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Assessment of Serological Techniques for Screening Patients Regarding COVID-19 (COVID-SER): a prospective multicentric study

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Assessment of Serological Techniques for Screening Patients Regarding COVID-19-COVID-SER: a prospective multicentric study

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ABSTRACT

Introduction

The COVID-19 pandemic caused by SARS-CoV-2 coronavirus threatens global public health. There is an urgent public health need to assess the acquired immunity to SARS-CoV-2. Serological tests might result complementary to confirm suspected COVID-19 cases and revealed a previous infection. Performances of serological assays have to be evaluated before their use in the general population. Besides the assessment of sensitivity and specificity of these assays, neutralization capacity of the produced antibodies has to be evaluated.

Methods and analysis

We set up a prospective, multicentric clinical study to evaluate serological kits´ performances among a population of healthcare workers presenting symptoms suggestive of SARS-CoV-2 infection. Four hundred symptomatic healthcare workers will be included in the COVID-Ser study. A control cohort included during the pre-pandemic time will be used as reference values. A workflow was set up to study serological response to SARS-CoV-2 infection and to evaluate the antibodies neutralization capacity in patients with a confirmed SARS-CoV-2 infection. The sensitivity and specificity of the tests will be assessed using the molecular detection of the virus as reference. The measurement of IgM and IgG antibodies, likely marker of immunization, will be performed once per week during 6 consecutive weeks and at 6 months post-diagnosis. The IgM and IgG apparition time will determine the optimal timing to use serological technique at the acute phase of the infection. As additional objective, proportion of PCR false negative in symptomatic subjects will be determined estimating the seroconversion rate.

Ethics and dissemination

Ethical approval has been obtained from the national review board for biomedical research in April 2020 (Comité de Protection des Personnes Sud Méditerranée I, Marseille, France) under the number ID RCB 2020-A00932-37. Results will be disseminated through presentations at scientific meetings and publications in peer-reviewed journals.

Trial registration number

Clinicaltrials.gov: NCT04341142.

ARTICLE SUMMARY

Strengths and limitations of this study

- → High-throughput evaluation of serological kits to detect antibodies against SARS-CoV-2 with best performances as urgent unmet public health need.
- → Prospective study to assess the mid-term immune memory against SARS-CoV-2 infection in a healthcare workers population.
- → Long-term memory follow-up will not be addressed here and should be examined in future studies.
- → Seroneutralization evaluation as quality assessment of the acquired immunity against SARS-CoV-2.
- → Assessment of false negative proportion of the gold standard qPCR in symptomatic subjects to consider in further diagnosis of SARS-CoV-2 infection.

INTRODUCTION

The outbreak of coronavirus disease (COVID-19) in December 2019 has rapidly spread worldwide and important efforts have been undertaken to contain the pandemic. The etiological agent of COVID-19 was identified as a SARS-related coronavirus known as SARS-CoV-2 coronavirus. Although the majority of SARS-CoV-2 infected individuals appear to have only mild to moderate symptoms, this virus is also responsible of severe and fatal cases. As of 3rd June 2020, 380.580 deaths have been reported worldwide (https://coronavirus.jhu.edu/map.html). The development of immunity is important to decrease the transmission rate of SARS-CoV-2 and the associated mortality [1]. There is an urgent public health need to assess the acquired immunity to SARS-CoV-2 [2].

Serological assays, including binding assays such as enzyme-linked immunosorbent assays (ELISAs) or lateral flow assays are essential tools in the management of infectious diseases, including diagnosis of infection, measurements of protective antibody titres upon vaccination, and seroprevalence assessment of immunity in a population [3]. In addition, serological tests are the way to understand the antibody responses mounted upon SARS-CoV-2 infection and to evaluate immune protection to reinfection [4]. How long antibody response lasts, is detection of binding antibodies correlated with virus neutralization and are antibody titres correlated with protection from reinfection are some of the questions that serological tests may provide an answer [5]. To reinforce the molecular testing, for which false negative PCR results has been reported, serological tests might have interest and result complementary in the management of COVID-19 patients to confirm suspected cases and revealed a previous infection [6]. All the raised points are important to assess the immune status to SARS-CoV-2 in the population and understand the conferred immunity after SARS-CoV-2 infection [7]. This will allow, in security, workers to resume work, especially those in contact with risk-populations in healthcare settings [8].

As there is an urgent need for a long-spectre testing method for SARS-CoV-2, an antigen-based system seems appropriate, besides meets the criteria of fast time-to-results and low-cost detection [9]. However, performances of serological kits have not been assessed. These tests might present

heterogeneous sensitivity and specificity between them [10] and assessment of the better performances is required in order to select a reliable kit to use in the general population.

