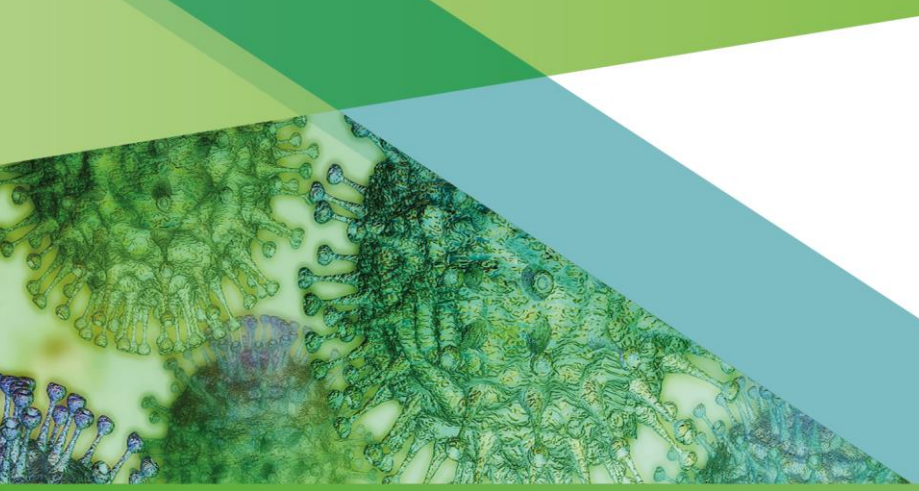

TECHNICAL REPORT

Rapid assessment of antigenic characterisation capability and capacity for SARS-CoV-2 viruses in EU/EEA laboratories

20 December 2021

Key messages

- EU/EEA countries and Switzerland have substantial capability and capacity in antigenic characterisation of SARS-CoV-2 viruses across the region.
- Multiple methods for antigenic characterisation of SARS-CoV-2 have been implemented in EU/EEA laboratories.
- 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, BSL3 facilities and training for laboratory personnel.
- ECDC is supporting EU/EEA countries through central laboratory testing activities, as well as information and protocol sharing and upcoming training activities.

Background and methods

ECDC has mapped the current laboratory practices and needs of Member States in terms of diagnostic testing and laboratory shortages across the EU/EEA and the UK during the COVID-19 pandemic due to knowledge gaps, for planning purposes, and due to Member States, external or European Commission requests. This is the fifth survey assessing the capacity and needs of laboratories in the EU/EEA, with a focus on antigenic characterisation capability and capacity for SARS-CoV-2 viruses. Antigenic characterisation means analysis of antigenic properties of a virus to help assess its relatedness to another virus (usually wildtype or vaccine strain). Antigens are molecular structures on the surface of viruses that are recognised by the immune system. Antigenic properties describe what type of antigen changes have occurred in the virus in comparison to a reference strain and what type of antibody or immune response is triggered by the current antigenic composition. Methods currently available for SARS-CoV-2 antigenic characterisation include the gold standard plaque reduction neutralisation (PRNT), microneutralisation (MNA) and pseudotyped virus (PSV) neutralisation assays [1].

A questionnaire[1] was sent to the 30 EU/EEA Member States on 19 October 2021, using the EU Survey Tool. The recipients included ECDC's Operational Contact Points for Influenza and COVID-19 (Microbiology), the National

1 The 'Antigenic characterisation capability and capacity for SARS-CoV-2 variants' survey is available at: https://www.ecdc.europa.eu/sites/default/files/documents/AntigenicCharSurvey2021_21_10_2021_EN.pdf

