GA 101003666 **Start date: 01/04/20** **End Date: 31/03/22**	
Project Title	OPENCORONA
WP number, deliverable number, and Title	WP 3 3.1 Established assay for determination of neutralizing antibodies to SARS-COV-2 that can be used to test mouse and rabbit sera
Responsible partner name and contact	Partner number: 3 Organisation: Public Health Agency of SWEDEN Name: Ali Mirazimi Email: Ali.Mirazimi@folkhalsomyndigheten.se
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
Delivery Month Planned	M 3
Actual delivery date (dd/mm/yy)	16/07/2020

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**

Detection of SARS-CoV-2 Neutralizing antibodies

Purpose

Detection of neutralizing antibodies against SARS-CoV-2 in serum.

Virus: SARS-CoV-2

Controls

- Negative control: Negative serum in equal dilution as the sample.
- Cell control: Cell culture medium alone
- Positive control: SARS-CoV2 Virus.

Procedure

- Vero E6 cells are plated in a 96 well plate. Plate $1.5x10^4$ cells/well and incubate in 37°C, 5% CO_2 over night.
- Cells are ready to be used when 90-95% confluence is reached.

- Dilute the serum sample and negative serum control
  - Make 2-fold serial dilutions of the serum samples and negative serum control.
  - All dilutions are made in quadruplicates

- On a round bottom 96 well plate add 55 ul of :
  - diluted serum samples to designated wells
  - diluted negative control serum to designated wells
  - only cell culture medium to designated wells (cell control)
  - only cell culture medium to designated wells (positive control)

- In a total of 55 ul, SARS-CoV-2 virus (500 pfu) to each designated well
  - serum samples and positive control wells

- Add 55 ul of medium to designated wells
  - negative control serum and cell control

- Incubate plate 1 hr in 37°C, 5% CO_2
- After 1 hr, remove cell culture medium from the Vero E6 plate
- add 100 ul of the serum/virus mix as well as the negative and positive controls to designated wells on the cell plate

- Incubate in 37°C, 5% CO_2 for 3 days.

- The cytopathogenic effect for each well is investigated

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

GA 101003666 **Start date: 01/04/20** **End Date: 31/03/22**	
Project Title	OPENCORONA
WP number, deliverable number, and Title	WP3, D3.2 Challange protocol
Responsible partner name and contact	Partner number: 3 Organisation: FOHM Name: Ali Mirazimi Email: Ali.Mirazimi@folkhalsomyndigheten.se
Nature R-Report P-Prototype D-Demonstrator O=-Other	Report
Dissemination level **PU**-public **PP**-restricted to otherprogramme participants RE-restricted to a group of partners **CO**-only for consortium members	Public
Delivery Month Planned	M6, September 2020
Actual delivery date (dd/mm/yy)	25 November 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**

Challenge protocols has been established in mice and ferrets.

In mice the hACE2 mice is susceptible to infection as described below. This model will be used to evaluate protection of infection after vaccination with the selected SARS-CoV-2 vaccine candidate/s.

In ferrets an infection study was performed to determine infectious dose and understand disease. The established model has thereafter been used to evaluate protection of infection after vaccination with the selected SARS-CoV-2 vaccine candidate/s.

Infection Protocols in mice:

SARS-CoV-2 infection of young and old BALB/c mice:

10 weeks (young) 7-8 months (old) BALB/c mice were Intranasally infected by either 10^5 or 10^3 pfu in a total of 20 ul medium (10 ul/nostril) of SARS-CoV-2. SARS-CoV-2 was isolated from a nasopharyngeal sample of a patient in Sweden on Vero E6 cells. Virus was titered using a plaque assay as previously described (Becker et al., 2008) with fixation of cells 72 hours post infection. The SARS-CoV-2 isolate was sequenced by Next-Generation Sequencing (Genbank accession number MT093571).

At 4 and/or 14 days post infection mice were sacrificed, and serum, lung, liver, spleen and kidney were collected for detection of infectious virus particles, viral RNA and antibodies against SARS-CoV-2.

No or very low levels of viral RNA detected in these mice. No clinical symptoms detected. This model will not be further evaluated within this project.

SARS-CoV-2 infection of hACE2 mice:

K18-hACE2 transgenic mice express human ACE2, the receptor used by severe acute respiratory syndrome coronavirus (SARS-CoV) to gain cellular entry. The human keratin 18 promoter directs expression to epithelia, including airway epithelia where infections typically begin. Because K18-hACE2 are susceptible to SARS-CoV-2 and SARS-CoV viruses, they are useful for studying antiviral therapies to COVID-19 and SARS. 14 weeks old K18 female mice (B6.CgTg(K18ACE2)2Prlmn/J, Hemizygot) were infected 10^5 pfu in a total of 40 ul medium (20 ul/nostril). SARS-CoV-2 was isolated from a nasopharyngeal sample of a patient in Sweden on Vero E6 cells. Virus was titered using a plaque assay as previously described (Becker et al., 2008) with fixation of cells 72 hours post infection. The SARS-CoV-2 isolate was sequenced by Next-Generation Sequencing (Genbank accession number MT093571).

Mice were sacrificed every 2 days post infection**.** At euthanasia, collection of nasal lavage sample, serum, lung, liver, spleen and kidney.

Viral RNA detectible in challenged animals. Clinical symptoms starting 4 days post infection. The hACE2 mice is a good model and will be used to evaluate protection against infection/disease after vaccination.

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