State-of-the-art SARS-CoV-2 vaccine testing services

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Introduction

Viroclinics-DDL is a leading diagnostic and clinical trial operation service company supporting clinical and preclinical studies for drug and vaccine development for prevention and treatment of virus infections. Our mission is to improve human and animal health by serving the biopharmaceutical industry with state-of-the-art diagnostics, operational and logistical services, custom-made models in preclinical and clinical drug testing, and expert advice on development of antivirals and vaccines. We offer a full range of virology services for new drug development programs and post-marketing surveillance of existing drugs and vaccines, ranging from traditional virology assays to the latest deep sequencing protocols for a very broad range of viruses. Our BSL2 and BSL3 labs enable us to perform analyses according to international ISO 15189 accreditation, GLP and GCLP.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), previously known as 2019 novel coronavirus (2019-nCoV) was discovered in China in December 2019. The virus can cause asymptomatic, mild or life-threatening disease in humans, designated coronavirus disease 2019 (COVID-19). On March 11th 2020, the WHO declared SARS-CoV-2 to be a pandemic. As of October 2021, nearly 5 million deaths have been reported as a result of COVID-19.

SARS-CoV-2 Virospot microneutralization (MN) assay

The SARS-CoV-2 Virospot MN assay developed at Viroclinics-DDL can be used to measure neutralizing antibody titers in serum and EDTA plasma samples, and can also be used to determine the potency of therapeutic mAbs against SARS-CoV-2 stocks (Ryu et al. Biochem Biophys Res Commun. 2021 Aug 20). Moreover, the assay can be used to determine the susceptibility of SARS-CoV-2 clinical isolates for neutralization by sera and EDTA plasma of COVID-19 patients, vaccinated individuals or therapeutic mAbs. In brief,

1. Twofold serial sample dilutions are mixed 1:1 with SARS-CoV-2 and incubated for 1 hour
2. The mixture is transferred to plates containing Vero E6 cells and incubated for 1 hour
3. Plates are aspirated, overlay medium containing CMC is added and incubated for ~20 hours
4. Cells are formalin fixed and immunostained with a primary antibody directed against the virus nucleocapsid protein, followed by a HRP-conjugated secondary antibody and TrueBlue substrate
5. Virus-positive microplaques (spots) are counted using a CTL Immunospot S6 Ultimate Analyzer equipped with Biospot software
6. The 50%, 80% and 90% microplaque reduction titers (MN50, MN80 and MN90, respectively) are calculated according to the method described by Zielinska et al. Virology journal vol. 2 84. 9 Nov. 2005. Therapeutic mAb results are typically expressed as IC50, IC80 or IC90 in µg/mL.
7. Titer results can be expressed in IU/mL, relative to the first WHO International Standard 20/136, to facilitate the comparison of results produced by different laboratories.

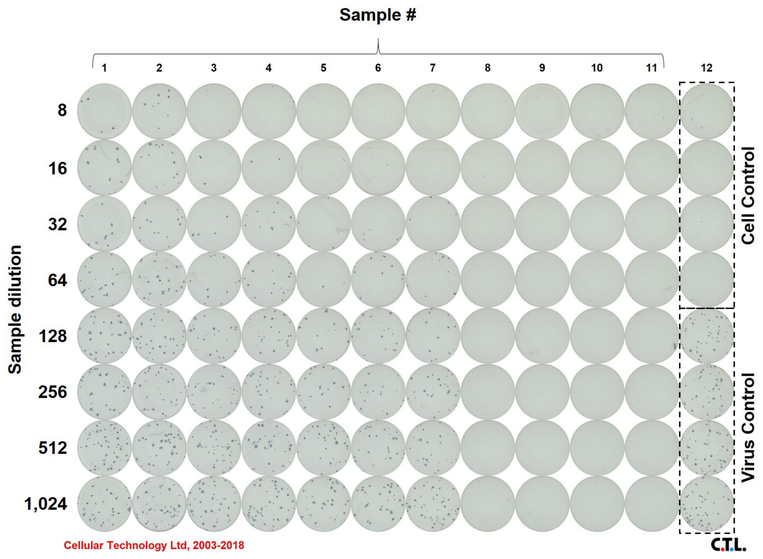

Figure 1 *Example plate layout used in the SARS-CoV-2 MN assay. Up to eleven samples can be vertically titrated per plate, on 3 replicate plates, starting with an 8-fold starting dilution. Each plate contains four cell control and four virus control wells.*

Since the start of the COVID-19 pandemic various virus variants have emerged for which increased transmissibility, increased virulence and/or increased resistance to neutralization is expected or has been demonstrated (https://www.cdc.gov/coronavirus/2019-ncov/variants/variant-info.html). These variants include Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2 / AY.4), Kappa (B.1.617.1) and Lambda (C.37). At present, SARS-CoV-2 Delta is the most dominant variant globally. Viroclinics-DDL has cultured stocks of these and other SARS-CoV-2 variants which are available for testing in the SARS-CoV-2 Virospot MN assay.