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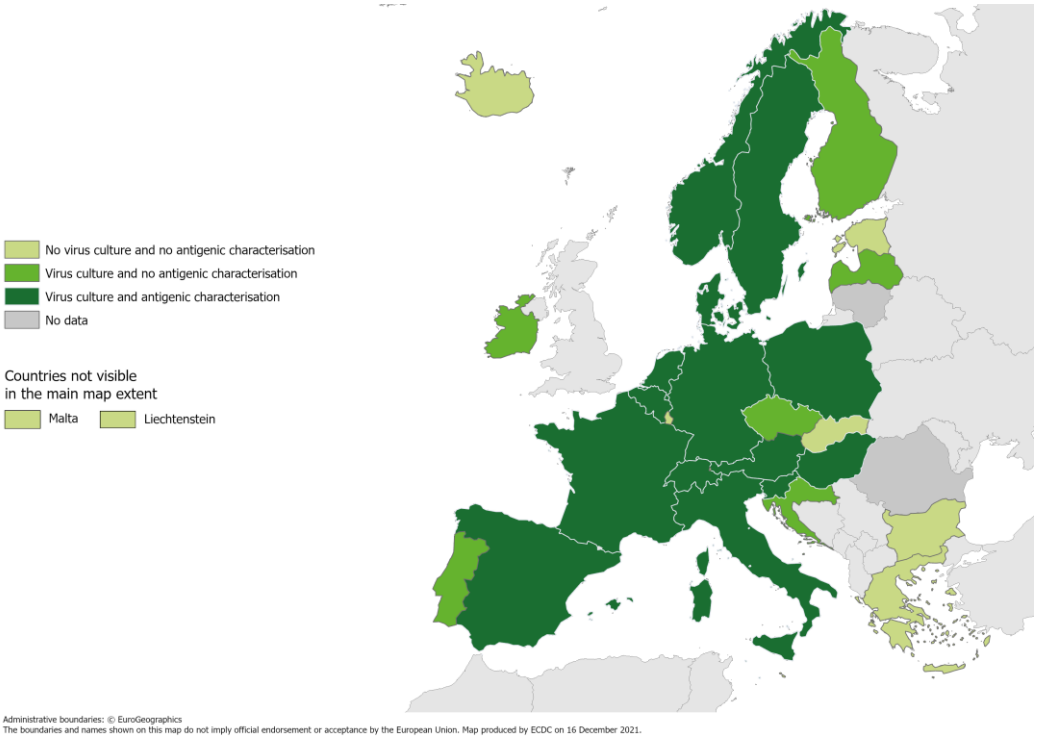
European Centre for Disease Prevention and Control. Stockholm, 2021.

Microbiology Focal Points, the National Focal Points for Viral Respiratory Diseases and the National Coordinators. Switzerland received the survey through an ECDC-WHO Regional Office for Europe Virus Characterisation Working Group and wanted to take part in the survey. Focal points were asked to answer the questions, where appropriate, and give answers for their whole country. If the contact point responded 'no' to question number 2 on if their country performs antigenic characterisation, the survey jumped to question number 11 on technical support needs. The survey responses are summarised in this report.

Results

As of 15 November 2021, 30 laboratories from 28 EU/EEA Member States and Switzerland replied to the survey (Figure 1). Twenty-three focal points reported being the Operational Contact Points for COVID-19 (Microbiology) and with that part of the European COVID-19 reference laboratory network (ECOVID-LabNet). Ten responders reported being ECDC National Focal Point for Viral Respiratory Diseases and eight ECDC National Focal Point for Microbiology. Eleven responders had multiple contact point roles. Five indicated that they had other roles that made them eligible to respond to the survey (e.g. being delegated by the operational or national focal point).

Figure 1. Performance of virus culture and/or antigenic characterisation, 28 EU/EEA countries and Switzerland, November 2021



Virus culture and antigenic characterisation capacities

The survey asked countries about their SARS-CoV-2 virus culture capacities and 20 of the 29 reporting countries indicated that they had established virus culture for SARS-CoV-2 (Figure 1). Virus culture is a prerequisite for antigenic characterisation technique, which requires testing of live virus. Fourteen of the twenty countries (15 laboratories in 14 countries) have established antigenic characterisation for SARS-CoV-2 (Figure 1).

