ANTIBODY RESPONSE TO THE SARS-COV-2 VIRUS IN STAFF WORKING AT INSTITUT BERGONIÉ IN THE CONTEXT OF THE COVID-19 PANDEMIC

PRO-SERO-COV study

NCT04426006

Interventional research protocol involving people
Category 2 for minimal risks and obligations

ID-RCB no. 2020-A01355-34 / IB no.: 2020-01
Version no. 2.0 of 25/09/2020

Principal investigator:
Dr Brice RICHEZ
Anaesthetist
Department of Anaesthesia and Intensive Care
Institut Bergonié
229, cours de l'Argonne – 33076 BORDEAUX Cedex
Phone: +33 (0)556 333 217 – Fax: +33 (0)556 333 330
E-mail: b.richez@bordeaux.unicancer.fr

Scientific managers
Dr Francoise DURRIEU
Doctor, clinical biologist
Department of Biopathology, Institut Bergonié

Prof. Simone MATHOULIN-PELISSIER
UREC, Institut Bergonié
Head doctor of the Research and Clinical Epidemiology Unit (UREC)

Dr Isabelle SOUBEYRAN
Pathology specialist
Department of Biopathology, Institut Bergonié

Prof. Isabelle BALDI
Occupational doctor
INSERM U1219/EPICENE team, Université Bordeaux

Research and Clinical Epidemiology Unit

Carine BELLERA
Bergonié
Biostatistician
Institut

Carine LALET
Bergonié
Head of Clinical Studies
Institut

SPONSOR INSTITUT BERGONIÉ

SUMMARY

TITLE OF THE STUDY	Antibody response to the SARS-CoV-2 virus in staff working at Institut Bergonié in the context of the COVID-19 pandemic.
ACRONYM	PRO-SERO-COV
SPONSOR	Institut Bergonié, Bordeaux
STUDY CATEGORY	RIPH2 (category 2 interventional research protocol involving people: minimal risks and obligations)
COORDINATING/PRINCIPAL INVESTIGATOR	Dr Brice RICHEZ, Institut Bergonié
SCIENTIFIC MANAGERS	Dr Françoise DURRIEU, Institut Bergonié Prof. Simone MATHOULIN-PELISSIER, Institut Bergonié Dr Isabelle SOUBEYRAN, Institut Bergonié Prof. Isabelle BALDI, INSERM U1219, EPICENE, Université Bordeaux
NUMBER OF SITES PLANNED	1
NUMBER OF PARTICIPANTS	Around 600 participants
STUDY DURATION	Inclusion period: around 3.5 months – until 30/09/2020 Follow-up: 12 months Study duration: around 16 months
INDICATIONS	Staff working at Institut Bergonié during the COVID-19 pandemic and with varying exposure levels at work: staff in treatment units and staff in other units (cf. protocol for a definition of exposure typologies).
STUDY RATIONALE	After having been infected with SARS-CoV-2, **the majority of individuals developed a measurable immune response through the production of antibodies against the virus.** Serological tests for the **SARS-CoV-2** virus are in development to detect antibodies **in order to determine whether an individual has been in contact with the virus.** These serological tests make it possible to detect specific (immunoglobulins: Ig) antibodies (Ac) produced by the body to fight SARS-CoV-2 and could also have multiple uses, as recently highlighted by the French National Authority for Health (*Haute Autorité de la Santé*, HAS). Serological tests will not be able to determine whether the person is contagious or not (HAS 16 April 2020 and 1 May 2020). Those who have been in contact with the virus, who fought off the virus and who have no more symptoms are considered to be recovered, as for all acute viral infections. However, the presence of antibodies is not the same as immune protection (= immunity). In fact, although the presence of neutralising antibodies can be observed in some patients, there is still no correlation with protection. Assured medium-term, lasting or definitive protection is not guaranteed. Therefore, a recurring reinfection or reactivation of the virus cannot be ruled out, as is the case for other coronaviruses. Although the risk of developing severe forms in the event of reinfection is very low, a person who has antibodies would be susceptible to reinfection and thus infecting their social circle. Consequently, to date, serological tests have had a place in epidemiological monitoring and in identifying those who were or have been in contact with

