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

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1 Assessment of Serological Techniques for Screening Patients Regarding 2 COVID-19-COVID-SER: a prospective multicentric study

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40 **performance**

41 **ABSTRACT**

42 **Introduction**

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

49 **Methods and analysis**

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

62 **Ethics and dissemination**

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

67 **Trial registration number**

68 Clinicaltrials.gov: NCT04341142.

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3 **69 ARTICLE SUMMARY**
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5 **70 Strengths and limitations of this study**
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- 8 71 → High-throughput evaluation of serological kits to detect antibodies against SARS-CoV-2
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10 72 with best performances as urgent unmet public health need.
11
12 73 → Prospective study to assess the mid-term immune memory against SARS-CoV-2
13
14 74 infection in a healthcare workers population.
15
16 75 → Long-term memory follow-up will not be addressed here and should be examined in
17
18 76 future studies.
19
20 77 → Seroneutralization evaluation as quality assessment of the acquired immunity against
21
22 78 SARS-CoV-2.
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24 79 → Assessment of false negative proportion of the gold standard qPCR in symptomatic
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26 80 subjects to consider in further diagnosis of SARS-CoV-2 infection.
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81 INTRODUCTION

82 The outbreak of coronavirus disease (COVID-19) in December 2019 has rapidly spread
83 worldwide and important efforts have been undertaken to contain the pandemic. The etiological
84 agent of COVID-19 was identified as a SARS-related coronavirus known as SARS-CoV-2
85 coronavirus. Although the majority of SARS-CoV-2 infected individuals appear to have only mild
86 to moderate symptoms, this virus is also responsible of severe and fatal cases. As of 3rd June 2020,
87 380.580 deaths have been reported worldwide (<https://coronavirus.jhu.edu/map.html>). The
88 development of immunity is important to decrease the transmission rate of SARS-CoV-2 and the
89 associated mortality ¹. There is an urgent public health need to assess the acquired immunity to
90 SARS-CoV-2 ².

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

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

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3 108 heterogeneous sensitivity and specificity between them ¹⁰ and assessment of the better
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5 109 performances is required in order to select a reliable kit to use in the general population.
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7 110 **RATIONALE**

8
9 111 The acquired immunity to SARS-CoV-2 to assess the protection face to reinfection still needs to
10
11 112 be determined. Serological assays are required to measure the detection of SARS-CoV-2
12
13 113 antibodies and its correlation with immune protection. The COVID-SER project aimed to assess
14
15 114 the performances of the serological kits in the detection of anti-SARS-CoV-2 antibodies and their
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17 115 seroneutralization capacity. The ultimate goal is the selection of the best kits with higher
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19 116 performances in the detection of IgM and IgG antibodies to measure the prevalence of SARS-
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21 117 CoV-2 infection and immunization in the screened general population. The detection of early IgM
22
23 118 antibodies could be complementary to the PCR test to diagnose the infection. It could allow a
24
25 119 broader screening of symptomatic subjects. The later but sustained production of IgG antibodies
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27 120 could enable to determine the immune protection of individuals against SARS-CoV-2.
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30 121 **OBJECTIVES**

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32 122 The objectives to the COVID-SER project are (1) to assess the performances of serological kits
33
34 123 to detect anti-SARS-CoV-2 antibodies in an infected healthcare workers population; (2) to assess
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36 124 the dynamic features of the production of antibodies against SARS-CoV-2; (3) to evaluate the
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38 125 seroneutralization of the produced antibodies, (4) to evaluate the false negative rate of the PCR
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40 126 test compared to the serological tests and (5) to assess the duration of the presence of SARS-CoV-
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42 127 2 in the nasopharyngeal sample and its potential infectious capacity.
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45 128 **DELIVERABLES**

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47 129 The assigned agenda is to determine the serological kits with better performances to offer to the
48
49 130 general population a reliable and rapid screening tool. Collective benefice from this study is
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51 131 expected to obtain in this major health crisis.
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133 **METHODS AND ANALYSIS**

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

137 **Study population**

138 COVID-SER will include healthcare workers (n=400) presenting presumed SARS-CoV-2
139 infection and associated symptoms for whom a SARS-CoV-2 PCR test on nasal-pharyngeal
140 sample will be performed to diagnose the presence of infection. Inclusion of 130 positive subjects
141 determined in the first visit is required to meet the expected objectives. Subjects have to be aged
142 18 years or more, giving their informed consent for the participation to the study and be affiliated
143 to a social security system in order to be eligible for the study. The only criteria of exclusion to
144 the study are pregnant or breastfeeding women. Subjects have the right to withdraw from the
145 study if desired.