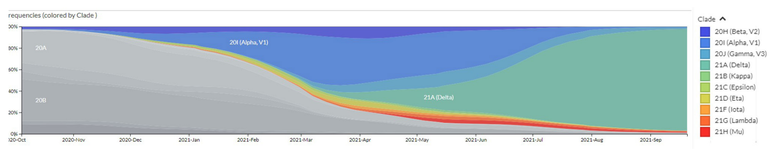

Figure 2 *Global prevalence of SARS-CoV-2 variants. Source https://nextstrain.org*

The SARS-CoV-2 Virospot MN assay is fully validated in 2020 using one of the earliest isolates of the virus, here designated wildtype (WT). Parameters such as accuracy, linearity, repeatability, intermediate precision, LLOQ, ULOQ and range were successfully assessed. Currently, the assay is undergoing optimization to make the assay suitable for measuring neutralization of SARS-CoV-2 variants. Figure 3 illustrates why optimization of the assay is needed when SARS-CoV-2 variants are used. Once optimization is completed the assay will be validated using Alpha, Beta, Gamma, Delta, Kappa and Lambda variants, which is expected to be completed by the end of 2021.

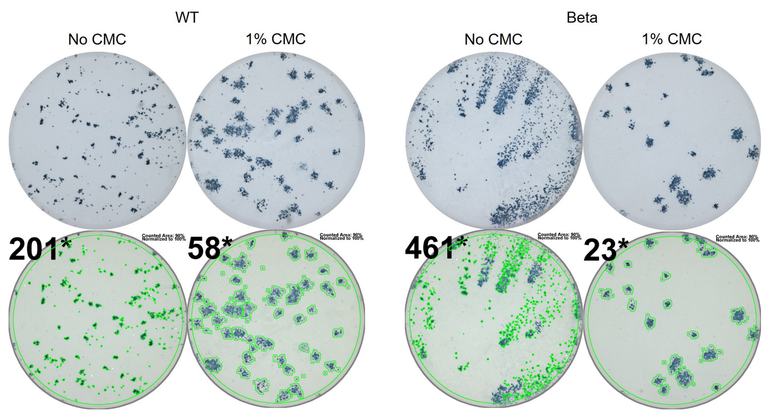

Figure 3 *SARS-CoV-2-positive foci and microplaques, i.e. spots, visualized using virus-specific immunostaining. The morphology of the spots varies between SARS-CoV-2 variants as shown here for WT and Beta variants. Whereas, in the absence of CMC overlay, the WT virus produces relatively small spots, the Beta variant has a "comet-shape" spot morphology. In both instances, due to secondary infections, determination of the virus input is challenging. By adding CMC overlay to the cell monolayer, progeny virus is confined to the initial site of infection, thereby causing the spots to become similar in size and morphology and allowing for a more accurate estimation of the virus input. Per variant, the wells depicted with and without CMC overlay are the same virus dilution.*

Viroclincs-DDL compiled a reference panel of serum and plasma samples originating from convalescent patients, vaccinated individuals and pre-pandemic donations. These samples are used for optimizing the assay and available for assay validation. Our data demonstrates that WT virus and Alpha and Gamma are similarly neutralized by convalescent sera/plasma (Figure 4). In contrast, a mean seven-fold reduction in the MN50 titers was observed against Beta variant, which is confirming earlier findings (Planas et al. Nature medicine vol. 27,5 (2021): 917-924).