Purpose of antigenic characterisation

Based on the survey, antigenic characterisation is used for multiple purposes in the reporting countries. Most laboratories are using it to detect variants of interest (VOIs) and concern (VOCs) [1], investigate breakthrough infections, perform vaccine effectiveness studies and investigate trends of VOCs by antigenic characterisation techniques. All laboratories are using antigenic characterisation for research purposes. One country commented that more comprehensive characterisation relies on whole genome sequencing and that antigenic characterisation is performed on a limited scale.

Sampling strategy

Responses from the reporting countries indicated that multiple sampling strategies are used for antigenic characterisation (countries could select more than one option). In seven and six countries, respectively, all VOCs or VOIs are antigenically characterised (a sequence first approach) based on genetic characterisation. Seven countries indicated a random selection of variants based on genetic characterisation and amino acid substitutions in the spike protein for antigenic characterisation. A random selection of SARS-CoV-2 viruses are investigated retrospectively in four countries and, in three countries, a random selection of SARS-CoV-2 RT-PCR-positive specimens are screened for antigenic variants on regular intervals. Two countries reported that systematic and convenience samples of SARS-CoV-2-positive specimens (e.g. the first 10 specimens of the week) are screened for antigenic variants. Ten of fourteen laboratories applied PCR cycling threshold values for selection for antigenic characterisation (these varied from Ct 25 to <32; one laboratory indicated a viral load threshold of 1x10e7 copies per mL). The survey also requested the number or proportion of viruses that are characterised. Most responders found that difficult to answer and gave answers such as 'very few' or 'one cell culture isolate per VOC/VOI'.

Methods and standards used in antigenic characterisation

Eleven of the fourteen responding countries (15 laboratories) that perform antigenic characterisation use MNA (Figure 2). The gold standard PRNT is used by six laboratories. PSV neutralisation assay is used in eight laboratories.

Figure 2. Methods of antigenic characterisation in use among the 15 laboratories in the 14 EU/EEA countries and Switzerland that perform antigenic characterisation of SARS-CoV-2, November 2021

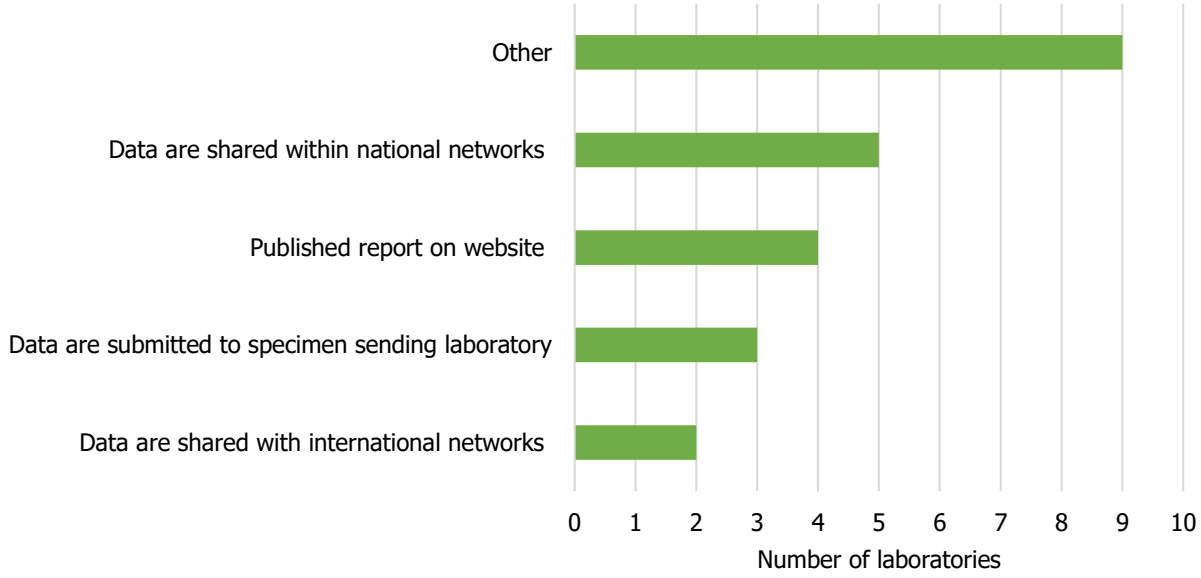
** Laboratories could select multiple choices.*

