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Technical Note

SARS-CoV-2

Impact To Biological Drug Products Safety

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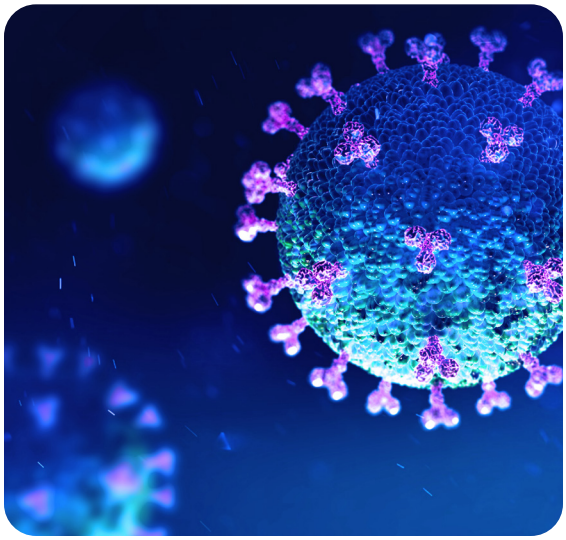
Executive Summary

It is our assessment that our regulatory compliant *in vitro* assays using Vero detector cells are a suitable method for the detection of adventitious SARS-CoV-2 in biological samples. We are also prioritizing the implementation of a GMP-compliant PCR assay for the detection of SARS-CoV-2 and offering GMP-compliant NGS analysis for broad spectrum adventitious agent screening as part of our testing packages.

As with any known or new/emerging viruses, a comprehensive testing strategy is required to address the risk of adventitious viruses through the production process, from starting materials to bulk harvest, and as identified through a virus safety risk assessment. This technical note is comprised of a review of currently available literature and our recommended SARS-CoV-2 virus detection test methods and susceptibility of common biological host cells to infection.

The scope of this document covers suitability of detection methods for SARS-CoV-2. Suitability of methods for removal / inactivation of SARS-CoV-2 are not included in this technical note.



The current global pandemic of coronavirus disease 2019 (COVID-19), is caused by a newly identified coronavirus (now classified as SARS-CoV-2). Currently, four genera of coronaviruses are recognized: Alphacoronaviruses, Betacoronaviruses, Gammacoronaviruses and Deltacoronaviruses. Alpha- and Betacoronaviruses generally infect mammals whereas Gamma- and Deltacoronaviruses are primarily found in birds. Whole genome sequence analysis places SARS-CoV-2 in the genus of Betacoronaviruses alongside severe acute respiratory syndrome coronavirus (SARS-CoV, now classified as SARS-CoV-1, the etiological agent responsible for the SARS epidemic 18 years ago), Middle East respiratory syndrome coronavirus (MERS-CoV) and SARS-like bat coronavirus[1]. This analysis revealed that SARS-CoV-2 has highest sequence similarity with SARS-like bat coronavirus and that SARS-CoV-2 is more closely related to SARS-CoV-1 than to MERS-CoV.

Review

Detector cell suitability

Similar to both SARS-CoV-1 and MERS-CoV, SARS-CoV-2 has been shown to replicate to high titres in Vero E6 cells[2,3]. SARS-CoV-2 produces clear cytopathic effect (CPE) in these cells where infected cells become damaged, clustered and ultimately are destroyed.

Vero E6 cells, African Green Monkey (*Cercopithecus aethiops*) kidney cells, are a subclone of Vero 76 cells which, in turn, are derived from the original Vero cells. We use Vero E6 cells (also known as Vero C1008 cells) in certain *in vitro* assays (i.e. the UK 'bovine' assays 032910GMP.BUK and 032930GMP.BUK, 'porcine' assay 033970GMP.BUK and they may be used in any of the veterinary vaccines *in vitro* assays in 060100GMP.BUK, and 060110GMP.BUK) but are a different lineage than the Vero cells (original clone, Vero CCL-81) we use for general *in vitro* assays (003 assay series globally).

003 assay series Vero cell suitability

Vero C1008 (Vero E6) cells have a lower saturation density than the original Vero clone and show a degree of contact inhibition after forming a monolayer and are generally considered better detector cells for slowly propagating viruses compared to the original Vero cells. For fast and moderately propagating viruses there is no known difference in virus susceptibility between the original and E6 clones. Considering the reported replication rate and rapid CPE formation of SARS-CoV-2 in Vero E6 cells it is a reasonable and justified assumption that the virus will be equally readily detected in the Vero CCL-81 cells used in the 003 *in vitro* assay series. This is supported by findings that SARS-CoV-1 replicated equally well in Vero CCL-81 cells as in Vero E6 cells[4] and more significantly, replication of SARS-CoV-2 in Vero CCL-81 cells has recently been confirmed in two separate publications[5,6]. Both publications report the excellent susceptibility of Vero CCL-81 cells to SARS-CoV-2 and clear CPE formation after viral propagation.

Our regulatory-compliant detector cell based *in vitro* assays (003 assay series) are 14- or 28-day culture assays that include the Vero CCL-81 detector cells at all three global sites (US, UK and Singapore). The time to first appearance of CPE in Vero detector cells reported in the literature was as short as 2 days but was commonly first noticed approximately 6-8 days, post-inoculation. Therefore, the use of Vero cells in cell-based *in vitro* assays, with a CPE endpoint, are considered a suitable test system for the detection of SARS-CoV-2 in starting materials and intermediates used in the production of biopharmaceuticals.

