Technical guidance for antigenic SARS-CoV-2 monitoring

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Key messages

- New SARS-CoV-2 variants of interest and concern have continued to emerge in the first few months of 2022 and monitoring their circulation in all countries through genomic surveillance remains important.
- The variants of interest and concern identified through genomic surveillance need to be characterised and assessed through antigenic characterisation of the viruses.
- Antigenic characterisation of SARS-CoV-2 variants is essential for evaluation of vaccine efficacy, selecting appropriate virus strain(s) for vaccine development and evaluating vaccine immunogenicity as well as for monitoring resistance to monoclonal antibody (mAb)-based antiviral treatments.
- Specimens originating from sentinel surveillance systems, as well as from targeted testing e.g. from immunocompromised patients, representing circulating SARS-CoV-2 (sub)lineages should be selected for further antigenic characterisation.
- SARS-CoV-2 virus isolation from clinical materials should be performed in BSL3 laboratories.
- Cell culture-adapted mutations during virus propagation present a challenge in antigenic characterisation as well as other analyses. Deep sequencing of the viruses pre- and post-isolation will help to detect any possible cell culture-adapted mutations. Sequencing should be of sufficient depth to detect the emergence of minor variants in which virus key features may have been ablated (e.g. the S1/S2 furin-cleavage site in spike).
- Antigenic properties of SARS-CoV-2 variants can be characterised using techniques such as plaque reduction neutralisation, microneutralisation and pseudovirus neutralisation assay. The antigenic cartography method is used for visualisation of antigenic properties.
- Standardisation and uniform laboratory methodologies and standardised output formats are key to be able to robustly interpret and compare data between studies so that national and international public health policies can be correctly informed.
- Laboratories should use antigenic characterisation to monitor resistance of circulating new variants to monoclonal antibody (mAb)-based antiviral treatments.
- Several WHO European Region countries have capacity for SARS-CoV-2 isolation and antigenic characterisation.
- ECDC, as well as WHO reference laboratories for COVID-19 can provide support to countries that do not have capacity to perform virus isolation in BSL3 laboratories and/or antigenic characterisation.
- Rapid sharing of clinical specimens and/or virus isolates and antigenic data is crucial when a new variant with potential antigenic drift emerges.
- Antigenic data should be shared immediately with ECDC, WHO Regional Office for Europe and European surveillance networks.
- Shipments of specimens to reference laboratories can be supported and expedited through the WHO shipment mechanism.

Introduction

Throughout the COVID-19 pandemic, SARS-CoV-2 has demonstrated continuous viral evolution, with emerging variants of concern (VOC) posing challenges to public health and healthcare services. Four VOCs, Alpha, Beta, Gamma and Delta, have widely circulated at various stages of the pandemic and are believed to be mainly associated with an increase in transmissibility, most likely driven by the adaptation of the virus to the human host, with only limited degree of escape from either natural or vaccine-derived immunity shown by the Beta variant [1-3]. On the other hand, the currently circulating Omicron variant and its sub-lineages (BA.1, BA.2, BA.3, BA.4 and BA.5) demonstrate significant degrees of immune evasion, which – coupled with increased transmissibility – provides these variants with a selective advantage in a population that is increasingly immune to the pre-Omicron VOCs and results in increased circulation and the rapid spread of the virus [4]. The rapid mutation rate necessitates continuous monitoring of circulating variants, their geographical distribution and their impact on virus characteristics such as tropism, transmissibility, infectivity, immune evasion, as well as susceptibility to existing vaccines and antiviral treatment.

The typical way to identify and characterise new variants and unambiguously type existing variants is accomplished through genomics. To be able to confirm infection with a specific variant, sequencing of the whole SARS-CoV-2 genome, or at least whole or partial S-gene for SARS-CoV-2 variants is required [5-7]. In case of recombinant viruses, only whole genome sequencing can confirm the composition and location of breakpoint(s) of a recombinant.

Antigenic characterisation of emerging and re-emerging viruses is necessary for understanding virus evolution, potential escape from immunity induced by vaccines and prior infection by other lineages of SARS-CoV-2, as well as for the selection of isolates for further in vitro and in vivo virus characterisation and vaccine development. Antigenic characterisation also supports decision-making on public health measures for prevention of and response to outbreaks as well as vaccine effectiveness studies. Antigenic characterisation of emerging SARS-CoV-2 variants links directly to the surveillance objectives and strategies recommended by ECDC and WHO [8-10], especially regarding the following:

- Rapidly detect and monitor SARS-CoV-2 variants at an early stage of local circulation in order to rapidly assess their characteristics and to recommend potential response measures;
- Detect and manage outbreaks of new SARS-CoV-2 variants and continue monitoring the trends of existing variants;
- Monitor locally circulating virus types/subtypes or lineages/sub-lineages and their relationship to global and regional circulation patterns;
- Provide candidate viruses for vaccine composition and production as well as risk assessment activities;
- Describe the genetic and antigenic characteristics of circulating respiratory viruses
- Monitor viruses for their susceptibility to antiviral treatments (i.e., monoclonal antibodies through antigenic characterisation in context of this guidance).