RATIONALE

The acquired immunity to SARS-CoV-2 to assess the protection face to reinfection still needs to be determined. Serological assays are required to measure the detection of SARS-CoV-2 antibodies and its correlation with immune protection. The COVID-SER project aimed to assess the performances of the serological kits in the detection of anti-SARS-CoV-2 antibodies and their seroneutralization capacity. The ultimate goal is the selection of the best kits with higher performances in the detection of IgM and IgG antibodies to measure the prevalence of SARS-CoV-2 infection and immunization in the screened general population. The detection of early IgM antibodies could be complementary to the PCR test to diagnose the infection. It could allow a broader screening of symptomatic subjects. The later but sustained production of IgG antibodies could enable to determine the immune protection of individuals against SARS-CoV-2.

OBJECTIVES

The objectives to the COVID-SER project are (1) to assess the performances of serological kits to detect anti-SARS-CoV-2 antibodies in an infected healthcare workers population; (2) to assess the dynamic features of the production of antibodies against SARS-CoV-2; (3) to evaluate the seroneutralization of the produced antibodies, (4) to evaluate the false negative rate of the PCR test compared to the serological tests and (5) to assess the duration of the presence of SARS-CoV-2 in the nasopharyngeal sample and its potential infectious capacity.

DELIVERABLES

The assigned agenda is to determine the serological kits with better performances to offer to the general population a reliable and rapid screening tool. Collective benefice from this study is expected to obtain in this major health crisis.

METHODS AND ANALYSIS

The COVID-SER project is a prospective longitudinal, multi-centre clinical study conducted in three Hospitals in Lyon, France: Hospital Lyon Sud, Hospital Edouard Herriot and Hospital Croix-Rousse.

Study population

COVID-SER will include healthcare workers (n=400) presenting presumed SARS-CoV-2 infection and associated symptoms for whom a SARS-CoV-2 PCR test on nasal-pharyngeal sample will be performed to diagnose the presence of infection. Inclusion of 130 positive subjects determined in the first visit is required to meet the expected objectives. Subjects have to be aged 18 years or more, giving their informed consent for the participation to the study and be affiliated to a social security system in order to be eligible for the study. The only criteria of exclusion to the study are pregnant or breastfeeding women. Subjects have the right to withdraw from the study if desired.

During March 2020, 30-50% of the symptomatic healthcare workers attending the screening centre were tested PCR positive for SARS-CoV-2 infection. Therefore, the period of inclusion was calculated up to 3 months, starting in April 2020, and the total duration of the study is expected to be 9 months. The participation of each COVID-19 positive subject is of 6 months.

Healthy volunteers´ serum samples (n=90) banked from a pre-pandemic period will be used as reference negative cohort for the COVID-SER project.

Sampling schedule

In the first visit (V1), explanations will be given to the patient and the first sampling will be performed. If the patient has a positive PCR result in V1, he/she will come back for the following visits at days 7 (V2), 14 (V3), 21 (V4), 28 (V5), 35 (V6), 42 (V7) and at 6 months (V8) for serum samples. PCR test will be performed at every visit until negative result. If the patient has a negative PCR result in V1, he/she will come back only at day 28 (V5) for a serum sample. Sampling schedule is illustrated in **Figure 1**.

Endpoints

To address the objectives mentioned above, the primary endpoint will be to assess the performances of the serological kits in the detection of anti-SARS-CoV-2 antibodies at the time of the symptoms (V1) upon positive PCR results. This will be done through the evaluation of IgM and IgG production kinetics in the infected population during the additional visits (V2 to V8) as well as the seroneutralization capacity of the produced antibodies. The second endpoint will evaluate the false PCR negative rate in symptomatic subjects (V1) with negative PCR results compared to their serological response at V5.

Biobanking

If patient agrees to the participation to the biocollection, a specific informed consent will be signed, and 5 types of blood samples will be collected with additional 18.5 mL of blood per visit and patient. Refusal to participate to the biocollection does not compromise participation to the study and only 8 mL of blood will be collected. This study will provide the opportunity to establish a biobank to preserve the material collected, enabling exploration of innovative biomarkers. (1) EDTA plasma biobank to study viral reactivation markers and soluble host biomarkers; (2) PBMC isolated from blood collected in EDTA tube (3) RNA biobank to study new transcriptomic host biomarkers and to complement the protein approach of the study (RNA will be extracted from whole blood collected in PAXgene tubes).

Sample size and data analysis plan

- Study design and sample size

A prospective cohort of healthcare workers will be used to assess the sensitivity of the serological tests in the detection of SARS-CoV-2 antibodies and in the production of the IgG and IgM kinetics. Moreover, healthy volunteers´ serum samples (n=90) banked from a pre-pandemic period will be used to assess the specificity of the serological tests. The inclusion of 130 positive PCR healthcare workers at V1 will provide 80% power to detect a sensitivity higher than 70% and the inclusion of 90 healthy volunteers´ serum samples will provide 80% power to detect a specificity higher than 80%. The number of subjects required to determine the expected threshold for the serological kits tested was defined using the function binDesign (binGroup package, R software) and the Wilson methodology to build the confidence range.

