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| **SOP Name** | Flow Cytometry based-Live SARS-CoV-2 Micro-Neutralisation assay |
| **SOP Identifier** | LAB007 Flow based neutralisation assay |
| **Edition** | Version 1 |
| **Effective Date** |  |
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1. **SCOPE**

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

# 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.

# 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.

# 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.

# DEFINITIONS

1. **RELATED DOCUMENTS**

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

# 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

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|  | 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.

# REVIEW AND REVISION

1. **DOCUMENT HISTORY**

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| --- | --- | --- | --- |
| **Version Number** | **Effective Date:** | **Summary of changes from****previous version:** | **Edited by: (name and role)** |
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1. **APPENDICES**