To achieve these objectives, surveillance systems rely on both primary care (or other dedicated community-based settings where testing of suspect COVID-19 cases takes place), and secondary care systems, to provide specimens through representative (from all spectrums of the disease and covering the different population groups and geographical locations of the epidemic) and targeted (from vaccine breakthrough infections and reinfections, outbreaks and clusters, travellers and unusual events) sampling to the laboratories for detection and virus characterisation [11].

Variants of interest and concern identified through genomic surveillance also need to be characterised and assessed more broadly through phenotypic characterisation of the virus. To assess how well antibodies elicited after natural infection or vaccination protect against circulating viruses, it is important to perform neutralisation assays, ideally using live virus, with convalescent plasma/sera from infected and vaccinated individuals. Further phenotypic characterisation is based on studies of transmissibility, receptor binding and *in vivo* and *in vitro* properties of viral infection.

Based on a survey conducted by ECDC in December 2021, EU/EEA countries and Switzerland have substantial capability and capacity in antigenic characterisation of SARS-CoV-2. Multiple methods for antigenic characterisation of SARS-CoV-2 have been implemented in 15 laboratories in 13 EU/EEA countries and Switzerland [12].

Furthermore, additional EU/EEA countries are in the process of adding antigenic characterisation to their laboratory methods. The main bottlenecks in this area are access to method protocols, reagents, the requirement of BSL3 facilities and training for laboratory personnel [12]. ECDC supports EU/EEA countries through central laboratory testing activities, as well as information and protocol sharing and upcoming training activities. WHO plans to support non-EU/EEA countries through provision of centralised access to antigenic characterisation services through a contract with one of the advanced European laboratories, as well as support for priority countries to build their capacity for antigenic characterisation.

Scope and objective

This document provides technical guidance to laboratories, microbiology experts and relevant stakeholders in making decisions on establishing or scaling up capability and capacity to isolate and antigenically characterise circulating SARS-CoV-2 variants, and in making decisions on which methods to use.

The objective of this document is to provide guidance on sampling for virus characterisation, and present available methods for isolation and antigenic characterisation of circulating SARS-CoV-2 viruses. The document also outlines quality assessment issues, as well as practical considerations on virus sharing, support for the laboratories and data sharing.

Use of antigenic characterisation data

Antigenic characterisation of emerging SARS-CoV-2 variants is an important tool that, in combination with other virological and epidemiological techniques, supports epidemic and pandemic preparedness and response. Analytic methods used for antigenic characterisation have been well established for influenza research and are now being mobilised and adapted for rapid characterisation of SARS-CoV-2 and its variants, allowing authorities to:

- Conduct surveillance of the virus, monitor emerging variants and their circulation and prevalence in countries and populations;
- Support the development of highly effective vaccines by providing important information about antigenic properties of circulating SARS-CoV-2 variants and informing on vaccine composition for manufacturers;
- Continuously monitor vaccine efficacy against circulating variants, sub-lineages and recombinants, which is a critical function in view of the emergence of the Omicron VOC, that have shown a significant degree of immune evasion;
- Closely monitor potential vaccine breakthrough variants and predict rapidly evolving antigenic profiles of SARS-CoV-2 in response to pressure from the host immune system;
- Monitor resistance of circulating new variants to monoclonal antibody (mAb)-based antiviral treatments to inform decisions on whether the use of some mAbs should be discontinued or different combinations of mAbs should be used;
- Support risk assessments and decisions on implementation of medical and non-medical countermeasures.

Sampling for virus characterisation

Respiratory specimens for detection

Upper respiratory tract (URT) specimens (e.g. nasopharyngeal swab, oropharyngeal swab, nasopharyngeal aspirate, nasal wash) are adequate for testing early-stage infections, especially in asymptomatic or mild cases. Testing nasopharyngeal and oropharyngeal swabs combined from one individual has been shown to increase sensitivity for detection of respiratory viruses and improve the reliability of the result [13]. Lower respiratory specimens should be preferred if samples are collected later in the course of COVID-19 disease or in patients with a negative URT sampling and if there is a strong clinical suspicion of COVID-19.

Lower respiratory specimens can consist of sputum, if spontaneously produced (induced sputum is not recommended as this poses an increased risk of aerosol transmission [14]) and/or endotracheal aspirate or bronchoalveolar lavage in patients with more severe respiratory disease [13]. Whilst a sample for sequencing can be stored at ambient temperatures once extracted, it is important to keep a frozen aliquot of unextracted sample in case live viral isolation is required.