- Statistical methods

Characteristics of the positive and negative PCR samples will be described and quantified as median (IQR). To evaluate the primary endpoint, sensitivity of the different serological tests on the infected population will be assessed according to the threshold established by the manufacturer´s instructions, with a confidence interval (CI) obtained by the Wilson method. Specificity of the serological tests will be assessed by the same method on the healthy serum samples. Sensitivities and specificities of the different serological kits will be compared using Mc Nemar test. Sensitivity of the most performant kits will be modelled through logistic regression to quantify the delay on the quantification of antibodies from the beginning of the symptoms or the exposition to specific therapy. Factors acting on the sensibility will be quantified as odds ratio (CI=95%). To evaluate the evolution of the antibody's titre (IgM and IgG) a mix-effects linear regression will be modelled. Analyses will be conducted with latest version of R.

Ethics and dissemination

- Ethics approval

The study is registered to the French Commission for Individual Data Protection and Public Liberties (CNIL) of Lyon's university hospital under the number 20-120. Ethical approval has been obtained from the national review board for biomedical research in April 2020 (Comité de Protection des Personnes Sud Méditerranée I, Marseille, France) under the number ID RCB 2020-A00932-37. The international trial registration number in ClinicalTrial.gov is NCT04341142.

- Informed consent

The identification of subjects included in the study will be kept anonymous and protected by a cryptographic code. Data will be anonymously extracted from medical records (HCL software). The informed and signed consent will be registered on the computerised record of each subject. Full information of the objectives and the workflow of the study will be given, and the possibility to refuse to participate or to withdraw from the study whenever chosen will be provided to the

subject. A comprehensive notice will be distributed to the subject summarising the protocol and the follow-up of the study.

- Dissemination

Results will be communicated at scientific meetings and submitted for publication in peer-reviewed journals.

Safety of participants

This study includes no serious foreseeable risk to the health of the subjects involved. The only potential risk is related to blood sample collection (maximum 212 mL collected over all time points — 6 months). However, this aspect of nursing is part of daily practice. Blood samples will be taken under the same conditions of safety as currently used for common diagnostic tests.

Patient and public involvement

No patient was involved in the design or implementation of this study. Study participants will be individually informed about their results during scheduled medical visits and will be given access, on demand, to the final publication of the study results.

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Authors' contribution

ST-A, AB, J-AN, PF, AP, AM-P, CA, VP, ML, AB, MD, CS, MR, FG and J-BF were involved in trial design and ST-A, CA-V, AB and J-BF were involved in the drafting of the manuscript. All authors were involved in critical revision of the article for important intellectual content and approved the final version.

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Competing interests

All the authors declare no conflict of interest.