Host cell tropism

Despite the very recent emergence of the virus, there already is a considerable amount of research data available on SARS-CoV-2, including *in vitro* studies. The scientific community has quickly responded to the outbreak and assumptions regarding the virology and pathogenesis of SARS-CoV-2 are extrapolated from the related MERS and SARS coronaviruses and rapidly tested in laboratories around the world. For instance, MERS-CoV RNA has been detected in a variety of human specimens, including urine, feces, and blood, and it is reasonable to assume this could be the case for SARS-CoV-2[7]. In addition, MERS-CoV and SARS-CoV-1 tissue tropism appears to be broad in humans and this is likely to be the case for SARS-CoV-2.

It has already been established that, at least *in vitro*, SARS-CoV-2 uses the same cellular entry receptor as SARS-CoV-1: angiotensin converting enzyme II (ACE2)[8], which is different than the entry receptor used by MERS-CoV. The study also showed that SARS-CoV-2 could utilize human, horseshoe bat, civet, and pig variants of ACE2 for viral entry, but not mouse ACE2, suggesting a potential broad host range.

Single cell RNA analysis identified ACE2 expression in human lung alveolar type 2 (AT2, main target cell of SARS-CoV-2), liver cholangiocyte, colon colonocytes, oesophagus keratinocytes, ileum enterocytes, rectum enterocytes, stomach epithelial cells, and kidney proximal tubules, suggesting broad ACE2 expression and host cell tropism[9,10] and could explain why some patients with COVID-19 exhibit non-respiratory symptoms, such as kidney failure. Interestingly, the respiratory epithelial cells from the respiratory tract contain approximately 2% of cells expressing ACE2 but almost no cells obtained from the nasal and bronchial samples showed ACE2 expression.

Susceptibility to SARS-CoV-2 contamination of common biological producer cells

Cell types commonly used for manufacturing biologics include Human Embryonic Kidney (HEK) 293 cells, Chinese Hamster Ovary (CHO) cells, Madin Derby Canine Kidney (MDCK) cells, certain insect cells, and many more. Prediction of the susceptibility of specific cell lines to SARS-CoV-2 contamination is difficult without empirical evidence. This is emphasized by the findings that SARS-CoV-2 could utilize human, horseshoe bat, civet, and pig ACE2 for viral entry, but not mouse ACE2[8] which was supported by analysis of the amino acid composition of the receptor binding motif of ACE2. It was predicted that murine ACE2 would not support SARS-CoV-2 infection but Chinese hamster ACE2 will support SARS-CoV-2 entry[11]. In contrast, it was shown that SARS-CoV-1, which uses the same entry receptor, did not replicate efficiently in HEK293 and CHO cells[3]. Therefore, it is likely that ACE2 expression is low in these cells which would render them refractory to SARS-CoV-2 contamination. One study showed that although HEK293 cells did support viral replication when inoculated with a high titer of SARS-CoV-2, replication was not very efficient[5], in line with a low expression of ACE2. The same study concluded that human liver cell line HUH7 also moderately supported SARS-CoV-2 replication and that human adenocarcinoma cell line A549 did not support SARS-CoV-2 replication. Of the cell lines tested, only Vero E6 and Vero CCL-81 cells showed CPE upon infection with SARS-CoV-2. Insect cells express a homologue of ACE2 that is genetically divergent enough to assume they are resistant to SARS-CoV-2 infection. In conclusion, the ability of SARS-CoV-2 to replicate in a host cell is determined by multiple factors, including the expression of an appropriate version of the ACE2 receptor, which can differ significantly between cell types from the same species. Without empirical evidence it is difficult to predict which cells will be susceptible to SARS-CoV-2 infection.

Our approach to SARS-CoV-2 testing

The available data indicates that our regulatory-compliant *in vitro* assays using Vero cells (both lineages) are suitable for the detection of adventitious SARS-CoV-2 in biological samples. These assays can be performed in our standard Biosafety Level 2 (BSL-2) facilities. However, it is advised that in case of a suspected SARS-CoV-2 positive culture, these are moved to the BSL-3 facility. All work involving cell culture and propagation of replication-competent SARS-CoV-2 should be conducted under BSL-3 conditions, in line with current guidance by the Centers for Disease Control[12] and Advisory Committee on Dangerous Pathogens (ACDP) of the Health and Safety Executive (HSE)[13].

In addition to our currently available and recommended *in vitro* assays suitable for the detection of SARS-CoV-2, we are currently prioritizing the implementation of a Good Manufacturing Practice (GMP) compliant PCR assay for the detection of SARS-CoV-2 to support rapid screening of products and to provide an orthogonal approach to testing where relevant. Our Next Generation Sequencing (NGS) platform is also available for holistic screening of materials and provides broad spectrum adventitious agent testing. Extraction of nucleic acids for these tests from suspected SARS-CoV-2 positive samples are performed in BSL-2 facilities, conforming with CDC and HSE guidelines.

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Please reach out to your local Project Manager or Account Manager to discuss your safety testing strategies.

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