	the virus (as a complement to RT-PCR, which remains the first-line test for diagnosing the acute stage of COVID-19), but not for identifying those who are potentially protected against the virus. **Serological tests are currently performed using diagnostic tests that are based on the ELISA (enzyme-linked immunosorbent assay) method that is available commercially. Other techniques, including immunochromatographic rapid tests and fully automated tests, are also in development and/or under evaluation. Furthermore, these tests have not yet been approved (awaiting approval from the National Reference Centre (CNR) at the Institut Pasteur).** At first glance, Nouvelle Aquitaine is a region that has not been severely affected in terms of COVID-19 cases (6% prevalence rate) and Institut Bergonié has put in place strict exclusion measures for staff who have been in contact with the virus and isolation measures for those who may be affected by the virus. There are currently no recommendations for carrying out systematic serological tests for staff. However, the question of immune response for the population, especially populations **in contact with vulnerable groups**, such as those suffering from a chronic disease, like cancer, remains an important one. Understanding the evolution of this response over time can make it possible to answer unresolved questions. In addition, due to the COVID-19 pandemic, the situation can seem particularly exceptional and worrying. This situation can lead to a considerable **state of anxiety**, in particular among vulnerable populations and the healthcare professionals who support them.
OBJECTIVES	**Primary objective** To evaluate the antibody immune response to infection by the SARS-CoV-2 virus in healthy members of staff who have volunteered to be included and work in an oncology healthcare facility, with different types of exposure: care unit staff and staff in other units. We are interested specifically in detecting IgG and IgM antibodies on inclusion using an ECLIA (electrochemiluminescence assay) or an ELISA (enzyme-linked immunosorbent assay) automated test. **Secondary objectives** 1. To evaluate antibody immune response (IgG and IgM through ELISA) in staff at 3 and 12 months (overall and depending on the type of exposure). 2. To evaluate the antibody immune response in staff using other antibody isotypes (IgA through ELISA) at inclusion, 3 and 12 months (overall and depending on the type of exposure) (subject to (i) approval of these tests by the National Reference Centre during the study period and (ii) a serum preservation period that is compatible with carrying out these tests). 3. To evaluate antibody immune response in staff using different antibody isotypes (IgG, IgM) at inclusion, 3 and 12 months (overall and depending on the type of exposure) using a rapid diagnostic test (RDT), known as a "rapid cassette test". 4. To identify the kinetics of producing antibodies (IgG, IgM using ELISA/ECLIA) against the SARS-CoV-2 virus over 12 months (overall and depending on the type of exposure). 5. To evaluate the immune response triggered against SARC-CoV-2 (IgG, IgM, possibly IgA, by ELISA/ECLIA) according to the biological criteria (rate of lymphocytes in particular), overall and depending on the type of staff exposure.

	6. To identify COVID-19 infections at inclusion, 3 and 12 months. 7. [Optional study] To evaluate the presence and evolution of anxiety disorders.
STUDY OUTLINE	A prospective, monocentric longitudinal cohort with blood samples and a self-questionnaire (at inclusion, 3 and 12 months).
INCLUSION CRITERIA	1. Being 18 years old or above. 2. Employed (temporary or permanent contract) since 17 March 2020 (at least), the date on which national lockdown measures were implemented in response to the COVID-19 pandemic. 3. Signed information consent form. 4. Those who are members of a French social security scheme in compliance with Article 1121-11 of the French Public Health Code (*Code de la Santé Publique*).
EXCLUSION CRITERIA	1. Declaration of a known, active SARS-CoV-2 viral infection in the 10 days before signing the consent form. A known, active SARS-CoV-2 viral infection is defined by a positive result from an RT-PCR diagnostic test. 2. Presence of known symptoms that indicate COVID-19 in the 10 days before signing the consent form: fever, tiredness, dry cough, shortness of breath, loss of taste and/or smell, headaches, stiffness, conjunctivitis or a cold, digestive problems (vomiting/diarrhea). 3. Those who are unable to follow and comply with the research procedures for geographic, social or psychological reasons. 4. Pregnant or breastfeeding women. 5. Individuals in detention and those who are unable or in no fit state to give their consent.
ROLL-OUT OF THE STUDY	• After the investigating doctor has verified the participant's eligibility criteria and information, each participant signs a written consent form. They are scheduled for an inclusion visit according to a timetable and in a unique collection point. Each participant is included with a unique study number. • A Registered Nurse takes the blood sample (two 5 ml tubes) and arranges the next appointment (3 and 12 months). • Demographic, clinical, professional and exposure data were collected on inclusion and at each visit: o Sex, age, weight, height (only at inclusion), o Biological parameters: complete blood count. o Job and exposure before and after the start of the crisis and the implementation of lockdown (16/03/2020): role, remote working, etc. o Tests for the SARS-CoV-2 virus, if carried out, and results, o If COVID-19 is detected (more than 10 days before inclusion or during follow-up): symptoms and duration. • [Optional study]: Collecting anxiety symptoms using a self-questionnaire during each visit. • Data collection will be carried out in a database (Redcap), under the administration of the EPICENE (cancer and environment team, Prof. I Baldi) team at the INSERM U1219 unit and the Clinical Investigation Centre CIC-EC 14.01.
EVALUATION CRITERIA	**<u>Primary evaluation criteria</u>** • The antibody immune response to a SARS-CoV-2 viral infection will be determined on inclusion by detecting specific antibodies (immunoglobulins: IgM and IgG) using the ELISA method, which enables