Of the fifteen laboratories performing antigenic characterisation, three indicated that they do not use standards and one did not know whether standards were used in their country. Eleven laboratories had access to a variety of standard materials, including the World Health Organization (WHO) International Standard from the National Institute for Biological Standards and Control (NIBSC) [2]; monoclonal antibodies developed at Institut Pasteur; NIBSC pooled serum product [3]; human convalescent serum from NIBSC (code 20/130) [4]; two separate JRC serum standards [5,6]; in-house standards based on pools of high, medium and low antibody titre sera; commercial assay reference sera and standards; a Wuhan-Hu-1 virus vaccinated rabbit serum that cross-reacts with all VOCs; and internal standards to check for assay-to-assay variation. For the viral strains, reference strain for B.1 (Bavarian strain Munich) [7] and an early wildtype virus control with D614G mutation from Denmark were mentioned as well.

Dissemination of results

Fourteen laboratories and countries responded to the question on the dissemination of antigenic characterisation results (Figure 3). Only two laboratories were sharing results to international networks and one indicated that this was for antigenic cartography purposes. Four indicated publishing the results on websites; however, only one provided a link to a report [8]. Five laboratories indicated sharing results within national laboratory or epidemiological networks and with national governmental structures or public health authorities (e.g. Chief medical officer) through presentations, emails and phone calls. Most of the responders commented that results are published through journal publications [9,10] or that sharing mechanisms were not yet established, which was indicated by the nine answers in the category 'other'.

Figure 3. Methods of distribution of antigenic characterisation results among the 15 laboratories in the 14 EU/EEA countries and Switzerland that perform antigenic characterisation of SARS-CoV-2, November 2021