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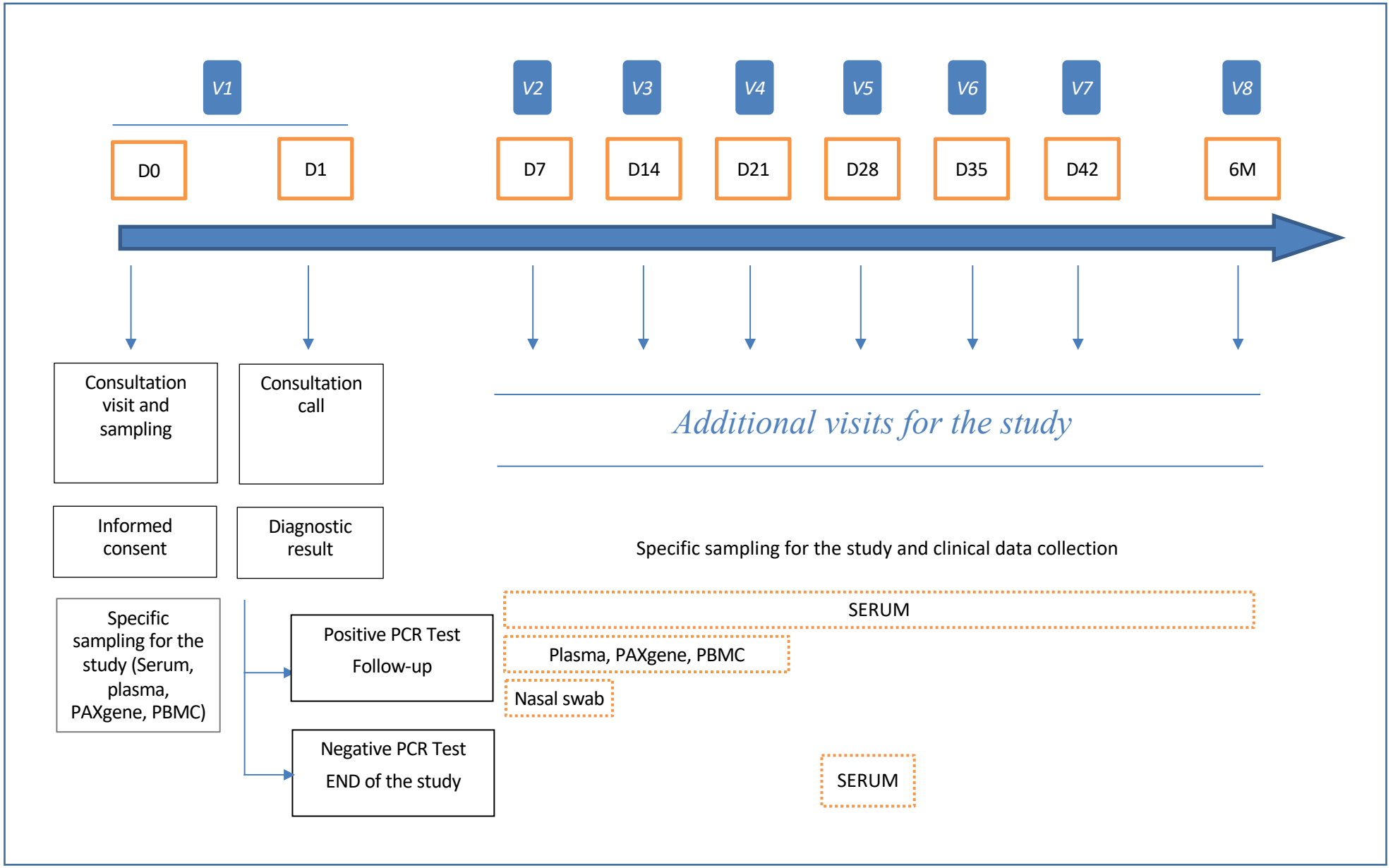
FIGURE LEGEND

Figure 1. Schematic design of the COVID-SER project illustrating the various time-points of the study and the type of collected sample at each visit.


V1
V2
V3
V4
V5
V6
V7
V8
D0
D1
D7
D14
D21
D28
D35
D42
6M
Consultation visit and sampling
Consultation call
Additional visits for the study
Informed consent
Diagnostic result
Specific sampling for the study and clinical data collection
Specific sampling for the study (Serum, plasma, PAXgene, PBMC)
Positive PCR Test
Follow-up
SERUM
Plasma, PAXgene, PBMC
Nasal swab
Negative PCR Test
END of the study
SERUM

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Assessment of Serological Techniques for Screening Patients Regarding COVID-19-COVID-SER: a prospective multicentric study

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ABSTRACT

Introduction

The COVID-19 pandemic caused by SARS-CoV-2 coronavirus threatens global public health. There is an urgent public health need to assess the acquired immunity to SARS-CoV-2. Serological tests might result complementary to confirm suspected COVID-19 cases and reveal a previous infection. Performances of serological assays (sensitivity and specificity) have to be evaluated before their use in the general population. Besides, the neutralization capacity of the produced antibodies has to be evaluated.

Methods and analysis

We set up a prospective, multicentric clinical study to evaluate serological kits´ performances among a population of healthcare workers presenting mild symptoms suggestive of SARS-CoV-2 infection. Four hundred symptomatic healthcare workers will be included in the COVID-Ser study. A control cohort included during the pre-pandemic time will be used as reference values. A workflow was set up to study serological response to SARS-CoV-2 infection and to evaluate the antibodies neutralization capacity in patients with a confirmed SARS-CoV-2 infection. The sensitivity and specificity of the tests will be assessed using molecular detection of the virus as a reference. The measurement of IgM and IgG antibodies will be performed once per week during 6 consecutive weeks, and then at 6, 12, 18, 24 and 36 months after the diagnosis. The kinetics of IgM and IgG will determine the optimal period to perform serological testing. The proportion of false negatives PCR tests in symptomatic subjects will be determined on the basis of subsequent seroconversions.

Ethics and dissemination

Ethical approval has been obtained from the national review board for biomedical research in April 2020 (Comité de Protection des Personnes Sud Méditerranée I, Marseille, France) under the number ID RCB 2020-A00932-37. Results will be disseminated through presentations at scientific meetings and publications in peer-reviewed journals.

Trial registration number

Clinicaltrials.gov: NCT04341142.