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

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

152 **Sampling schedule**

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

158 Sampling schedule is illustrated in **Figure 1**.

159 **Endpoints**

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3 160 To address the objectives mentioned above, the primary endpoint will be to assess the
4
5 161 performances of the serological kits in the detection of anti-SARS-CoV-2 antibodies at the time
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7 162 of the symptoms (V1) upon positive PCR results. This will be done through the evaluation of IgM
8
9 163 and IgG production kinetics in the infected population during the additional visits (V2 to V8) as
10
11 164 well as the seroneutralization capacity of the produced antibodies. The second endpoint will
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13 165 evaluate the false PCR negative rate in symptomatic subjects (V1) with negative PCR results
14
15 166 compared to their serological response at V5.

167 **Biobanking**

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

177 **Sample size and data analysis plan**

178 - Study design and sample size

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

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3 188 - Statistical methods
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5 189 Characteristics of the positive and negative PCR samples will be described and quantified as
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7 190 median (IQR). To evaluate the primary endpoint, sensitivity of the different serological tests on
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9 191 the infected population will be assessed according to the threshold established by the
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11 192 manufacturer's instructions, with a confidence interval (CI) obtained by the Wilson method.
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13 193 Specificity of the serological tests will be assessed by the same method on the healthy serum
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15 194 samples. Sensitivities and specificities of the different serological kits will be compared using Mc
16
17 195 Nemar test. Sensitivity of the most performant kits will be modelled through logistic regression
18
19 196 to quantify the delay on the quantification of antibodies from the beginning of the symptoms or
20
21 197 the exposition to specific therapy. Factors acting on the sensibility will be quantified as odds ratio
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23 198 (CI=95%). To evaluate the evolution of the antibody's titre (IgM and IgG) a mix-effects linear
24
25 199 regression will be modelled. Analyses will be conducted with latest version of R.
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28 **200 Ethics and dissemination**
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31 201 - Ethics approval
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34 202 The study is registered to the French Commission for Individual Data Protection and Public
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36 203 Liberties (CNIL) of Lyon's university hospital under the number 20-120. Ethical approval has
37
38 204 been obtained from the national review board for biomedical research in April 2020 (Comité de
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40 205 Protection des Personnes Sud Méditerranée I, Marseille, France) under the number ID RCB 2020-
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42 206 A00932-37. The international trial registration number in ClinicalTrial.gov is NCT04341142.
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45 207 - Informed consent
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48 208 The identification of subjects included in the study will be kept anonymous and protected by a
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50 209 cryptographic code. Data will be anonymously extracted from medical records (HCL software).
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52 210 The informed and signed consent will be registered on the computerised record of each subject.
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54 211 Full information of the objectives and the workflow of the study will be given, and the possibility
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56 212 to refuse to participate or to withdraw from the study whenever chosen will be provided to the
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3 213 subject. A comprehensive notice will be distributed to the subject summarising the protocol and
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5 214 the follow-up of the study.
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8 215 - Dissemination
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10 216 Results will be communicated at scientific meetings and submitted for publication in peer-
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12 217 reviewed journals.
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14 218 **Safety of participants**

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17 219 This study includes no serious foreseeable risk to the health of the subjects involved. The only
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19 220 potential risk is related to blood sample collection (maximum 212 mL collected over all time
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21 221 points — 6 months). However, this aspect of nursing is part of daily practice. Blood samples will
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23 222 be taken under the same conditions of safety as currently used for common diagnostic tests.
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25 223 **Patient and public involvement**

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27 224 No patient was involved in the design or implementation of this study. Study participants will be
28
29 225 individually informed about their results during scheduled medical visits and will be given access,
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31 226 on demand, to the final publication of the study results.
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28
29 272 in trial design and ST-A, CA-V, AB and J-BF were involved in the drafting of the manuscript.

30
31 273 All authors were involved in critical revision of the article for important intellectual content and

