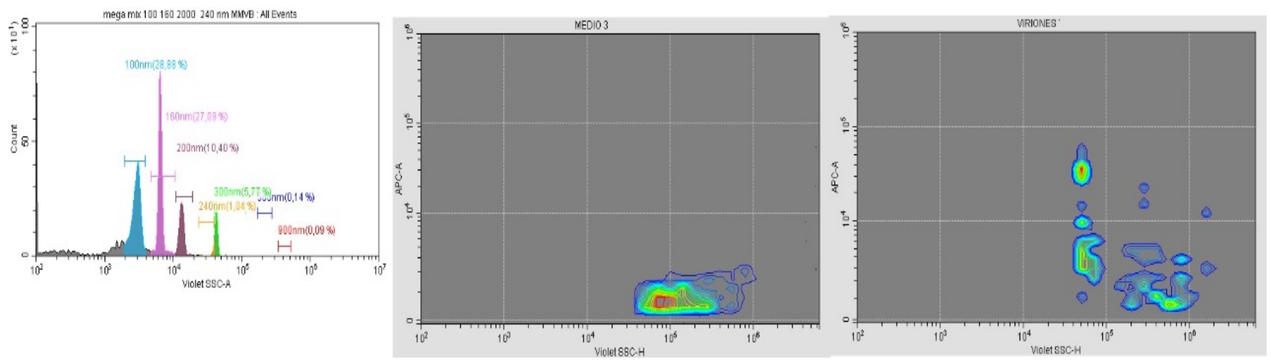
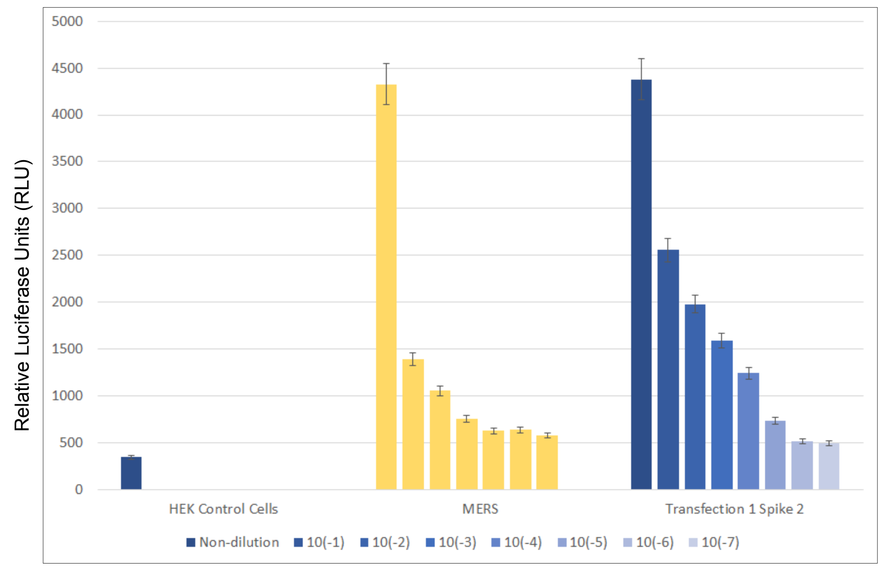


Figure 2. Characterization of pseudovirions by flow cytometry. Detection of particles of around 80 nm.

Infectivity quantification

The infectivity quantification of the pseudovirions was assessed by transduction of VERO-E6 cell using 10-fold serial dilutions. The Luciferase activity was detected 72 horas post transduction (Fig 3).

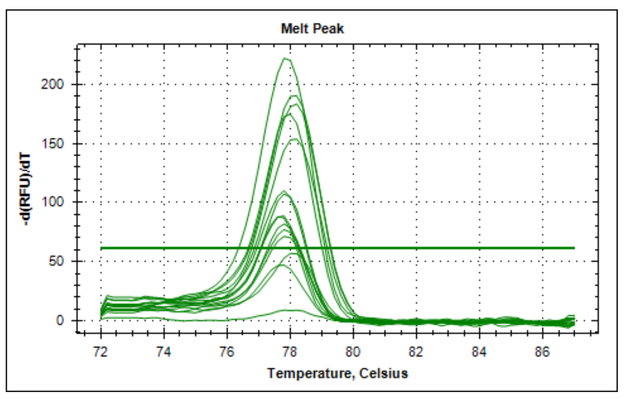


Pseudovirus Stock	Titer RLU/ml
SARS-CoV-2 Spike	7,76 x 10^8
MERS CoV Spike	5,66 x 10^7

Figure 3. Quantification of the pseudovirions expressing the Spike protein of SARS-CoV-2 and the Spike protein of MERS CoV after transduction of VERO-E6 cells by luciferase activity detection.

RT-pPCR quantification

The stock of pseudovirions expressing the Spike protein of SARS-CoV-2 and the one obtained by cotransfection of MERS-CoV Spike plasmid were quantifed by Real Time qPCR.



Pseudovirus Stock	Titer (copies/ml)
SARS-CoV-2 Spike	144.315,479
MERS CoV Spike	166.399,734

Figure 4. Quantification of copy number of the plasmid Firefly luciferase gene/ MLV Ψ RNA / 5'/3' MLV LTR by Real Time qPCR.

Current activities

Current experiments are directed to detect the pseudovirions by the interaction with a thiolated ACE2-reactive peptide linked to maleimide-coated magnetic particles, which electrochemical reaction will be followed by chronoamperometry by a biotinylated anti-ACE2 antibody conjugated with Streptavidin-Horseradish peroxidase (HRP).

CONTACT

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REFERENCES

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2. Kong YW, Cannell IG, de Moor CH, Hill K, Garside PG, Hamilton TL, et al. The mechanism of micro-RNA-mediated translation repression is determined by the promoter of the target gene. PNAS July 1, 2008 105 (26) 8866-8871;

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