ARTICLE SUMMARY

Strengths and limitations of this study

- → High-throughput evaluation of serological kits to detect antibodies against SARS-CoV-2 with best performances
- → Prospective study to monitor the development of humoral response against SARS-CoV-2 infection in a healthcare workers population
- → Long-term memory follow-up will be addressed until 3 years post-diagnosis
- → Seroneutralization techniques will assess the acquired immunity against SARS-CoV-2.
- → Assessment of false negative proportion of the gold standard qPCR in subjects with mild symptoms and detected seroconversion

INTRODUCTION

The outbreak of coronavirus disease (COVID-19) in December 2019 has rapidly spread worldwide and important efforts have been undertaken to contain the pandemic. The etiological agent of COVID-19 was identified as a SARS-CoV related (severe acute respiratory syndrome) coronavirus known as SARS-CoV-2. Although the majority of SARS-CoV-2 infected individuals appear to have only mild to moderate symptoms, this virus is also responsible of severe and fatal cases. As of 25th August 2020, 813,207 deaths have been reported worldwide (https://coronavirus.jhu.edu/map.html). The development of immunity is important to decrease the transmission rate of SARS-CoV-2 and the associated mortality [1]. There is an urgent public health need to assess the acquired immunity to SARS-CoV-2 [2].

Serological assays, including binding assays such as enzyme-linked immunosorbent assays (ELISAs) or lateral flow assays are essential tools in the management of infectious diseases, including diagnosis of infection, measurements of protective antibodies after vaccination, and immunity assessment in a population [3]. In addition, serological tests allow to understand the antibody responses after SARS-CoV-2 infection and to evaluate immune protection against reinfection [4]. The duration of antibody response, the correlation of binding antibodies with virus neutralization assay and with protection against reinfection are some of the questions that serological tests may provide an answer to [5]. Serological tests may have additional value in the management of patients with COVID-19 to confirm suspected cases or to reveal a past infection in situations of false-negative PCR results [6]. All of these elements are important for understanding the immunity conferred after infection and the immune status of the population against SARS-CoV-2[7]. They may also allow workers at high risk of coronavirus exposure (such as in healthcare settings) to be able to work safely knowing their immunity against the risk of re-infection [8].

As there is an urgent need for a long-spectre testing method for SARS-CoV-2, an antigen-based system meeting the criteria of fast time-to-results and low-cost detection [9] seems appropriate. However, performances of serological kits have not been assessed. These tests might present heterogeneous sensitivity and specificity [10] and assessment of their performance is required in order to select a reliable kit to use in the population.

In brief, there are many uncertainties associated with serological testing [11]. They are less efficient than RT-PCR for diagnosis in the acute phase of the disease. Antibodies are detectable in a time-delayed manner after the onset of symptoms, and their persistence over time is variable. Antibody kinetics have been studied mostly in hospitalized populations with signs of severity, but appear to be less rapid and with less amounts of antibodies in populations with mild symptoms. Most notably, it is still unknown to what extent detectable antibodies imply immunity [11].

RATIONALE

The acquired immunity to SARS-CoV-2 against reinfection still needs to be determined. Serological assays are required to measure the presence of SARS-CoV-2 antibodies and its correlation with immune protection. The COVID-SER project aims to assess the performances of different serological kits aimed at detecting anti-SARS-CoV-2 antibodies and their neutralizing capacity. The ultimate goal is to identify the kits with higher performances in the detection of IgM and IgG antibodies to measure the prevalence of SARS-CoV-2 infection and immunization in the population. The detection of early IgM antibodies could be complementary to the PCR test to diagnose the infection. It could allow a broader screening of symptomatic subjects. The later but sustained production of IgG antibodies could enable to determine the immune protection of individuals against SARS-CoV-2.

OBJECTIVES

The objectives to the COVID-SER project are (1) to assess the performances of different serological kits to detect anti-SARS-CoV-2 antibodies in an infected healthcare workers population; (2) to monitor the development of humoral response against SARS-CoV-2 infection up to 36 months after diagnosis; (3) to evaluate the neutralizing capacity of the antibodies produced (4); to evaluate the false negative rate of the PCR tests and (5) to assess the duration of the presence of SARS-CoV-2 in the nasopharyngeal sample and its infectious potential.

DELIVERABLES

The assigned agenda is to determine the serological kits with the best performances to provide the population with a reliable and rapid screening tool. The expected collective benefits are to be able to better understand and manage the progression of the pandemic in the population.

METHODS AND ANALYSIS

The COVID-SER project is a prospective longitudinal, multi-centre clinical study conducted in a consortium of 13 University Hospitals in Lyon, France (*Hospices Civils de Lyon*; 23,000 workers; https://www.chu-lyon.fr/en).

Study population

COVID-SER will include healthcare workers (n=400) with symptoms suggesting SARS-CoV-2 infection for whom a SARS-CoV-2 PCR test on nasal-pharyngeal sample will be performed to diagnose the infection. It is expected to include 130 positive subjects to meet the expected objectives. Participants must be over 18 years of age, give their informed consent and be affiliated to a social security system. The only criteria of exclusion are pregnancy or breastfeeding, in accordance with French research regulations. Subjects have the right to withdraw from the study at any time if desired.

