**Next-generation sequencing (NGS) and PCR-based assays for SARS-CoV-2 variants tracking**

**Research Project**

In January 2020, a previously unknown coronavirus strain was identified as the cause of a respiratory infection and death in humans. The first viral genome was sequenced using high throughput sequencing (HTS) from a sample collected in Wuhan, China. This virus, belonging to the viral species *Severe acute respiratory syndrome– related coronavirus*, has been subsequently named SARS-CoV-2 and the associated disease coronavirus disease 2019 (COVID-19).

Since the first described case of SARS-CoV-2 infection in Wuhan, China in December of 2019, over 107 million confirmed infections have been documented, enabling viral diversification and the emergence of several distinct lineages with numerous variants. The emerged variants were classified by the Centers for Disease Control and Prevention (CDC), based on the threat level they pose to the public health, in variants of concern (VOCs), variants of interest (VOIs) and variants of high consequence. To be able to confirm infection with a specific variant, sequencing of the whole SARS-CoV-2 genome is required. Combining information of virus characteristics with clinical and epidemiological data is important to guide public health actions. Genetic characterization of SARS-CoV-2 is used to monitor viral evolution and to timely identify potential markers of increased transmissibility, severity of disease or altered antigenicity. Sequence data will become increasingly important to assess the impact of mutations on the performance of antiviral drugs and to monitor the match of the circulating variants with the vaccine. Currently, whole genome sequencing (WGS) is being used as the main tool for epidemiological monitoring of SARS-CoV-2 variants. However, the relatively long turn-around-time for WGS makes identification of concerning variants lagged far behind laboratory COVID diagnosis.

Recently, faster and simpler screening tool, such as PCR-based assays, have been developed for the identification of signature mutations of VOCs/VOIs that can be used in a high throughput fashion to increase the capacity of SARS-CoV-2 variant detection.

This research project involves the establishment of a workflow for SARS-CoV-2 variants testing by WGS and PCR-based assays to provide epidemiological data regarding the circulation and spread of current annotated VOCs/VOIs and for identification of new emerging viral variants.

**Research Activities**

- Sample selection.In order to more effectively monitor virus evolution, samples will be selected from patients of varied demographics, across the disease severity spectrum, and from possible cases of reinfections and/or vaccine failures.

- Identification of SARS-CoV-2 variants. Implementation of Next-generation sequencing (NGS) methodology to analyze complete SARS-CoV-2 genomes. Evaluation and implementation of RT-PCR based assays for the identification of signature mutations of VOCs/VOIs.

- Data-sharing and reporting. Consensus sequences will be shared in the GISAID EpiCov database (www.gisaid.org) to enable global phylogenetic analysis as well as in regional and national platforms for a prompt monitoring of virus variants circulation.

**Figura 1. Piano delle attività**

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|  | MESI | | | | | | | | | | | |
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| Selezione random o mirata dei campioni |  |  |  |  |  |  |  |  |  |  |  |  |
| Esecuzione dei test (RT-PCR Real Time & NGS) |  |  |  |  |  |  |  |  |  |  |  |  |
| Analisi dei dati e caricamento sulle piattaforme ICOGEN e GISAID |  |  |  |  |  |  |  |  |  |  |  |  |