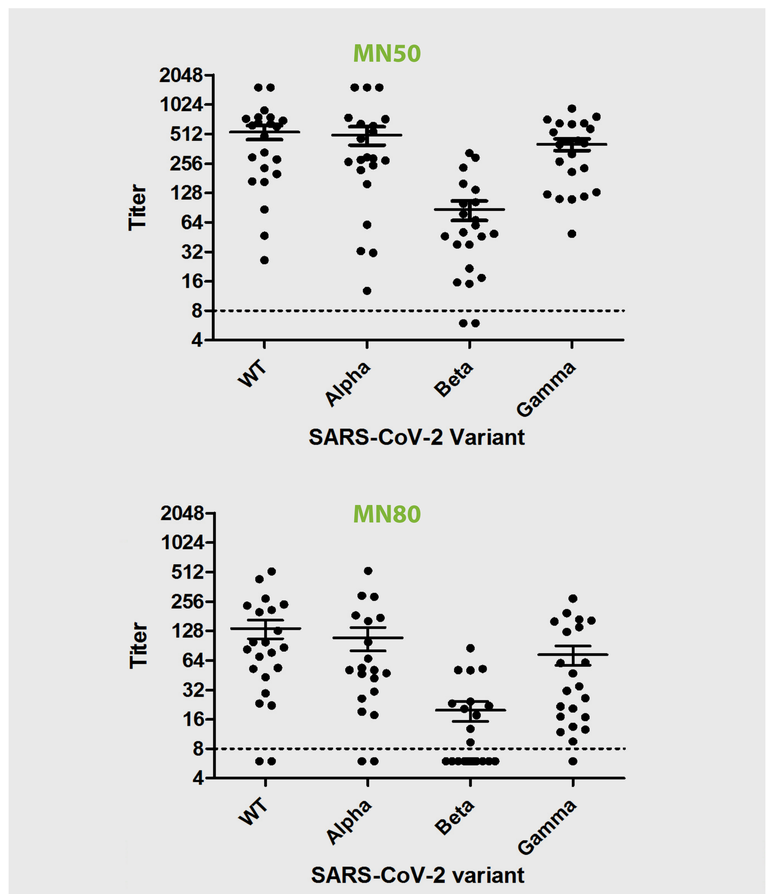

Figure 4 *MN titer determination of 22 serum and plasma samples against four SARS-CoV-2 variants using the SARS-CoV-2 Virospot MN assay. Results can be expressed as MN50, MN80 and MN90 titers, which are the reciprocal of the sample dilution at which 50%, 80% and 90% inhibition of infection is observed. SARS-CoV-2 Beta is less susceptible to neutralization by sera and plasma from convalescent individuals compared to WT, Alpha and Gamma variants. Each result is based on a technical triplicate. Horizontal dashed line indicates the lowest sample dilution tested.*

Multiplex binding assays for SARS-CoV-2 immunogenicity testing

Viroclinics-DDL offers multiplex assays for the detection and quantitation of binding antibodies to SARS-CoV-2 antigens, using the Meso Scale Discovery (MSD) platform. The assays allow for measuring up to ten analytes in a single well using only small sample volumes up to 25 µL, using ready-to-use kits. Plates are provided with antigens coated on spots in the wells of 96-wells plates (Figure 5). Antibodies in samples bind to the antigens on the spots and anti-human antibodies conjugated with MSD SULFO-TAG are used for detection. The plates are read on an MSD instrument, which measures the light emitted from the MSD SULFO-TAG. Various kits are available to measure IgA, IgM and IgG against N, RBD and Spike proteins of SARS-CoV-2 variants. MSD SARS-CoV-2 Panel 2 (IgG) Kit (Cat# K15383U) is currently undergoing verification and implementation at Viroclinics-DDL (Figure 6).

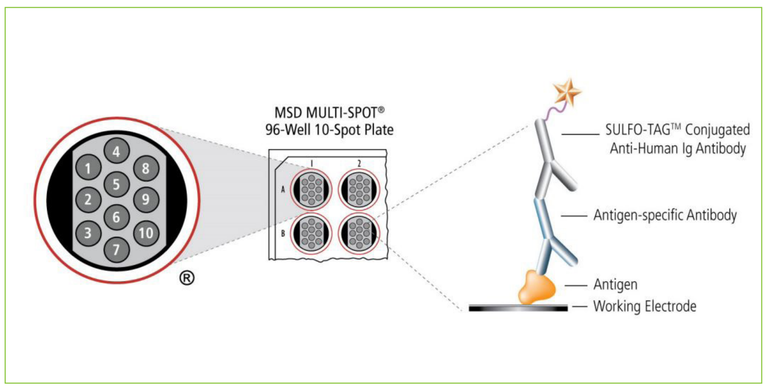

Figure 5 *MSD assay principle. Up to 10 antigens are coated at designated positions i.e. "spots" in the wells. Serum antibodies bind the antigen which are subsequently detected by a SULFO-TAG conjugated anti-human antibody. When electricity is applied to the plates the electrochemiluminescence (ECL) reaction starts and intensity of the light emitted at 620 nm is proportional to the amount of antibody bound to the antigen.*

Figure 6 *N, RBD and Spike IgG binding results of 9 convalescent and 10 pre-pandemic samples using the validated V-PLEX SARS-CoV-2 Panel 2 (IgG) Kit (Cat# K15383U). Each result is based on a technical duplicate. Results are expressed as binding antibody units per mL (BAU/mL) relative to the first WHO International Standard 20/136. Horizontal dashed lines are the cutoff values for each antigen. Sample results below cutoff (i.e. negative) are shown as 8 BAU/mL.*

Conclusion

The SARS-CoV-2 Virospot assay platform for whole virus neutralization and the MSD SARS-CoV-2 assay kits for antibody binding are available for large scale testing of clinical trial samples and are continually updated to accommodate testing of novel virus variants that arise in the field.

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