



<b>SOP Name</b>	Flow Cytometry based-Live SARS-CoV-2 Micro-Neutralisation assay
<b>SOP Identifier</b>	LAB007 Flow based neutralisation assay
<b>Edition</b>	Version 1
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## 1. SCOPE

Evaluation of the humoral immunity of participants in COVID-19 vaccination trials

## 2. PURPOSE

This assay is design to quantify and compare the neutralization capacity of plasma/serum samples (convalescent/vaccinated) against wild type and VoCs SARS-CoV-2.

## 3. POLICY

VACCELERATE works within the guidelines and regulations of the EU CT Directive 2001/20/EC, GCP Commission Directive 2005/28/EC. ICH/GCP and with all the other local and international applicable regulatory requirements.

## 4. ROLES AND RESPONSIBILITIES

Clinical/laboratory sites: Collection, labeling and immediate storage of plasma samples (300ul) at -80C .

Clinical/laboratory sites: Shipment (dry ice) of batch of plasma samples (300ul) within two weeks of collections

Research laboratory with Containment Level 3 facility: Flow Cytometry-based Live SARS-CoV-2 Micro-Neutralisation assay, data collection and analysis.

## 5. DEFINITIONS

## 6. RELATED DOCUMENTS

SOPs for collection of blood samples, processing plasma samples, labeling, recording, storage and shipment of plasma samples.

## 7. PROCEDURES

SARS-CoV-2 is classified as a Risk Group 3 Biological Agents. All procedures involving the isolation, preparation of viral stock, titration and manipulation of live SARS-CoV-2 for research work require authorisation from national authorities and must take place in a Containment Level 3 facility according to the biosafety level 3 guidelines, code of practice and SOPs in place.

### Equipment

Containment Level 3 laboratory

Class two Biosafety cabinet (BSC)

Table-top centrifuge with swinging bucket rotors and sealed buckets for 96 well-plates

Flow cytometer

### Materials

	Product	Manufacturer	Reference
Cells	Vero E6 TMPRSS2 cells	National Institute for Biological Standards and Control	100978
Cell Culture	DMEM	Thermo Fisher Gibco	61965-026
	Foetal Bovine Serum	Thermo Fisher Gibco	10500-064
	Geneticin	Thermo Fisher Gibco	10131-027

	Phosphate Buffered Saline	Thermo Fisher Gibco	14190-094
	Trypsin	Thermo Fisher Gibco	25300-054
	Penicillin/Streptomycin	Thermo Fisher Gibco	15140-122
	Amphotericin B	Thermo Fisher Gibco	15290-026
Fixation and Permeabilisation	Formaldehyde solution	Sigma	F8775
	Perm/Wash	BD	554723
	Phosphate Buffered Saline	Thermo Fisher Gibco	14190-094
	Deionised Water	NA	NA
Nucleocapsid Staining	SARS/SARS-CoV-2 Coronavirus Nucleocapsid Antibody	Invitrogen	MA1-7403
	FITC Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody	Invitrogen	F-2761

## Methods

Neutralisation assays are performed in a 96 well plate format using VERO E6-TMPRSS2 cells and wild type SARS-CoV-2 and VOCs. Each participant sample (convalescent/vaccinated plasma) is first heat inactivated @ 56°C for 30 min and then serial diluted (half-log) starting at 1/20 with 8 dilutions. Plasma dilutions are incubated with virus for 1 h @ 37°C. Virus-plasma mixture are added in duplicate wells onto monolayer of VERO E6-TMPRSS2. After 18h incubation @ 37°C cells are trypsinised and fixed in 4% formaldehyde overnight. Cells are then permeabilised (BD perm/wash) and stained for SARS-CoV-2 Nucleocapsid protein (NP) in 96-well plate (round bottom). Percentage of SARS-CoV-2 infected cells (NP+) is analysed by flow cytometry. % cells infected with virus alone (positive control) should reach between 30-50%. The half maximal Neutralisation Titers (NT<sub>50</sub>) are determined using four-parameter logistic regression using GraphPad Prism.

## 8. REVIEW AND REVISION

## 9. DOCUMENT HISTORY

Version Number	Effective Date:	Summary of changes from previous version:	Edited by: (name and role)
01			

## 10. APPENDICES