During March 2020, 30-50% of the symptomatic healthcare workers attending the screening centres were tested PCR positive for SARS-CoV-2 infection. Therefore, the initial period of inclusion was calculated up to 3 months, starting in April 2020, and the total duration of the study was initially expected to be 9 months. From 20 April 2020, amendments to the research protocol have been proposed to adapt to the evolution of the pandemic. The duration of inclusion was extended to 12 months, and the duration of follow-up (participation of each COVID-19 positive subject) was extended to 36 months. Healthy volunteers´ serum samples (n=90) banked from a pre-pandemic period will be used as reference negative cohort for the COVID-SER project.

Sampling schedule

At the first visit (V1), explanations will be given to the patient and the first sample will be taken. Patients with a positive PCR result at V1 will come back for the following visits at day 7 (V2), 14 (V3), 21 (V4), 28 (V5), 35 (V6), 42 (V7), and at 6, 12, 18, 24 and 36 months (V8 to 12) for serum samples. PCR tests will be performed at each visit until negative result. Patients with a negative PCR result at V1 will come back only at day 28 (V5) for a serum sample. Sampling schedule is illustrated in **Figure 1**.

Endpoints

To address the objectives mentioned above, the primary endpoint will be to assess the performances of the serological kits in the detection of anti-SARS-CoV-2 antibodies at the time of the symptoms (V1) upon positive PCR results. This will be done through the evaluation of IgM and IgG production kinetics in the infected population during the additional visits (V2 to V12) as well as the seroneutralization capacity of the produced antibodies. The second endpoint will evaluate the PCR false negative rate in symptomatic subjects (V1) with negative PCR results compared to their serological response at V5.

Biobanking

If patient agrees to the participation to the biocollection, a specific informed consent will be signed, and 5 types of blood samples will be collected with additional 18.5 mL of blood per visit and patient. Refusal to participate to the biocollection does not compromise participation to the study and only 8 mL of blood will be collected. This study will provide the opportunity to constitute a biobank enabling further exploration of innovative biomarkers: (1) EDTA (ethylenediaminetetraacetic acid) plasma biobank to study viral reactivation markers and soluble host biomarkers; (2) PBMC (peripheral blood mononuclear cell) isolated from blood collected in EDTA tube (3) RNA (ribonucleic acid) biobank to study new transcriptomic host biomarkers and to complement the protein approach of the study (RNA will be extracted from whole blood collected in PAXgene tubes).

Serological tests

Description of the serological tests evaluated in the study is available in **Table 1**.

- Antibodies titers assessment

Titers will be assessed by the ratio of patient signal to cut-off or calibrator value depending on the tests, as mentioned in **Table 1**.

- Virus neutralisation assay

A ten-fold dilution of each serum specimen in Dulbecco modified Eagle medium containing antibiotics and 2% foetal calf serum will first be heated for 30 min at 56°C in order to avoid

complement-linked reduction of the viral activity. The virus used in these experiments (RoBo strain) will be a clinical strain isolated on VERO-E6 cells from a patient hospitalized at the University Hospital of Saint-Etienne for severe COVID-19 infection; it will be diluted in the same medium so that to obtain 100 to 500 tissue culture infectious doses 50% (TCID50) per 150 µl. Virus infectivity controls will be included in each test. Serial two-fold dilutions (tested in duplicate) of the specimens will be mixed with the diluted virus at equal volume (100 µl each). After gentle shaking and a contact of 30 minutes at room temperature in plastic microplates, 150 µl of the mixt will be transferred to 96-well microplates covered with Vero-E6 cells. The plates will be placed at 37°C in a 5% CO2 incubator. The reading will be evaluated microscopically 5 to 6 days later when the cytopathic effect of the virus control reaches 100 TCID50/150 µl. A seroprotection will be recorded if more than 50% of the cells are preserved. The protection titer will be expressed as the inverse of the higher serum dilution that spared the cells. The threshold of positivity for protective antibodies will be 10. All the experiments will be performed in an L3 facility.

Sample size and data analysis plan

- Study design and sample size

A prospective cohort of healthcare workers will be used to assess the sensitivity of different serological tests in the detection of SARS-CoV-2 antibodies and to monitor the IgG and IgM kinetics. Healthy volunteers´ serum samples (n=90) banked from a pre-pandemic period will be used to assess the specificity of the serological tests. The inclusion of 130 positive PCR healthcare workers at V1 will provide 80% power to detect a sensitivity higher than 70% and the inclusion of 90 healthy volunteers´ serum samples will provide 80% power to detect a specificity higher than 80%. The number of subjects required to determine the expected threshold for the serological kits tested was defined using the function binDesign (binGroup package, “R” software) and the Wilson methodology to build the confidence range.

