NEWSLETTER

A proposal for sharing viruses and sera with AURORAE to contribute to a collaborative study: Genetic and antigenic characterization of new circulating SARS-CoV-2 variants.

SARS-CoV-2 continues to circulate and evolve which results in genetic and antigenic evolution of the virus. Newly emerging Omicron lineages are gaining substitutions at a fast rate, resulting in escape from neutralization by antibodies that target the spike protein. To assess the implications of SARS-CoV-2 evolution for COVID-19 vaccine antigen composition it is important to determine the phenotypic and antigenic characteristics of the variants that circulate. In addition, the neutralizing capacity of sera from individuals previously vaccinated against SARS-CoV-2 needs to be evaluated in different geographic regions. However, there are persistent and increasing gaps in genetic and serological surveillance of SARS-CoV-2, including low numbers of samples sequenced and limited geographic diversity.
The scope of the ECDC-funded AURORAE centralized laboratory support for influenza and SARS-CoV-2 viruses is to ensure surveillance and in depth genetic and antigenic characterization of circulating influenza and SARS-CoV-2 viruses for risk assessment and possible need for vaccine revisions.

We therefore propose a collaborative study to investigate the circulation of novel SARS-CoV-2 variants in the EU/EEA, Western Balkans and Türkiye. Purpose of the study is to support risk assessment and the interpretation of surveillance data. The study materials would be used for public health and surveillance purposes and not for research purposes. The study will focus on variant SARS-CoV-2 viruses emerging in 2024 and serum samples from individuals vaccinated in 2023, to further map the antigenic evolution of SARS-CoV-2 and the current population antibody landscape against these variant viruses in Europe.

More specifically:

1. The first aim is to characterize variant SARS-CoV-2 viruses that may not be completely protected against by natural immunity or the current vaccines and are likely to cause increased numbers of infection or impact public health response measures. Laboratories are asked to share swab specimens or virus isolates that were collected through national surveillance or response systems, especially those from recent vaccine breakthrough infections and SARS-CoV-2 viruses that caused an increase in the number of cases in the region. The specimens should be obtained in 2024 and shall be selected after initial genetic characterization to make sure that these were not circulating in 2023.

2. The second aim is to collect sera during the period of May-August 2024 from individuals vaccinated in 2023. Given the fact that most countries used vaccination programs targeting only high-risk patients in 2023 and the fact that these will most likely be targeted again for vaccination in 2024, sera from these individuals are of high priority. In addition, we would like to receive sera also from populations with a history of possibly multiple infections and previous vaccinations.

Place for laboratory analysis: The study will be conducted at ERASMUS MC, Rotterdam, the Netherlands.
Laboratories invited: ECDC focal points for COVID-19 laboratories in the EU/EEA, Western Balkans and Türkiye

Timeline of the study:
- Please register your interest for the study by 1 May 2024.
- Submission of samples obtained during the period May-August 2024.
- Laboratory analysis of the samples at ERASMUS MC and other AURORAE labs if needed.
- Analysis of the data, approximately by September 2024.
- If possible, serum samples will also be collected after the vaccination campaign in autumn 2024 (this will be communicated later).

Samples shipment and analysis costs:
Study samples shipment and analysis costs will be covered by ECDC through framework contract with AURORAE consortium

Dissemination of the study results:
- Dissemination of sequence results will be through GISAID and GenBank.
- The results will be presented to and discussed with the study participants in an online meeting before finalization of the report. The contributing countries can review the report.
- **The study will be aimed for publishing in an international journal.** Contributors will co-author the publication.
- The results will be presented in the ECDC respiratory viruses laboratory network meeting and in any appropriate scientific conferences.

Methods that will be used:
The viruses from samples that are submitted, will undergo whole genome sequencing and virus neutralization assays. Furthermore, antigenic cartography mapping will be provided for the different variants strains included in the study.
Antigenic cartography is currently used routinely to facilitate selection of seasonal influenza vaccine strains and studies are underway to see if the evolutionary path of influenza viruses can be predicted. A similar strategy is now also developed for SARS-CoV-2. Future maps will

will likely elucidate the direction of SARS-CoV-2 antigenic evolution, aid in vaccine strain selection, and provide insight into population immune profiles to newly emerging variants. However, a continuous inclusion of novel variants is needed.

Registration of SARS-CoV-2 samples

Please register your interest to the study by 1 May 2024 by email to Bart Haagmans (b.haagmans@erasmusmc.nl; cc: info.aurorae@rivm.nl; eeva.broberg@ecdc.europa.eu).
SARS-CoV-2 samples and sera for phenotypic characterization can be registered at the website portal after May 1.
https://ec.europa.eu/eusurvey/runner/RegistrationSARS-CoV-2Samples

Detailed process

A specific form to register samples for the study will be put online before May 1st.
The laboratories submitting specimens can ship either:
1) virus isolates from clinical specimens characterized by whole genome sequence, with known infectious titers, determined by e.g. plaque assay, on which the contractor will perform additional characterization based on virus neutralization assay.
2) clinical specimens of defined quality for which the contractor will attempt to isolate the virus. For successfully isolated viruses, the contractor will perform genetic and antigenic characterization.
3) sera collected during the period of May-August 2024 from individuals vaccinated in 2023 and also from populations with a history of possibly multiple infections and previous vaccinations.