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42
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44
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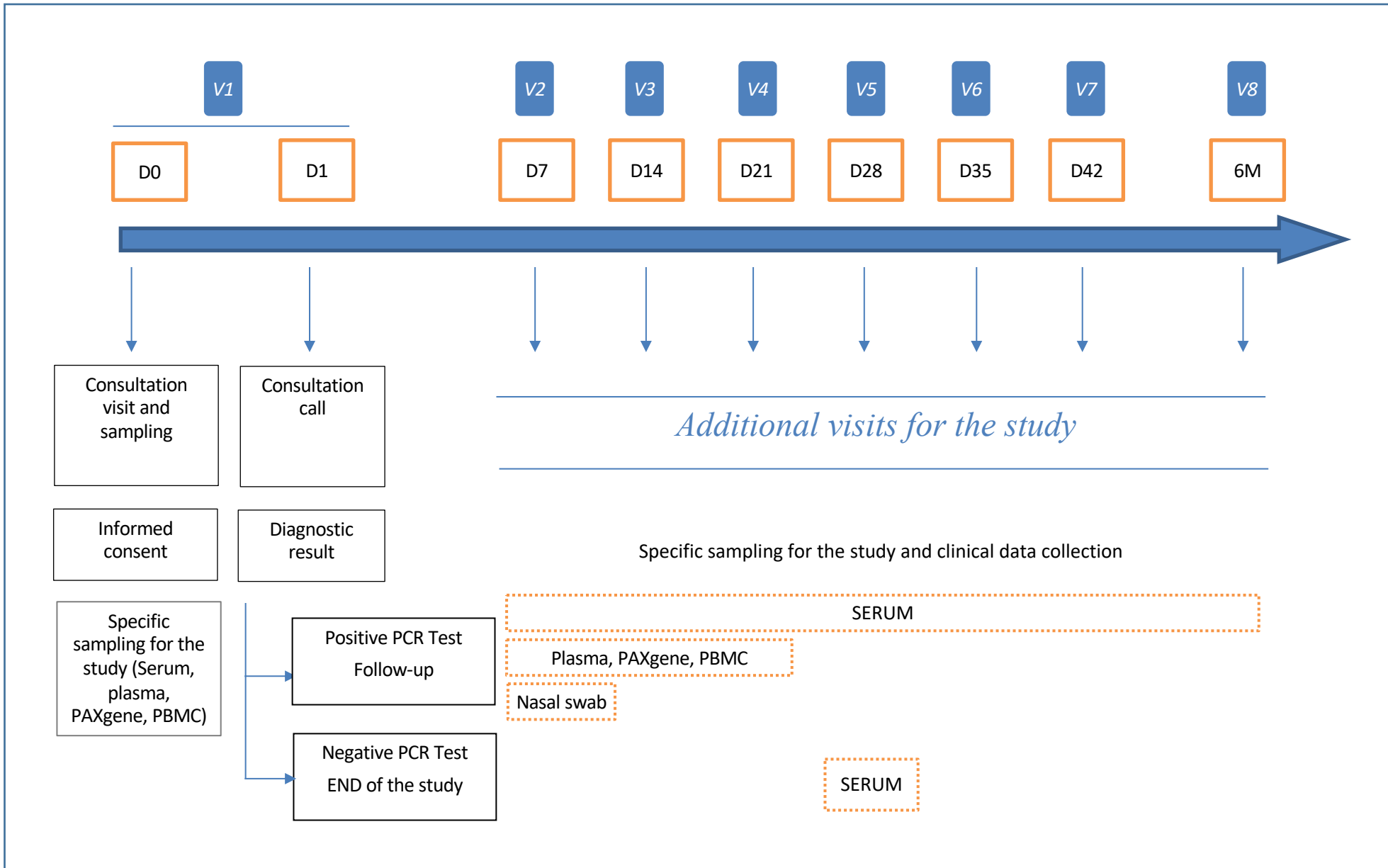
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47 281 We thank HCL Covid Task Force for their helpful advices.

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3 283 **FIGURE LEGEND**
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5 284 **Figure 1.** Schematic design of the COVID-SER project illustrating the various time-points of the
6
7 285 study and the type of collected sample at each visit.
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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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42 **ABSTRACT**

43 **Introduction**

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

50 **Methods and analysis**

51 We set up a prospective, multicentric clinical study to evaluate serological kits' performances
52 among a population of healthcare workers presenting mild symptoms suggestive of SARS-CoV-
53 2 infection. Four hundred symptomatic healthcare workers will be included in the COVID-Ser
54 study. A control cohort included during the pre-pandemic time will be used as reference values.
55 A workflow was set up to study serological response to SARS-CoV-2 infection and to evaluate
56 the antibodies neutralization capacity in patients with a confirmed SARS-CoV-2 infection. The
57 sensitivity and specificity of the tests will be assessed using molecular detection of the virus as a
58 reference. The measurement of IgM and IgG antibodies will be performed once per week during
59 6 consecutive weeks, and then at 6, 12, 18, 24 and 36 months after the diagnosis. The kinetics of
60 IgM and IgG will determine the optimal period to perform serological testing. The proportion of
61 false negatives PCR tests in symptomatic subjects will be determined on the basis of subsequent
62 seroconversions.

