

PROCEDURES

Detection of SARS-CoV-2 RNA with naso-pharyngeal swab specimens

During the first study period (September 21, 2020 to January 6, 2021; symptomatic patients), naso-pharyngeal (NP) swab specimens were analyzed by RT-PCR using the ORF1ab and N genes Da An Gene DA0992-Detection Kit for 2019-nCoV (Da An Gene Co., Ltd. Sun Yat-sen University, Guangzhou, Guangdong, China). NP swabs were eluted into a vial containing 400 µl of viral RNA extraction buffer (RNA/DNA purification kit, Da An Gene, ref DA0940); and 5 µl was then processed on an AGS 4800 Thermocycler (Hangzhou, Zhejiang, China). According to the guidelines of the French Society for Microbiology [reference 30 Avis du 25 septembre 2020 de la Société Française de Microbiologie (SFM) relatif à l'interprétation de la valeur de Ct] the result was considered as positive when the Ct (cycle threshold) value for the N and/or Orf1b genes was equal or less than 36.

During the second study period (February 6 to March 6, 2021; close contacts) NP samples were analysed with the Aptima[®] SARS-CoV-2 assay (Hologic, Inc. 10210 Genetic Center Drive San Diego, CA 92121 USA), which combines Transcription Mediated Amplification (TMA), and Dual Kinetic Assay (DKA) that amplifies and detects two conserved regions of the ORF1ab gene. NP swabs were eluted into a vial containing 710 µl of medium (Hologic Specimen Lysis Tubes, ref PDD-06554) before being tested on the Panther system. Assay results were determined with a cut-off based on the total Relative Light Units (RLU). According to the guidelines of the French Society for Microbiology the results were considered as positive when the RLU was above 850.

Detection of SARS-CoV-2 RNA with oral sponge (OS) specimens at the Synlab Barla laboratory

25 µl of the OS eluates transferred fresh from the Nice COVID-19 biobank were put into a vial containing 400 µl of viral RNA extraction buffer (RNA/DNA purification kit, Da An Gene,

ref DA0940); and 5 μ l was then processed on an AGS 4800 Thermocycler (Hangzhou, Zhejiang, China).