Specimens for laboratory testing should be collected early on in the infection and within the first five days after the onset of symptoms. This ensures that there is sufficient virus in the specimen for successful detection and further characterisation of the virus. Most commonly, viral loads peak around the time of symptom onset and then gradually decrease. Ideally, samples for viral isolation should be frozen immediately, but extracted samples for genetic analysis can be stored at 2-8°C for several days after collection. For handling or shipping, storage at -70°C is recommended in WHO's interim guidance for laboratory testing [15]. Where possible, specimens should not be repeatedly frozen and thawed, because this results in degradation of the virus, reducing detectability and viability for further isolation/characterisation.

Testing strategies for virus characterisation

Testing strategies should be flexible and rapidly adaptable to change, depending on the local epidemiology, population dynamics and resources. For genetic characterisation, whole genome sequencing (WGS) or Sanger sequencing approaches can be used to identify, monitor and assess virus variants. More information on the methods for detecting and characterising SARS-CoV-2 variants can be found in the ECDC/WHO Euro technical report 'Methods for the detection and characterisation of SARS-CoV-2 variants' [16].

For genomic surveillance of SARS-CoV-2, ECDC and WHO recommend the following complementary sampling approaches:

- Representative sampling of SARS-CoV-2 RT-PCR positive cases from existing, population-based surveillance systems. All specimens from sentinel reporting sites should ideally be sequenced.
- Targeted sampling of SARS-CoV-2-positive cases occurring in special settings or populations, including cases with vaccine breakthrough infections, specimen from immunocompromised patients, from outbreaks and clusters, with travel history in areas where VOCs or variants of interest (VOIs) are endemic, and from unusual events.
- Specimen from patients with an unusually severe outcome.

ECDC and WHO recommend that to accurately estimate and monitor the prevalence of VOCs in the community, a representative number of positive samples needs to be sequenced on a weekly basis [11]. It is not possible to give universally appropriate recommendations for the number or proportion of samples subjected to SARS-CoV-2 sequencing, as decisions will depend on the epidemic context and questions to be answered [6]. For novel or emerging variant detection, ECDC recommends a detection threshold minimum which is a relative proportion of 2.5% of a particular variant among all variants within one unit of time [11]. With a reduction in SARS-CoV-2 case numbers and testing in general, a sentinel primary care surveillance system will provide the most efficient approach for obtaining representative samples; all positive sentinel case specimens should be sequenced. All positive samples from hospitalised patients with severe outcome should ideally be sequenced as well. Early detection of emerging variants provides a time window to implement public health measures (e.g. vaccination of high-risk groups) which may delay the onset of widespread community transmission in the European Region. For that purpose, ECDC/WHO recommend that countries enhance their sequencing efforts and sequence a number of samples that allow for the detection of an emerging variant, ideally at 1% prevalence level. It is also important to prioritise targeted sampling. Targeted sampling covers vaccine breakthrough infections and reinfections, outbreaks and clusters, confirmed cases with travel history in areas where VOCs or VOIs are endemic and unusual events. Specimens from sentinel severe acute respiratory infection (SARI) cases, immunocompromised patients, patients with poor response to antiviral treatment and specimens from cases associated with animal-human interface situations should also be sequenced.

Guidance on sampling and sequencing strategy to ensure representativeness and reliability of findings can be found in ECDC and WHO guidance documents [9,11,17,18].

Testing strategies for antigenic characterisation of viruses

When selecting samples for antigenic characterisation, a sequencing-first approach is optimal. After genetic characterisation, viruses that carry three or more amino acid substitutions in key areas of the genome known to contribute to immune escape (eg. Receptor-binding domain (RBD) and receptor-binding motif (RBM) of the spike protein) or substantial changes elsewhere (eg. large deletions, frameshifts or substantial substitution) in comparison to the index virus should be further characterised antigenically. It is also essential to perform antigenic characterisation of specimens if an altered clinical or epidemiological picture is observed, e.g. increased morbidity or enhanced transmission in the community. The number of specimens that should be collected for antigenic characterisation on a weekly/monthly basis depends on the number of routinely tested and positive samples, specimens indicated for antigenic characterisation genetically, as well as the available resources. ECDC and WHO recommend characterising all sentinel specimens genetically and specimen that fulfil the above genetic criteria even antigenically.

For the sampling strategy for antigenic characterisation, several approaches can be considered:

- selection of VOCs, VOIs and VUMs based on genetic characterisation and covering relevant (sub-) lineages (ideally at least one virus per variant should be isolated and antigenically characterised);
- selection of variants based on genetic characterisation and new amino acid substitutions in the spike protein compared to their ancestor strains;
- random selection of SARS-CoV-2 RT-PCR-positive specimens at regular intervals if sequencing is not performed.