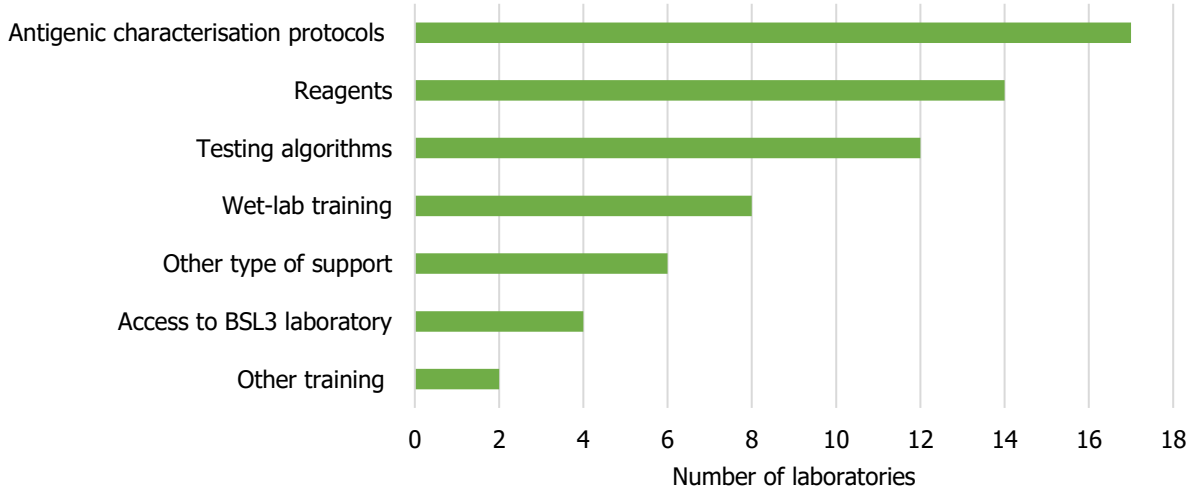


** Laboratories could select multiple choices.*

Technical support needs and awareness of existing support

Responders were asked to identify technical support needs for setting up or performing existing antigenic characterisation in their country. Seventeen of the twenty-four responding countries needed antigenic characterisation method protocols (Figure 4). Various reagent needs were also expressed by the majority of the countries (n = 14), which could include panels of characterised antibodies; reference, pooled sera from vaccinated individuals or animal antisera for important variants; prototypic virus strains; reagents or reagent kits; and equipment for the implementation of the method. Testing algorithms were mentioned as a need by half of the laboratories and wet-lab training by a third of them. Four laboratories lacked access to a BSL3 laboratory. Other types of support and training included external quality assessment (EQA) panels and discussion around the number of reference strains for antigenic characterisation.

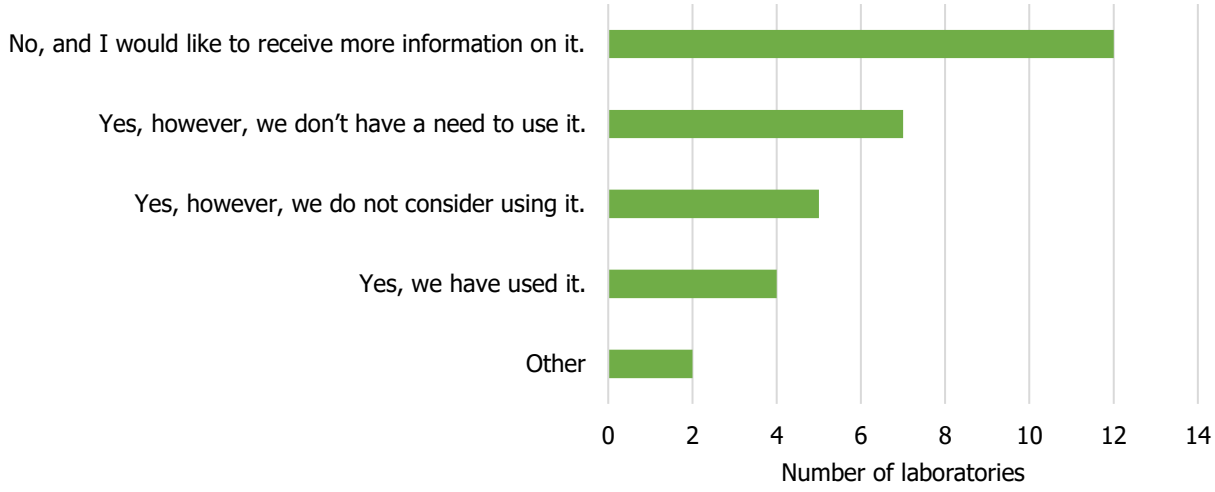
Figure 4. Technical support needs for antigenic characterisation, 28 EU/EEA countries and Switzerland, November 2021



** Laboratories could select multiple choices.*

Despite multiple announcements by email and presentations during the COVID-19 laboratory network calls, the survey revealed that 12 of the 30 responding laboratories were not aware of the outsourced ECDC central laboratory support that was offered to EU/EEA countries and laboratories from May to August 2021 (Figure 5). Seven laboratories indicated that they did not need to use the central laboratory support and five others were aware of the support but did not consider using it. Only four laboratories had used the support during summer 2021. One laboratory explained that they did not accept the offer but may when it is available again in future and another did not have time to finish the sampling before the deadline for sending specimens. Some comments gave further insight into the challenges of using the central testing. These included, for example, using a sampling media that inactivates the virus and 'logistical problems both accessing and referring samples of interest to an ECDC designated laboratory.' Support and advice were said to be appreciated; however, some commented that 'relationships and expectations between national and international reference laboratories should be developed and clarified.'