qualitative and semi-quantitative measures. These ELISA tests will be performed in the Institut Bergonié's medical biology laboratory.

- First and foremost, ***the antibody status for total antibodies (IgG + IgM) by ECLIA*** will be categorised according to the following methods (cf. "Biological tests" section):
  - "Positive" serological status:
    - optical density above the ECLIA positivity level,
    - *or* optical density between the ECLIA positivity and negativity levels and optical density above the ELISA positivity level (IgG or IgM),
    - *or* positive or negative response controls not available in ECLIA and optical density above the ELISA positivity level (IgG or IgM).
  - "Negative" serological status:
    - optical density below the ECLIA negativity level,
    - *or* optical density between the ECLIA positivity and negativity levels and optical density below the ELISA negativity level (IgG and IgM).
  - "Uncertain" serological status: optical density between the ECLIA positivity and negativity levels and optical density between the ELISA positivity and negativity levels (IgG and IgM).
  - "Non-interpretable" serological status: positive or negative response controls not available in ECLIA and positive or negative response controls not available in ELISA.
- ***The IgG serological status*** will be categorised according to the following methods:
  - "Positive" IgG serological status:
    - optical density above the ECLIA positivity level and optical density above the ELISA IgG positivity level,
    - *or* optical density between the ECLIA positivity and negativity levels and optical density above the ELISA IgG positivity level,
    - *or* positive or negative response controls not available in ECLIA and optical density above the ELISA IgG positivity level.
  - "Negative" IgG antibody status:
    - optical density below the ECLIA negativity level,
    - *or* optical density between the ECLIA positivity and negativity levels and optical density below the ELISA IgG negativity level,
    - *or* positive or negative response controls not available in ECLIA and optical density below the ELISA IgG negativity level.
  - "Uncertain" IgG serological status: optical density between the ECLIA positivity and negativity levels and optical density between the ELISA IgG positivity and negativity levels.
  - "Non-interpretable" IgG serological status: positive or negative response controls not available in ECLIA and IgG positive or negative response controls not available in ELISA.
- ***The IgM serological status*** will be categorised according to the following methods:
  - "Positive" IgM serological status:
    - optical density above the ECLIA positivity level and optical density above the ELISA IgM positivity level,