- targeted sampling of specimens that are known to originate from:
  - vaccine breakthrough infections and reinfections, as well as samples from cases with poor response to antiviral treatments: comprehensive sampling to detect and characterise variants causing infection in the presence of SARS-CoV-2 antibodies;
  - outbreaks and clusters: a representative sample, with a minimum of five specimens per event to investigate virus transmission dynamics; detect novel antigenic variants; assess the relatedness of viral strains within epidemiological clusters, and support public health interventions;
  - unusual events: a representative sample, with a minimum of five specimens from superspreading events or settings with unusually high transmission; for cases with unusual clinical presentations or increased morbidity, ECDC and WHO recommend comprehensive sampling to support investigations of virus transmission dynamics and detection of novel antigenic variants.

Inclusion of the above specimens will facilitate the identification of genetic variants with deviating antigenic phenotypes. The specimens for antigenic characterisation would need to be collected in virus transportation medium (VTM) that allows virus isolation. Therefore the use of inactivating VTM for these specimens is discouraged, especially in sentinel specimens. All sentinel specimens (General Practitioner, GP, and hospital-based) should ideally be collected in non-inactivating VTM.

Serum specimens for neutralisation assays

For antigenic characterisation and to assess the neutralisation capacity of sera from infected individuals with different serological backgrounds, the serum panels should preferably include serum from asymptomatic, symptomatic and convalescent individuals after severe disease with different sampling intervals (e.g. 14 days either post symptom onset or post initial identification of asymptomatic infections, and after three to six months, or later). For sera from vaccinated individuals, different sampling regimens could include 14 days, and/or three to six months post-second dose, and 14 days and three to six months post-booster vaccination. Serum specimens collected following heterologous prime-boost vaccination regimen or infection before or after any vaccination would also be beneficial for comparison purposes.

Specimens for monoclonal antibody treatment resistance monitoring

Ideally, sentinel specimens which are representative of the population as well as specimens from COVID-19 patients under treatment should be selected for monitoring SARS-CoV-2 antiviral resistance and assessment of neutralising potency of monoclonal antibodies (see below). Specimens from immunocompromised patients under treatment should be selected for antigenic characterisation, given the higher probability to acquire mutations associated with resistance and antigenic drift due to prolonged viral shedding [19] [20].

Virus shipment

Countries with limited or no capacity to perform antigenic characterisation of SARS-CoV-2 variants are strongly encouraged to send clinical specimens or virus isolates to WHO reference laboratories for COVID-19 [21]. Shipments of specimens to reference laboratories can be supported and expedited through the WHO shipment mechanism (see the section on Laboratory Support).

According to WHO's biosafety guidance, patient specimens from suspected or confirmed cases should be transported as UN3373, 'Biological Substance, Category B', Packing Instruction 650 Viral cultures or isolates should be transported as Category A UN2814, 'infectious substance, affecting humans', Packing Instruction 620 [22]. All specimens being transported (whether UN3373 or UN2814) should have appropriate packaging, labelling, and documentation, as required by the applicable national or international regulations for the mode of transport used. More information can be found in the WHO Guidance on regulations for the transport of infectious substances 2021–2022 [23]. Specimens intended for further PCR analysis and with an expected duration of shipment time of up to 12 days can be stored at 2-8°C and then shipped on ice packs. If the expected duration of shipment exceeds 12 days, the specimens should be stored and shipped on dry ice (-70°C) [24]. Specimens intended for virus isolation should be frozen at -80°C as quickly as possible and shipped on dry ice to avoid loss of viability.

Virus isolation for SARS-CoV-2

Based on the WHO document, 'Laboratory biosafety guidance related to coronavirus disease 2019 (COVID-19)' [22], routine diagnostic testing procedures, such as molecular analysis of extracted nucleic acid preparations or generally any analysis of inactivated specimens, should be conducted at a facility using heightened control measures similar to Biosafety Level 2 (BSL-2). SARS-CoV-2 isolation in cell culture should be handled in a containment laboratory with inward directional airflow (heightened control measures/BSL-3).

Before initiating virus isolation and to determine appropriate biosafety measures, a biosafety risk assessment should be performed evaluating the methodology, the laboratory facilities on site, staff resources and training level and available safety equipment and measures. Biosafety professionals, laboratory management, and scientific and safety experts should be involved in the risk assessment process.