- Statistical methods

Characteristics of the positive and negative PCR samples will be described and quantified as median (IQR). To evaluate the primary endpoint, sensitivity of the different serological tests on the infected population will be assessed according to the threshold established by the manufacturer´s instructions, with a confidence interval (CI) obtained by the Wilson method. Specificity of the serological tests will be assessed by the same method on the healthy serum samples. Sensitivity and specificity of the different serological kits will be compared using Mc Nemar test. Sensitivity of the best performing kits will be modelled through logistic regression to quantify the delay on the quantification of antibodies from the beginning of the symptoms or the exposition to specific therapy. Factors acting on the sensibility will be quantified as odds ratio (CI=95%). To evaluate the evolution of the antibody's production (IgM and IgG) a mix-effects linear regression will be modelled. Antibodies production will be assessed by optical density ratios determined according to manufacturer recommendation. Analyses will be conducted with the latest version of "R" software.

Ethics and dissemination

- Ethics approval

The study is registered to the French Commission for Individual Data Protection and Public Liberties (CNIL) of Lyon's university hospital under the number 20-120. Ethical approval has been obtained from the national review board for biomedical research in April 2020 (Comité de Protection des Personnes Sud Méditerranée I, Marseille, France) under the number ID RCB 2020-A00932-37. The international trial registration number in ClinicalTrial.gov is NCT04341142.

- Informed consent

The identification of subjects included in the study will be kept anonymous and protected by a cryptographic code. Data will be anonymously extracted from medical records (HCL software). The informed and signed consent will be registered on the computerised record of each subject. Full information of the objectives and the workflow of the study will be given, and the possibility to refuse to participate or to withdraw from the study whenever chosen will be provided to the

subject. A comprehensive notice will be distributed to the subject summarising the protocol and the follow-up of the study. Participants will be informed at the time of inclusion in the study that the interpretation of results is limited by the current state of knowledge, and that a positive serological test does not mean that they are immune to the virus.

- Dissemination

Results will be communicated at scientific meetings and submitted for publication in peer-reviewed journals.

Safety of participants

This study includes no serious foreseeable risk to the health of the subjects involved. The only potential risk is related to blood sample collection (maximum 212 mL collected over all time points — 6 months). However, this aspect of nursing is part of daily practice. Blood samples will be taken under the same conditions of safety as currently used for common diagnostic tests.

Patient and public involvement

No patient was involved in the design or implementation of this study. Study participants will be individually informed about their results during scheduled medical visits and will be given access, on demand, to the final publication of the study results.

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Authors' contribution

ST-A, AB, J-AN, PF, AP, AM-P, CA, NG, VP, ML, AB, MD, CS, MR, MAT, FG and J-BF were involved in trial design and ST-A, CA-V, AB and J-BF were involved in the drafting of the manuscript. All authors were involved in critical revision of the article for important intellectual content and approved the final version.

Competing interests

Antonin Bal has received grant from bioMérieux and has served as consultant for bioMérieux for work and research not related to this manuscript. The other authors declare no conflict of interest.