63 **Ethics and dissemination**

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

68 **Trial registration number**

69 Clinicaltrials.gov: NCT04341142.

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2
3 70 **ARTICLE SUMMARY**
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5 71 **Strengths and limitations of this study**
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8 72 → High-throughput evaluation of serological kits to detect antibodies against SARS-CoV-2
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10 73 with best performances
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12 74 → Prospective study to monitor the development of humoral response against SARS-CoV-
13

14 75 2 infection in a healthcare workers population
15

16 76 → Long-term memory follow-up will be addressed until 3 years post-diagnosis
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18 77 → Seroneutralization techniques will assess the acquired immunity against SARS-CoV-2.
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20 78 → Assessment of false negative proportion of the gold standard qPCR in subjects with mild
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22 79 symptoms and detected seroconversion
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80 INTRODUCTION

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

90 Serological assays, including binding assays such as enzyme-linked immunosorbent assays
91 (ELISAs) or lateral flow assays are essential tools in the management of infectious diseases,
92 including diagnosis of infection, measurements of protective antibodies after vaccination, and
93 immunity assessment in a population ³. In addition, serological tests allow to understand the
94 antibody responses after SARS-CoV-2 infection and to evaluate immune protection against
95 reinfection ⁴. The duration of antibody response, the correlation of binding antibodies with virus
96 neutralization assay and with protection against reinfection are some of the questions that
97 serological tests may provide an answer to ⁵. Serological tests may have additional value in the
98 management of patients with COVID-19 to confirm suspected cases or to reveal a past infection
99 in situations of false-negative PCR results ⁶. All of these elements are important for understanding
100 the immunity conferred after infection and the immune status of the population against SARS-
101 CoV-2⁷. They may also allow workers at high risk of coronavirus exposure (such as in healthcare
102 settings) to be able to work safely knowing their immunity against the risk of re-infection ⁸.

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

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2
3 108 In brief, there are many uncertainties associated with serological testing¹¹. They are less efficient
4
5 109 than RT-PCR for diagnosis in the acute phase of the disease. Antibodies are detectable in a time-
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7 110 delayed manner after the onset of symptoms, and their persistence over time is variable. Antibody
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9 111 kinetics have been studied mostly in hospitalized populations with signs of severity, but appear
10
11 112 to be less rapid and with less amounts of antibodies in populations with mild symptoms. Most
12
13 113 notably, it is still unknown to what extent detectable antibodies imply immunity¹¹.

14 114 **RATIONALE**

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16
17 115 The acquired immunity to SARS-CoV-2 against reinfection still needs to be determined.
18
19 116 Serological assays are required to measure the presence of SARS-CoV-2 antibodies and its
20
21 117 correlation with immune protection. The COVID-SER project aims to assess the performances of
22
23 118 different serological kits aimed at detecting anti-SARS-CoV-2 antibodies and their neutralizing
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25 119 capacity. The ultimate goal is to identify the kits with higher performances in the detection of
26
27 120 IgM and IgG antibodies to measure the prevalence of SARS-CoV-2 infection and immunization
28
29 121 in the population. The detection of early IgM antibodies could be complementary to the PCR test
30
31 122 to diagnose the infection. It could allow a broader screening of symptomatic subjects. The later
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33 123 but sustained production of IgG antibodies could enable to determine the immune protection of
34
35 124 individuals against SARS-CoV-2.

36 125 **OBJECTIVES**

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38
39 126 The objectives to the COVID-SER project are (1) to assess the performances of different
40
41 127 serological kits to detect anti-SARS-CoV-2 antibodies in an infected healthcare workers
42
43 128 population; (2) to monitor the development of humoral response against SARS-CoV-2 infection
44
45 129 up to 36 months after diagnosis; (3) to evaluate the neutralizing capacity of the antibodies
46
47 130 produced (4); to evaluate the false negative rate of the PCR tests and (5) to assess the duration of
48
49 131 the presence of SARS-CoV-2 in the nasopharyngeal sample and its infectious potential.

50 132 **DELIVERABLES**

51
52
53 133 The assigned agenda is to determine the serological kits with the best performances to provide
54
55 134 the population with a reliable and rapid screening tool. The expected collective benefits are to be
56
57 135 able to better understand and manage the progression of the pandemic in the population.

136 METHODS AND ANALYSIS

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

140 Study population

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

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

156 Sampling schedule

157 At the first visit (V1), explanations will be given to the patient and the first sample will be taken.
158 Patients with a positive PCR result at V1 will come back for the following visits at day 7 (V2),
159 14 (V3), 21 (V4), 28 (V5), 35 (V6), 42 (V7), and at 6, 12, 18, 24 and 36 months (V8 to 12) for
160 serum samples. PCR tests will be performed at each visit until negative result. Patients with a
161 negative PCR result at V1 will come back only at day 28 (V5) for a serum sample. Sampling
162 schedule is illustrated in **Figure 1**.

