



SOP Name	Flow Cytometry based-Live SARS-CoV-2 Micro-Neutralisation assay
SOP Identifier	LAB007 Flow based neutralisation assay
Edition	Version 1
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Author	Dr Virginie Gautier, Associate professor in Virology, Centre for Experimental Pathogen Host Research (CEPHR), School of Medicine, University College Dublin (UCD), Ireland

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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 (NT₅₀) 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)
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10. APPENDICES