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TABLES

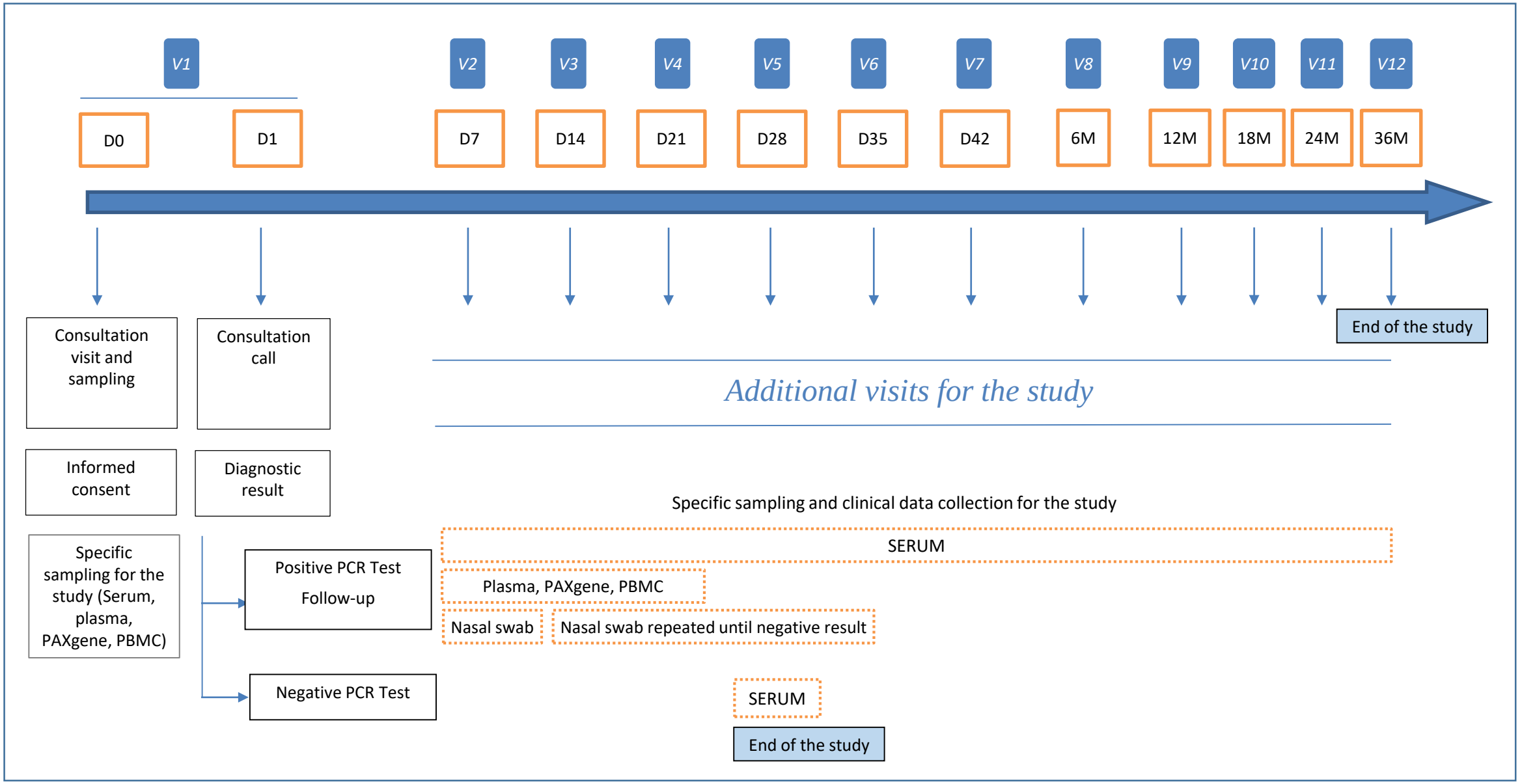
Table 1. Description of the serological tests evaluated in the study

Manufacturer	System	Product	Principle	Titer assement
ABBOTT	ARCHITECT	SARS-COV-2 IgG	CMIA	Index: Sample/Calibrator RLU
BIOMERIEUX	VIDAS®	VIDAS SARS-COV-2 IgG VIDAS SARS-COV-2 IgM	ELFA	Ratio: patient RFV/standard RFV
BIORAD	Manual or automated ELISA systems	Platelia SARS-CoV-2 Total Ab	ELISA	Ratio: sample OD / mean cut-off control OD
DIASORIN	LIAISON® XL	LIAISON SARS-COV-2 S1/S2 IgG	CLIA	UA/ml
EUROIMMUN	Manual or automated ELISA systems	ELISA SARS-CoV-2 IgA	ELISA	Ratio: sample OD / calibrator OD
SIEMENS	Atellica® IM	SARS-CoV-2 Total	CLIA	Index: Sample/Calibrator RLU
WANTAI	Manual or automated ELISA systems	WANTAI SARS-CoV-2 Ab ELISA WANTAI SARS-CoV-2 IgM ELISA	ELISA	Ratio: sample OD / cut-off OD
AAZ	none	COVID-PRESTO (RT COVID-19 IgG/IgM)	LFIA	Qualitative
BIOSYNEX	none	BIOSYNEX COVID-19 BSS (IgG/IgM)	LFIA	Qualitative
SD BIOSENSOR	none	STANDARD Q COVID-19 IgM/IgG combo	LFIA	Qualitative

CMIA: Chemiluminescence Microparticule luminescence ImmunoAssay
CLIA: Chemiluminescence Luminescence ImmunoAssay
ELFA: Enzyme Linked Fluorescence Assay
LFIA: Lateral Flow Immunochromatographic Assay
RLU: Relative Light Unit
RFV: Relative Fluorescence Value
OD: Optical density
AU: Arbitrary Unit

FIGURE LEGEND

Figure 1. Schematic design of the COVID-SER project illustrating the various time-points of the study and the type of collected sample at each visit.


V1
V2
V3
V4
V5
V6
V7
V8
V9
V10
V11
V12
D0
D1
D7
D14
D21
D28
D35
D42
6M
12M
18M
24M
36M
End of the study
Consultation visit and sampling
Consultation call
Additional visits for the study
Informed consent
Diagnostic result
Specific sampling and clinical data collection for the study
Specific sampling for the study (Serum, plasma, PAXgene, PBMC)
Positive PCR Test
Follow-up
SERUM
Plasma, PAXgene, PBMC
Nasal swab
Nasal swab repeated until negative result
Negative PCR Test
SERUM
End of the study