	▪ *or* optical density between the ECLIA positivity and negativity levels and optical density above the ELISA IgM positivity level, ▪ *or* positive or negative response controls not available in ECLIA and optical density above the ELISA IgM positivity level. o "Negative" IgM serological status: ▪ optical density below the ECLIA negativity level, ▪ *or* optical density between the ECLIA positivity and negativity levels and optical density below the ELISA IgM negativity level, ▪ *or* ECLIA unavailable positive or negative response controls and optical density below the ELISA IgM negativity level. o "Uncertain" IgM serological status: optical density between the ECLIA positivity and negativity levels and optical density between the ELISA IgM positivity and negativity levels. o "Non-interpretable" IgM serological status: positive or negative response controls not available in ECLIA and IgM positive or negative response controls not available in ELISA. • The choice of defining the principal evaluation criteria is based on HAS recommendations: the serological tests recommended should, preferably, make it possible to detect the IgM and IgG specific to viral antigens separately using the same test. Detecting IgA is optional based on current knowledge. This IgM/IgG configuration makes it possible to cover a larger antibody production period (earlier with IgMs and later with IgGs). Furthermore, each combination of isotype is likely to provide additional information on the infection status. **Secondary evaluation criteria** 1. The antibody immune response to a SARS-CoV-2 viral infection at 3 months and 12 months will be evaluated like the principal evaluation criteria (ELISA method). 2. The antibody immune response to a SARS-CoV-2 viral infection will also be determined using other antibody isotypes (IgA) (subject to (i) approval of these tests by the National Reference Centre during the study period and (ii) a serum preservation period that is compatible with carrying out these tests). The IgA antibody response will be evaluated according to the same methods for IgG and IgM antibody responses (cf. principal evaluation criteria). 3. The antibody immune response to a SARS-CoV-2 viral infection will also be determined using other antibody isotypes (IgG and IgM) using a rapid diagnostic test (RDT), known as a "rapid cassette test". The "by RDT" serological status will be categorised according to the following methods: o "Positive" RDT serological status: presence of antibodies and subtypes (IgG and/or IgM) o "Negative" RDT serological status: no antibodies o Uninterpretable RDT antibody result: non-contributive internal controls that do not make it possible to determine the result. The test will have to be repeated. 4. With regard to the kinetics of producing antibodies (IgG, IgM, IgA) against the SARS-CoV-2 virus over 12 months, the concentration of each antibody will be described in longitudinal terms at inclusion, 3 and 12 months.

	5. The immune response that fights SARS-CoV-2 (IgG, IgM and IgA by ELISA) will also be evaluated according to the following biological criteria: rate of lymphocytes, lymphocyte subgroups T, NK and B, as well as the rate of white blood cells, polymorphonuclear neutrophils and eosinophils and the haemoglobin rate. 6. An active COVID-19 infection is defined by a positive result to molecular tests that detect the SARS-CoV-2 coronavirus genome through RT-PCR. It will be identified by the reporting of active COVID-19 infections (positive PCR test) at month 3 and month 12 for participants included (can be performed in lockdown or in the event that the virus has a resurgence at the end of the year). The study will complete information on exposure between the two periods depending on the epidemic context at the time. 7. [Optional study]: Anxiety symptoms will be collected using a GAD-7 self-questionnaire (Spitzer et al. 2006), the score of which varies from 0 to 21. The recommended level for estimating generalised anxiety is 10 points.
BIOLOGICAL TESTS	The platform for carrying out biological tests will be the biology department at Institut Bergonié (Dr F Durrieu) and the biopathology department (Dr I Soubeyran). **ELISA and ECLIA IgM, IgG and possibly IgA tests** • The ELISA IgG tests will be performed with EuroImmun kits that detect SARS-CoV-2 spicule antigens. The reading is carried out at 450 nm on a Multiskan FC (ThermoScientific) microplate photometer. The ELISA IgM tests will be carried out using Epitope Diagnostic kits (EDI: awaiting validation by the CNR). The ELISA IgA tests can be carried out as soon as possible and once validated by the CNR. • The positivity and negativity levels are determined by the response-related optical density obtained through a positive control and at least one negative control, according to the kit. • The intensity of the response to each serum tested is compared to these negativity and positivity levels and, in particular, for each antibody, the antibody response will be determined as follows: o "Positive" serological status: optical density above the positivity level, o "Negative" serological status: optical density below the negativity level, o "Uncertain" serological status: optical density between the positivity and negativity levels, o Uninterpretable result: unavailable positive or negative response control • The total Ig automated test (IgG + IgM) by ECLIA (electrochemiluminescence immunoassay) will be performed through the Cobas Pro E801 (Roche Diagnostic) module using the Elecsys Ac Anti-SARS-CoV-2 reagent. The positivity level is determined using a reactive optical indication compared to a positive calibrator and a negative calibrator and is expressed as a sample/level connection (S/L connection). o S/L: < 1.0: non-reactive (negative for anti-SARS-CoV-2 antibodies) o S/L: ≥ 1.0: reactive (positive for anti-SARS-CoV-2 antibodies) • These different tests are validated by the CNR in line with HAS recommendations and are approved in a laboratory in line with recommendations from Cofrac, the French accreditation committee (SH-GTA-04).