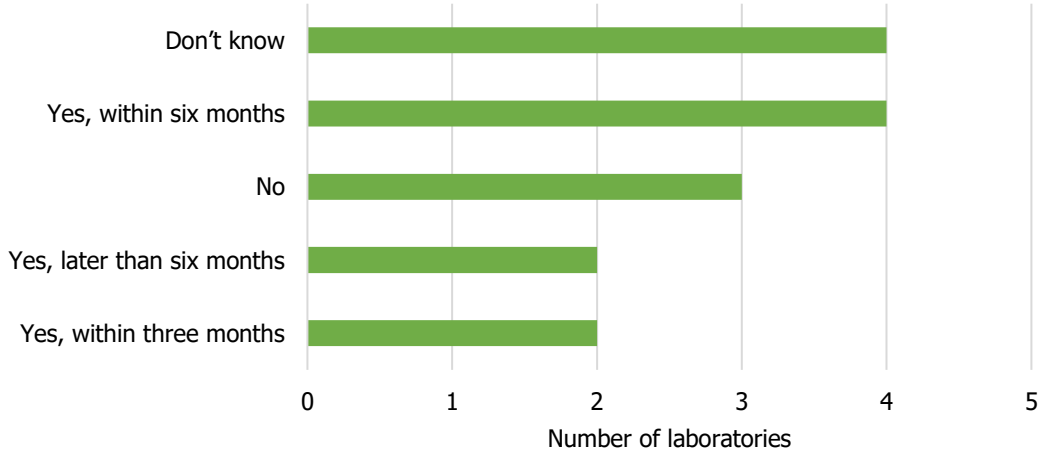
Figure 5. Awareness of ECDC-offered central testing of clinical specimens and/or SARS-CoV-2 virus isolates for antigenic characterisation, 28 EU/EEA countries and Switzerland, November 2021



Plans for implementing antigenic characterisation

The survey asked about plans to implement antigenic characterisation in their country, if they had not already done so. Four out of fifteen countries who had not yet implemented antigenic characterisation did not know and another four had plans to implement it within six months (Figure 6). Three countries have decided not to implement the method, two have plans to implement it within two months and another two plan to implement it in more than six months.

Figure 6. Response to the question: 'Do you plan to start antigenic characterisation for SARS- CoV-2 variants?', 28 EU/EEA countries and Switzerland, November 2021



Conclusions

Laboratory capability and capacity to perform SARS-CoV-2 antigenic characterisation was assessed through a short survey sent to all EU/EEA Member States and Switzerland. Thirty laboratories from twenty-eight EU/EEA Member States and Switzerland replied to the survey, providing a thorough representation of the present situation for antigenic characterisation in the EU/EEA. Although surveys on RT-PCR, the use of rapid antigen tests and other laboratory capabilities and capacities [11] have been conducted, previous surveys did not assess laboratories' capability and capacity to perform antigenic characterisation. This report is the first to indicate capability and capacity in the EU/EEA for this method. Two thirds (n = 20) of the responding countries have capability for virus culture and approximately half (n = 14) of the responding laboratories for antigenic characterisation. It is noteworthy that a similar number of laboratories have established an antigenic characterisation method for SARS-CoV-2 as for influenza (approximately 15 to 20 laboratories in the WHO European Region, in seasons 2018/19 and 2019/20 [12]), even though SARS-CoV-2 has only circulated in the EU/EEA and Switzerland from 2020 and influenza virus characterisation has been established for decades.

Objectives for applying antigenic characterisation include conducting research on SARS-CoV-2 viruses, detecting VOIs and VOCs, investigating breakthrough infections, performing vaccine effectiveness studies and investigating trends of VOCs. Many of the countries use a 'sequence first' approach for virus characterisation and select specimens for antigenic characterisation from the pool of specimens already genetically characterised. ECDC has recommended both representative and targeted sampling of SARS-CoV-2-positive specimens for sequencing [1]. Targeted sampling includes cases with vaccine breakthrough infections, from outbreaks and clusters, with travel history in areas where VOCs or VOIs are endemic, and from unusual events. The representative sampling should be performed in such a way that detection of a particular variant among all variants is possible within one unit of time (e.g. week).