A strong relationship between Ct value and the ability to isolate infectious virus has been observed [25-27]. Consequently, specimens with a high viral load should be selected for virus isolation, as virus isolation or cell culture propagation is typically unsuccessful when specimens have a Ct value above 34 [28]. It is important to note however, that although Ct values have been used as a surrogate marker for viral load for research purposes, there are many different RT-PCR assays with different gene targets and Ct values may differ significantly between testing platforms [29,30].

SARS-CoV-2 has been shown to grow in a variety of cell lines [31], and the development of culturing systems for SARS-CoV-2 has progressed further, providing a continuously increasing number of sensitive methods for virus isolation [32,33]. It has been observed that different viral variants replicate differently in different cell lines [34]. This underscores the importance of the evaluation and characterisation of each SARS-CoV-2 variant to establish the replication patterns before performing tests, and of the consideration of the ideal SARS-CoV-2 genotype–cell type pair for each assay.

Generally, cell lines based on Vero cells are most widely used, particularly Vero E6 cells ectopically expressing TMPRSS2 [27,33]. Vero/hSLAM [35], which seem to improve the overall genetic stability of the virus stocks and have supported growth of more recent variants like Omicron. The laboratories cultivating SARS-CoV-2 viruses should consider that serial propagation of SARS-CoV-2 variants in VeroE6 or other cell types (i.e. cells not naturally or artificially expressing human TMPRSS2) may lead to mutations or deletions in important regions like the furin cleavage site which affects how the virus grows and behaves in vitro. Propagation of unwanted mutations can be mitigated by growth in cells such as Vero/hSLAM and by frequent sequence confirmation [35,36].

Cytopathic effects (CPE) are used as an indicator for successful propagation of SARS-CoV-2 and are commonly observed after three days post inoculation [31], but is likely to take longer in first isolation from clinical specimens. Different variants can produce different forms of visible pathogenic changes including rounding and/or syncytia before detachment.

Sequencing pre- and post-isolation

Sequencing of (partial) genes and whole genomes (WGS) has proven to be a powerful method to investigate viral pathogen genomes, understand outbreak transmission dynamics and screen for mutations that potentially have an impact on transmissibility, pathogenicity, and/or countermeasures (e.g. diagnostics, antiviral treatments and vaccines). Whole genome sequencing can be used efficiently to detect VOCs as it represents an unbiased approach without the need for prior knowledge on the presence of certain mutations in the viral genome. Whole genome sequencing is a relatively resource-intensive method that can take from hours to several days to generate results, depending on the protocol, although it provides high sequencing yields and high accuracy. Using either a tiled amplicon approach or shotgun sequencing, the entire genome of the virus can be sequenced and compared with other circulating strains [16].

Conventional sequencing (Sanger sequencing) is widely accessible and cost-effective, however, it has a very low throughput and lacks the depth necessary to detect minor changes and small proportional differences. If Sanger sequencing is the preferred method, the region to be sequenced should cover at least the entire N-terminal and RBD (amino acid 1-541, 1623 bp) to reliably differentiate between the circulating variants. Ideally, S-gene amino acids 1-800 (2400 bp) or the entire S-gene should be sequenced to also monitor the S1/S2 cleavage site and other regions of interest [16].

For most genomic surveillance objectives, a consensus sequence of the complete or almost complete genome is sufficient. This can be achieved in a cost-effective way by using multiplex amplicon assays, for example the open-source ARTIC protocol [37], commercial kits (available for both Illumina and Ion Torrent platforms), or in-house protocols [5]. Guidance on the implementation of WGS can be found in guidance documents produced by ECDC and WHO [5,6].

Cell culture-adapted mutations during virus propagation continue to present a challenge in antigenic characterisation. Deep sequencing (more than Sanger or consensus sequencing) of the viruses pre- and post-isolation would be optimal to detect any possible culture-adapted mutations [38], especially if they are present at a low proportional level [35]. Moreover, it is advised to work with low-passage-number stocks that are fully sequenced, to avoid selecting mutants that are better adapted to grow in the cell line used for virus amplification. If, due to certain limitations, pre- and post-isolation sequencing is not possible, the virus should be sequenced every few passages to ensure that no major mutations have occurred from the passaging of virus in cell culture. Deep sequencing of viral stocks after isolation is critical for obtaining relevant results and assessing the presence and frequency of viral variants. Deep sequencing allows for detection of variants with frequency of 1-3% if the obtained sequence data is of high quality (average Q30 >90%). In general, minor variants in the virus stocks should be present at frequencies <10-20%.

Antigenic characterisation

The term 'antigenic properties' is used to describe the antibody or immune response triggered by the antigens to a particular virus [39]. 'Antigenic characterisation' refers to the analysis of a virus' antigenic properties to help assess how related it is to other strains of the virus [39].