	• In the event of detecting IgM, an RT-qPCR and PCR digital (ddPCR) test will be offered to each person. **<u>Rapid Diagnostic Test (RDT)</u>** • The rapid test will be carried out on a COVID-19 Sign IgM/IgG cassette (ServiBio), which is awaiting approval. • An EDTA tube makes it possible to have the results of the complete blood count to ensure that there has been no interference in the test. • In the event of detecting IgM, an RT-qPCR and PCR digital (ddPCR) test will be offered to each person. **<u>Test by RT-PCR and test by digital PCR (Dr I Soubeyran)</u>** • The sample will be taken with a nasopharyngeal swab. After extracting the viral RNA with the QIAamp Viral RNA Mini kit (Qiagen), an RT-qPCR analysis will be carried out with the TAQPATH 1-STEP RT-qPCR kit on the QuantStudio 5 system in line with the supplier recommendations. • The RT-PCR test is currently the only one recommended for diagnosing a COVID-19 infection (HAS, 16/04/2020). • The digital PCR is a recent and decisive technology. It makes it possible to obtain the absolute quantification of DNA by dividing it into several subunits (around 20,000) in the form of emulsion droplets. In addition, it will be more sensitive than the RT-qPCR and suited to samples with a low viral load. The utility of this technology compared to RT-qPCR has recently been highlighted in two publications (Suo T. et al. 2020; Dong L. et al. 2020), indicating the benefit of using ddPCR in uncertain or negative cases obtained through qPCR. Based on the same RNA, the ddPCR will be performed on the Bio-Rad system with 2019-nCoV CDC ddPCR Triplex Probe Assay kits in line with supplier recommendations. Comparing the results obtained from RT-qPCR and ddPCR makes it possible to assess the suitability of this latest technology in evaluating the contagiousness of staff carrying IgM.
STATISTICAL CONSIDERATIONS	**<u>Number of subjects</u>:** The inclusion duration will be a maximum of 6 months (we are aiming for 2-3 months), thus making it possible to offer the study to all staff (temporary and permanent contracts) at the Institut (1,087 people). We are hoping for a maximum of 1,000 participants, but are estimating a possible participation rate of 75% in view of societal demands. A minimum of 460 participants is needed on inclusion to estimate a 5% proportion (low estimate) of people with a positive serological test (G or M) with a 2% exactness of estimates (Wald test method); 811 participants on inclusion would allow us to have an estimate of this proportion with 1.5% exactness. **<u>Analyses</u>** • The demographic, clinical, professional and lifestyle characteristics at different points (inclusion, month 3 and month 12) will be described. • A flow diagram of the participants will be given. • The qualitative variables will be described using figures and percentages. The figures and percentages will be presented in the event of missing data. • The quantitative variables will be described in terms of non-parametric statistics (minimum, first quartile (Q1), median, third quartile (Q3) and maximum), as well as based on the average and standard deviation in the event of normality of distribution.

IDMC	• Not applicable.
OTHER POINTS	• The signed information consent form will be stored by the Occupational Medicine department at Institut Bergonié in a secure location. • Individual results cannot be communicated to the staff directly until the tests have been officially validated by the health authorities; in this case, the results will be given confidentially by the occupational doctor or the principal investigator to those who want them. • If an RT-PCR test is necessary, the result will be given by the prescriber of the examination in accordance with the national strategy that will be validated by HAS (report of 1 May 2020).

Timetable of evaluation of volunteers in the PRO-SERO-COV study

	Inclusion	3 months (+/-1 month from the initial sample)	12 months (+/-1 month from the initial sample)
Consent Inclusion and exclusion criteria	+		
Blood sample (2 tubes: dry + EDTA)	+	+	+
Short questionnaire	+	+	+
Self-questionnaire GAD-7 (optional)	+	+	+

https://www.inspq.qc.ca/boite-outils-pour-la-surveillance-post-sinistre-des-impacts-sur-la-sante-mentale/instruments-de-mesure-standardises/questionnaires/symptomes-d-anxiete