Looking at the methods in use for SARS-CoV-2 antigenic characterisation, MNT was most commonly used, followed by PSV neutralisation assay and then PRNT. MNT and the gold standard PRNT methods require a BSL3 level laboratory, as the tests use live virus [13]. PSV neutralisation assay does not require live virus and can therefore be performed at a BSL2 level laboratory. PSV can be performed faster than PRNT and can be performed in a high throughput system. PSV usually has a low intra-assay variability and can be adapted to new variants even if the live virus is not available. All systems require highly standardised ways of working and standardisation of cell lines is important.

Use of international standards is highly recommended for all antigenic characterisation assays. The intra- and inter-assay variability can be reduced with international standards. Most laboratories had at least one type of a standard material available, but for comparison of results at the European level, all laboratories would need to have the same type of standard in use. Laboratories also need to set up in-house (secondary) standard material for each run, as the international standards are used only for calibration of the systems on a regular basis, but not for each assay run.

Most of the responding countries have not yet established an antigenic characterisation method for surveillance and are performing it only for research purposes. Therefore, it is understandable that the dissemination of results is also mainly through research articles. Only one laboratory indicated an online surveillance report that integrated antigenic characterisation results [8]. ECDC, the WHO Regional Office for Europe and WHO headquarters – together with the ECOVID-LabNet members in the virus characterisation working group – will soon begin discussing sampling strategies, types of antigenic data and a regular report that could be used for surveillance purposes and facilitating vaccine composition decisions on top of the already existing surveillance and genetic characterisation data.

Many countries are willing to and interested in adding antigenic characterisation methods to their arsenal of laboratory methods used for detection and characterisation of SARS-CoV-2 viruses. In this survey, some countries indicated that they were planning to implement the new method within three months of the survey, while others indicated that they may do so during 2022. The main limiting factor seems to be a lack of BSL3 facilities, reagents, standards, protocols and training of laboratory staff. It is understandable that all laboratories do not need to set up and perform this type of sophisticated virus characterisation, but other laboratories could perform it for them through central testing opportunities.

In the survey results, EU/EEA Member States expressed a need for support from the European Union services. The necessary support varies from information on appropriate assay protocols to EQAs. ECDC performed a pilot study for central testing for antigenic characterisation of SARS-CoV-2 viruses in summer 2021 and is setting up another contract for similar outsourced activities. These activities will also include training and guidance development. The European Commission's Joint Research Centre has produced and provided a standard for antibody tests [5,6,14] that can also be used in antigenic characterisation. NIBSC has produced the international standard and is currently developing a new one to replace the first, which has already been depleted. Since February 2021, ECDC has provided whole genome sequencing support to 11 EU/EEA countries and three Western Balkan countries. More than 95 000 specimens have been sequenced. From September 2021 onward, ECDC will allocate EU 77.1 million to 24 EU/EEA countries for whole genome sequencing and RT-PCR infrastructure building, under HERA Incubator action area one. ECDC has already performed a molecular detection EQA of SARS-CoV-2 detection [15] and has performed an EQA on serology. EQAs are part of ECDC capacity building for laboratories, as they provide quality assessment and guide training. Additional EQAs for SARS-CoV-2 are planned for 2022 and some are planned to include virus characterisation as a topic.

ECDC and the WHO Regional Office for Europe continue with the regular ECOVID-LabNet information sharing mechanisms. ECOVID-LabNet has been seen as a valuable interaction platform and therefore activities within the network will be continued and expanded towards training (through study visits and working groups), development of guidance and sharing of laboratory protocols.

Contributors (in alphabetical order)

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Table. 'Antigenic characterisation capability and capacity for SARS-CoV-2 variants' survey contributors, November 2021

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The 'Antigenic characterisation capability and capacity for SARS-CoV-2 variants' survey is available at: https://www.ecdc.europa.eu/sites/default/files/documents/AntigenicCharSurvey2021_21_10_2021_EN.pdf

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