


<p><b>GA 101003666</b>  <b>Start date: 01/04/20</b>  <b>End Date: 31/03/22</b></p>	
<p><b>Project Title</b></p>	<p>OPENCORONA</p>
<p><b>WP number, deliverable number, and Title</b></p>	<p>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</p>
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<p><b>Nature</b>          R-Report          P-Prototype          D-Demonstrator          O=-Other</p>	<p>R</p>
<p><b>Dissemination level</b>  <b>PU</b>-public  <b>PP</b>-restricted to otherprogramme participants          RE-restricted to a group of partners  <b>CO</b>-only for consortium members</p>	<p>PU</p>
<p><b>Delivery Month Planned</b></p>	<p>M 3</p>
<p><b>Actual delivery date (dd/mm/yy)</b></p>	<p>16/07/2020</p>



# 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  $1.5 \times 10^4$  cells/well and incubate in 37°C, 5% CO<sub>2</sub> 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<sub>2</sub>
- 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<sub>2</sub> for 3 days.
  
- The cytopathogenic effect for each well is investigated