Further information on antigenic cartography:

Tools to map the impact of substitutions on the further antigenic evolution of SARS-CoV-2, such as antigenic cartography, may be helpful to update SARS-CoV-2 vaccines. The role of spike substitutions and insertion-deletions alone or in combination in a single variant has been described in detail in the context of neutralizing antibody escape, but mostly without quantifying the antigenic relationships of multiple variants simultaneously. The most commonly used technique to compare the antigenic properties of variants involves determining either binding or neutralizing antibody titers in serum. These assays provide critical data on antigenic differences post infection or vaccination, but quantification of antigenic distances between multiple variants is difficult. In addition, antigenic cartography can be used to quantify and visualize the antigenic properties of multiple antigens and antisera in antigenic

space, providing reliable antigenic distances between multiple variants. This technique was first described for mapping the antigenic properties of influenza A(H3N2) virus, where multidimensional scaling was used to position antigens and antisera based on hemagglutinin inhibition (HI) titers, such that the distance between each antigen and antiserum is inversely proportional to the HI titer. In the case of SARS-CoV-2, neutralizing antibody titers are used.

Multiple approaches exist to generate antisera to build a SARS-CoV-2 antigenic map. Human post-infection or post-vaccination sera can be obtained, but with increasing population immunity due to infection or vaccination, sera obtained after a single exposure to recent variants are increasingly difficult to obtain.

As an alternative to human sera, susceptible animals such as Syrian golden hamsters can be inoculated to generate mono-specific sera. Upon obtaining sera from hamsters, neutralizing titers are determined using either live SARS-CoV-2 or pseudotyped viruses. An example of an antigenic map is shown in figure 1. In this map, all Omicron variants were positioned distantly from the pre-Omicron cluster. Omicron BA.5 was positioned within one antigenic unit from BA.2, with an antigenic unit representing a two-fold dilution in neutralisation titres. All remaining Omicron variants were positioned 2.3 to 7.0 antigenic units from each other. Omicron BQ.1.1, BM.1.1.1, and XBB.1 mapped the furthest from the pre-Omicron variants. More recent analyses include other Omicron variants, including BA.2.86 and JN.1.

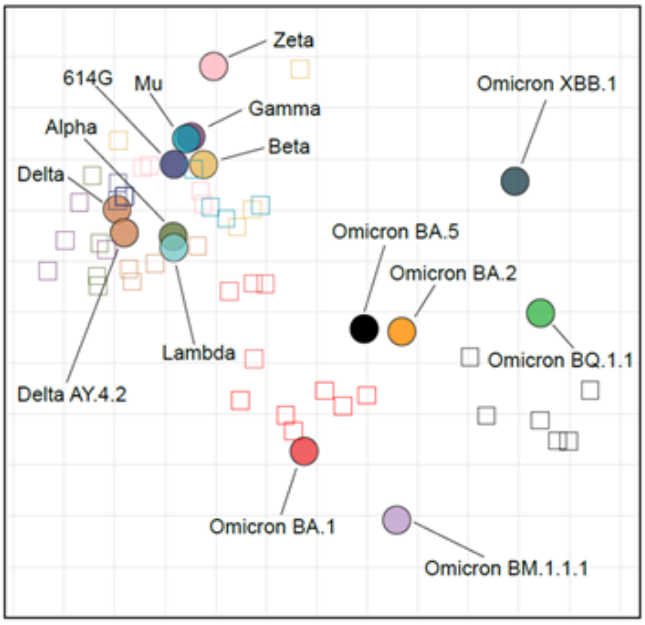

Figure 1. Antigenic cartography of newly emerging SARS-CoV-2 variants. Multidimensional scaling was used to generate an antigenic map from PRNT50 titres generated against 614G, Alpha, Beta, Gamma, Zeta, Delta, Delta AY.4.2, Lambda, Mu, Omicron BA.1, BA.2, BA.5, BQ.1.1, BM.1.1.1 and XBB.1. Viruses are shown as circles and anti-sera as squares of matching colour. All generated antisera are displayed; against all viruses except Delta AY.4.2, Lambda, Omicron BA.2, BQ.1.1, BM.1.1.1 and XBB.1. Distances between viruses and antisera in the map are inversely related to PRNT50 titres with minimized error. The grid represents two-fold dilutions in titrations. PRNT50 = plaque reduction neutralization titre resulting in 50% plaque reduction.