Neutralisation assays

Multiple laboratory-based methods to determine virus neutralisation capacity have been developed. Some examples are plaque reduction neutralisation (PRNT; seen as gold standard method), microneutralisation (MN) and pseudovirus (PSV) neutralisation assay [36,40,41]. Assays with replication of competent SARS-CoV-2 isolates are normally either plaque reduction/focus forming assays or TCID50 (Median Tissue Culture Infectious Dose)-based assays. However, they have the disadvantage that they require biosafety level (BSL)-3 laboratories and are often labour intensive. Alternatively, assays using replication-defective pseudoviral particles can be performed under BSL-2 conditions.

In a typical PRNT assay, the experiments are performed in duplicate or triplicate using cell culture plates. Serial dilutions of serum samples are incubated with a defined number of plaque-forming units or a defined tissue culture infectious dose of virus. The virus-serum mixtures are added onto Vero E6 cell monolayers in most instances and incubated for 3-5 days before the plates are fixed and stained. Antibody titres are defined as the highest serum dilution that results in either >50% (PRNT50) or >90% (PRNT90) reduction in the number of plaques [42]. PRNT has been shown to be more sensitive in detecting neutralising antibodies than MN [42].

Microneutralisation assays represent a cluster of methods based on the read-out of the method (inhibition of CPE, foci reduction or luciferase/fluorescent reporter change). In a typical MN assay, serum dilutions are mixed with defined quantities of virus, incubated, and added on cell monolayers in 96-well microtitre plates. After adsorption of the virus, cell culture medium is added, and the plates are incubated for 3-5 days. A virus back-titration is performed to assess input virus dose. In a standard MN assay, cytopathic effect (CPE) is read as the outcome. In modified assays, cells can be stained with virus-specific antibodies after 24 hours post-infection and infectious foci are counted [43]. For example, in a focus forming assay with replication competent SARS-CoV-2, the number of infected cells can be counted using an ImmunoSpot reader after staining the infected cells with a horseradish peroxidase-conjugated anti-human secondary antibody and developing the signal with a 3-amino-9-ethylcabazole substrate or other combinations of antibodies and signal molecules [43]. Due to the staining, focus forming assays offer a time advantage over either PRNT or MN-CPE based methods.

For a PSV neutralisation assay, plasmid constructs expressing SARS-CoV-2 spike protein, generation of human ACE2 over-expressing cells and production, generation, purification and quantification of pseudotyped particles expressing SARS-CoV-2 and reporter genes are needed. For the neutralisation assay, pseudotyped viruses are incubated with serially diluted serum/antibody specimens. Thereafter the ACE2-expressing cells are seeded into the virus-serum mixture on 96-well plates. After 1–4 days, the neutralisation percentage can be calculated from measurement of relative reporter gene activity, e.g. firefly luciferase, in infected cells [44,45]. Usually, half-maximal inhibitory concentration (IC50) is calculated after normalisation to no antibody controls [45]. Advantages of PSV neutralisation assays in comparison to PRNT assays are that PSV can be performed in BSL-2. It can be modified to a high throughput assay and to be performed in a shorter two-day time regimen. It is highly reproducible and can use any highly permissive cell lines and is readily adaptable to new variants. Pseudovirus assays have been shown to correlate well with those from live virus assays [44].

Several studies have already looked at VOI and VOC antigenic properties through various neutralisation assays, e.g. on SARS-CoV-2 501Y.V2 [46], Alpha [47], Beta [48], Delta [49], Eta [48], Gamma [50], Lambda [47], and Omicron variants [51,52]. Further assay details have been shared from scientists without peer review on Omicron variant as well [46,53,54].

Unlike for mAbs and immune escape, changes leading to antiviral drug escape are not likely to be constrained to the spike glycoprotein (more likely to be in the replication complex in ORF1ab) [55].

Antigenic cartography

Antigenic cartography is the process of creating maps of antigenically variable pathogens [56]. Two-dimensional maps can be produced which reveal information about the antigenic evolution of a pathogen. Antigenic cartography is used to quantify and visualise the antigenic relationships among SARS-CoV-2 variants titrated against serum samples taken post-vaccination and post-infection with different variants [57] [58] [59] (preprints).

In such illustrations, sera are often shown as open squares and viruses are shown as coloured circles, labelled by strain name. Each grid-square corresponds to a two-fold dilution in neutralisation assay. Antigenic distance is interpretable in any direction [60].

The antigenic maps have shown the close antigenic relatedness of the SARS-CoV-2 Alpha VOC to the original strain, which can explain the maintained vaccine effectiveness for that variant. In contrast, the Beta, Gamma, and Delta VOCs, which are known to reduce vaccination effectiveness, are antigenically distinct from the index strain. Antigenic clustering of the VOCs is associated with mutations at positions 452 and 484 in the spike protein. Furthermore, the maps have highlighted the unique antigenicity of the Omicron variant [61] (preprint). Until now, of all SARS-CoV-2 VOCs, Omicron VOC has shown the highest escape from all sera tested [59] (preprint). Major antigenic differences were caused by substitutions at positions 417, 452, 484, and possibly 501 [57] (preprint).

