GA 101003666 Start date: 01/04/20 End Date: 31/03/22	
Project Title	OPENCORONA
WP number, deliverable number, and Title	WP 2 D 2.1: Assays to measure cytokine induction and IFN suppression
Responsible partner name and contact	Partner number: 2 Organisation: JLU Name: Friedemann Weber Email: friedemann.weber@vetmed.uni-giessen.de
Nature R-Report P-Prototype D-Demonstrator O=-Other	**R**
Dissemination level **PU**-public **PP**-restricted to otherprogramme participants RE-restricted to a group of partners **CO**-only for consortium members	**PU** (to be published)
Delivery Month Planned	3
Actual delivery date (dd/mm/yy)	14/07/2020

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101003666

Description of deliverable

- **COMPLETED**

D 2.1: : Assays to measure cytokine induction and IFN suppression

RT-qPCR analyses

Interferon and cytokine induction by DNA vaccine candidates are measured by quantitative real-time PCR (qRT-PCR). For this, A549 cells are seeded into 24-well plates and transfected with 500 ng DNA using EndoFectin™ Max transfection reagent (GeneCopoeia, 3 µl per 1 µg DNA). Cell culture medium is changed 4 h after transfection. RNA is extracted from lysates of transfected cells using the RNeasy mini kit (Qiagen). For this, cells are lysed in 350 µl RLT buffer and lysates are processed according to manufacturer's instructions. Cellular RNA is then transcribed into copy DNA (cDNA) using the prime Script RT reagent kit (Takara). For initial genomic DNA elimination, 200 ng of RNA are used in a 1X reaction consisting of 1 µl gDNA eraser and 2 µl 5X gDNA eraser buffer in a final volume of 10 µl. Reaction is incubated for 2 min. at 42°C. For reverse transcription, 1 µl PrimeScript RT Enzyme Mix 1, 4 µl 5X PrimeScript buffer 2 and 4 µl RT primer mix are added to 10 µl gDNA eraser reaction in a final volume of 20 µl and incubated for 15 min. at 37°C and 5 sec. at 85°C.

Differential regulation of cellular genes is assayed using TB Green™ Premix Ex Taq™ II (Tli RNase H Plus; Takara) according to manufacturer's instructions with QuantiTect primer assays (Qiagen). For this, 10 ng of cDNA are used in a 1X reaction consisting of 12.5 µl TB Green Premix Ex Taq II (Tli RNaseH plus) (2X), 2 µl 10X QuantiTect primer assay, and 0.5 µl 50X ROX reference dye in a final reaction volume to 25 µl total. qPCR reactions are performed in a StepOne Plus Instrument (Thermo Fisher) with the protocol listed in table 1. QuantiTect primer assays are listed in table 2. 18S rRNA is used as housekeeping gene. Fold gene induction over mock treated control is calculated by the $\Delta\Delta C_T$ method.

Table 1. qPCR protocol

Plate setup: reporter FAM, quencher NFQ-MGB, dye as passive reference: ROX

Polymerase activation	20 sec	95°C	
Denaturation	1 sec	95°C	x 40 cycles
Annealing & extension	20 sec	60°C	

Table 2. List of QuantiTect primers assays.

Assay Name	Cat.no.:	Assay Name	Cat.no.:
Hs_CCL4_1_SG	QT01008070	**Hs_IFNL1_2_SG**	QT01033564
Hs_CCL5_1_SG	QT00090083	**Hs_IFNL2_1_SG**	QT00222488
Hs_CXCL10_1_SG	QT01003065	**Hs_IL6_1_SG**	QT00083720
Hs_CXCL8_1_SG	QT00000322	**Hs_RR18s**	QT00199367
Hs_IFNB1_1_SG	QT00203763	**Hs_TNF_3_SG**	QT01079561

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101003666

Reporter assays

Luciferase reporter assays are used to assess interference of the DNA vaccine candidates with the antiviral innate immune responses. For this, HEK293 cells were seeded into 96-well plates (1.5×10^4 cells per well) one day prior to transfection. Transfection mixes comprised the indicated firefly and Renilla luciferase reporter constructs (40 ng each; firefly luciferase under the control of IFNB-, ISG56-, MX1-, or NFκB-promoter; constitutively expressed Renilla luciferase as internal expression and transfection control), as well as expression constructs for the protein of interest or the control proteins ΔMx (negative control) or RVFV NSs (positive control) (10 ng). Plasmid DNA was transfected via TransIT®-LT1 (Mirus Bio LLC, 3 µl per 1 µg DNA). Cells were stimulated by transfection of VSV RNA (50 ng per well) or addition of IFN-α (50 IU per well) 24 h post transfection. Lysis and analysis of luciferase activities was performed 18 h after stimulation with the Dual Luciferase Reporter Assay System (Promega) according to the manufacturer's instructions. Firefly and Renilla luciferase were normalized to the stimulated control sample within each biological replicate, then mean and SD were calculated across three biological replicates.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101003666