Use of neutralisation assays for assessment of therapeutic antibodies

Several neutralising monoclonal antibodies (mAbs) to SARS-CoV-2 have been developed and authorised for treatment of COVID-19 [62]. It is known that amino acid substitutions that alter the antigenic properties of SARS-CoV-2 variants may impact the effectiveness of certain treatments [63,64]. In COVID-19 patients who receive treatment with mAbs, resistant variants may emerge, which are selected under the pressure of the treatment [65]. Genotypic and phenotypic testing need to be implemented in the laboratories to monitor the emergence and spread of resistant strains. Neutralisation assays can be used to examine the neutralising ability of therapeutic antibodies, while WGS data can be used in combination to assess the association of the different amino acid substitutions with resistance. It needs, however, to be noted that not all mutations are considered clinically relevant, even if lower neutralising activity is observed against some variants than against earlier variant strains. There are still several open questions on the optimal use of these treatments, such as the kinetics of viral load and its correlation with clinical outcomes. Therefore, there is a need to link laboratory generated genotypic and phenotypic data to clinical data. The use of international standards to convert titres into International Units will greatly assist scientific and regulatory comparisons of data and accelerate the development of candidate treatments and therapeutics.

Quality assessment

Standards and harmonisation of methods

Standardised and uniform laboratory methodologies are key to robustly interpret and compare data between studies so that national and international public health institutions and policy makers can be correctly informed. Currently, PRNT, MN and PSV neutralisation are the most commonly used laboratory-based techniques for measuring the neutralisation capacity of an individual's vaccine-induced antibody response to SARS-CoV-2. However, inherent variability exists between laboratories in their methodology, virus strains and reagents, that may hamper the ability to compare data. Thus, there is a need for standardisation to overcome such challenges.

Testing the robustness across types of neutralisation assays or between laboratories is a crucial step to harmonisation [43]. In one study, a comparison of four different types of neutralisation assays has shown that these SARS-CoV-2 neutralisation assays were robust, as results were comparable and produced highly reproducible neutralisation titres [43,66]. Excellent concordance between the surrogate virus neutralisation test and the gold standard PRNT has also been demonstrated [40]. In addition, comparison of MN assay in twelve laboratories showed that neutralisation titres had a linear relationship with dilution and very high correlation coefficients between log transformed values [67].

The WHO International Antibody Standard (WHO IS) or the National Institute for Biological Standards and Control (NIBSC) working reagent (21/234) [68] or high titre reference serum (20/150) [69], should be used for calibration of neutralisation assays [40,69-71]. These standards act as a reference system to calibrate the neutralising antibody responses between laboratories and across assays to compare quantitative results in international units (IU). As demonstrated in one study, the NIBSC working reagent was used to normalise results from 12 different laboratories and showed that reproducibility could be enhanced, and showed a more than 10-fold reduction in variability across laboratories compared to raw titres [67]. A study comparing PRNT assays across 44 laboratories was carried out in November 2020. This study showed that calibration against the WHO IS was able to reduce inter-laboratory variation

by over 50 times [72]. It should be noted that the WHO IS performs differently for each variant and therefore, any data presented comparing the WHO IS should always specify the tested variant. It is important to include representatives of different variant strains (index virus, D614G, Alpha, Beta, Gamma, Delta and Omicron with sublineages) in the neutralisation assays. The assays should also ideally be performed in duplicate or triplicate. Technical guidance on the calibration of secondary antibody standards to the International Standard is also provided by the WHO Manual ('WHO manual for the preparation of reference materials for use as secondary standards in antibody testing', *in press*).

External quality assessment

In general, laboratories should have a quality assurance system in place and are encouraged to participate in external quality assessment (EQA) schemes or perform result comparison between laboratories, of a subset of samples [13]. ECDC will conduct virus characterisation EQAs for national COVID-19 reference laboratories in the coming years. WHO is working on establishing a global EQA program for SARS-CoV-2 sequencing and bioinformatics, with specific objectives to assess: (1) identification of single nucleotide polymorphisms (SNPs) and indels; (2) identification of lineages and variants; and (3) assessing genomic relatedness. For more information, please contact euinfluenza@who.int.

Result reporting

Isolation techniques and antigenic characterisation results are presented and discussed on a regular basis in the joint ECDC/WHO Regional Office for Europe ECOVID-laboratory network and Virus Characterisation Working Group meetings. Many network members are publishing data and reports on websites, pre-print servers and through ECDC and WHO mechanisms to share laboratory protocols with the wider network. Laboratories are asked to share antigenic characterisation results, preliminary results and laboratory assessments with ECDC and WHO Euro as soon as they become available to improve understanding of the potential impact on medical and non-medical countermeasures.

Currently, data on isolation success or antigenic characterisation are not reported to The European Surveillance System (TESSy). Public Health laboratories are encouraged to actively share information and preliminary findings on VOCs and VOIs. ECDC has set up functionalities in EpiPulse to support effective information sharing [73].

Laboratory support

ECDC and the WHO Regional Office for Europe coordinate their support to countries in the WHO European Region. ECDC is supporting scaling up of sequencing and neutralisation assay capacity in EU/EEA Member States. Please contact covid.microbiology@ecdc.europa.eu for more information. Countries wishing to receive support from WHO Regional Office for Europe may contact euinfluenza@who.int.

Reference viruses for neutralisation assays, constructs for pseudovirus assays and control material for NAAT assays can be found through the European Virus Archive Global (EVAg) [74] and National Institute for Biological Standards and Control (NIBSC) [75]. WHO is currently also setting up a platform, called WHO BioHub [76], for the purpose of material sharing.

Operational laboratory support

ECDC will provide laboratory support for surveillance, preparedness and response to COVID-19 and influenza. The aim is to strengthen the laboratory and public health competencies and capabilities for early detection, surveillance and response to influenza and SARS-CoV-2 viruses in the Member States.

The supported activities under this contract will include EQAs of diagnostic assays (e.g. RT-PCR, rapid antigen tests, antibody tests), virus neutralisation tests (VNT) as well as ring trials for the validation of the bioinformatic workflows. Furthermore, this contract will support laboratories fulfilling national reference functions and ensure that information on circulating viruses is collected, validated, analysed and shared in a timely manner with the participating laboratories. This contract will also facilitate the swift alerting of public health authorities at national and international level on the detection of variant viruses that may not be completely protected against by natural immunity or the current vaccines and are likely to cause increased number of COVID-19 cases or impact any public health response measures. Finally, training activities will support capacity building relevant for detection, characterisation and response, focusing primarily on influenza and SARS-CoV-2.

Platform for sharing of variants of concern material

To facilitate rapid sharing of virus material, ECDC has complemented the online portal EpiPulse [73] with special events for newly emerging variants. Via this platform, EpiPulse users from Member States assigned by National Coordinators can report virus isolate access and information regarding ongoing investigations related to variant severity, transmissibility, immune evasion, effect on diagnostics and therapeutics and seroprevalence.

Other sources for sharing and depositing live virus stocks include the European Virus Archive Global [74], WHO BioHub (WHO) [76], National Institute for Biological Standards and Control (United Kingdom) [75] and the BEI Resources (USA) [77].

WHO can provide financial, administrative and logistical support for the shipment of clinical specimens and virus isolates through the WHO Shipping Fund Project [78]. The shipment booking form and guidance for specimen shipping are available at WHO webpage [21]. Please contact euinfluenza@who.int for more information.

Omicron variant assays and animal models study tracker

WHO and its international network of experts and partners have been monitoring the evolution of SARS-CoV-2 to identify consequential changes to the virus and to track variants of concern [79]. In addition, the WHO SARS-CoV-2 Omicron variant assays and animal models study tracker is available [80].

To add, remove, or update information in the table please download the current version, highlight changes, and email the new file to euinfluenza@who.int. A high-level document comprising references to studies that are published, in preprint, or in the press related to SARS-CoV-2 Omicron variant assays and animal models is also available from this webpage, with results updated every two weeks.

Additionally, a list of major tools and initiatives that have been established to track and visualise SARS-CoV-2 variants and/or mutations and their effects can be found in the WHO's 'Guidance for surveillance of SARS-CoV-2 variants: interim guidance, 9 August 2021' [17].

Protocol and information sharing

The WHO Regional Office for Europe together with ECDC has set up a protocol/information sharing platform EZCollab for 'COVID-19 protocol sharing'. Registration can be done in: https://ezcollab.who.int/euroflu/flulab/covid19_protocols. Information sharing at international level can take place in various forms such as presentations at joint ECDC and WHO network meetings and working groups on COVID-19 or sharing of national publications or reports. Rapid sharing of preliminary data is necessary for public health decision making.

Contributors

This document was developed by technical experts from ECDC and the WHO Regional Office for Europe by summarising knowledge on SARS-CoV-2 antigenic characterisation objectives and methods. The document was reviewed by experts from the WHO COVID-19 reference laboratories and the SARS-CoV-2 Characterisation Working Group.

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