163 Endpoints

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3 164 To address the objectives mentioned above, the primary endpoint will be to assess the
4
5 165 performances of the serological kits in the detection of anti-SARS-CoV-2 antibodies at the time
6
7 166 of the symptoms (V1) upon positive PCR results. This will be done through the evaluation of IgM
8
9 167 and IgG production kinetics in the infected population during the additional visits (V2 to V12) as
10
11 168 well as the seroneutralization capacity of the produced antibodies. The second endpoint will
12
13 169 evaluate the PCR false negative rate in symptomatic subjects (V1) with negative PCR results
14
15 170 compared to their serological response at V5.

171 **Biobanking**

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

182 **Serological tests**

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

184 - Antibodies titers assessment

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

187 - Virus neutralisation assay

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

1
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3 190 complement-linked reduction of the viral activity. The virus used in these experiments (RoBo
4
5 191 strain) will be a clinical strain isolated on VERO-E6 cells from a patient hospitalized at the
6
7 192 University Hospital of Saint-Etienne for severe COVID-19 infection; it will be diluted in the same
8
9 193 medium so that to obtain 100 to 500 tissue culture infectious doses 50% (TCID50) per 150 µl.
10
11 194 Virus infectivity controls will be included in each test. Serial two-fold dilutions (tested in
12
13 195 duplicate) of the specimens will be mixed with the diluted virus at equal volume (100 µl each).
14
15 196 After gentle shaking and a contact of 30 minutes at room temperature in plastic microplates, 150
16
17 197 µl of the mixt will be transferred to 96-well microplates covered with Vero-E6 cells. The plates
18
19 198 will be placed at 37°C in a 5% CO2 incubator. The reading will be evaluated microscopically 5
20
21 199 to 6 days later when the cytopathic effect of the virus control reaches 100 TCID50/150 µl. A
22
23 200 seroprotection will be recorded if more than 50% of the cells are preserved. The protection titer
24
25 201 will be expressed as the inverse of the higher serum dilution that spared the cells. The threshold
26
27 202 of positivity for protective antibodies will be 10. All the experiments will be performed in an L3
28
29 203 facility.

204 **Sample size and data analysis plan**

205 - Study design and sample size

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

215 - Statistical methods

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3 216 Characteristics of the positive and negative PCR samples will be described and quantified as
4
5 217 median (IQR). To evaluate the primary endpoint, sensitivity of the different serological tests on
6
7 218 the infected population will be assessed according to the threshold established by the
8
9 219 manufacturer's instructions, with a confidence interval (CI) obtained by the Wilson method.
10
11 220 Specificity of the serological tests will be assessed by the same method on the healthy serum
12
13 221 samples. Sensitivity and specificity of the different serological kits will be compared using Mc
14
15 222 Nemar test. Sensitivity of the best performing kits will be modelled through logistic regression to
16
17 223 quantify the delay on the quantification of antibodies from the beginning of the symptoms or the
18
19 224 exposition to specific therapy. Factors acting on the sensibility will be quantified as odds ratio
20
21 225 (CI=95%). To evaluate the evolution of the antibody's production (IgM and IgG) a mix-effects
22
23 226 linear regression will be modelled. Antibodies production will be assessed by optical density
24
25 227 ratios determined according to manufacturer recommendation. Analyses will be conducted with
26
27 228 the latest version of "R" software.

229 **Ethics and dissemination**

230 - Ethics approval

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

236 - Informed consent

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

1
2
3 242 subject. A comprehensive notice will be distributed to the subject summarising the protocol and
4
5 243 the follow-up of the study. Participants will be informed at the time of inclusion in the study that
6
7 244 the interpretation of results is limited by the current state of knowledge, and that a positive
8
9 245 serological test does not mean that they are immune to the virus.
10

11
12 246 - Dissemination
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15 247 Results will be communicated at scientific meetings and submitted for publication in peer-
16
17 248 reviewed journals.
18

19 249 **Safety of participants**
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21 250 This study includes no serious foreseeable risk to the health of the subjects involved. The only
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23 251 potential risk is related to blood sample collection (maximum 212 mL collected over all time
24
25 252 points — 6 months). However, this aspect of nursing is part of daily practice. Blood samples will
26
27 253 be taken under the same conditions of safety as currently used for common diagnostic tests.
28

29 254 **Patient and public involvement**
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31 255 No patient was involved in the design or implementation of this study. Study participants will be
32
33 256 individually informed about their results during scheduled medical visits and will be given access,
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35 257 on demand, to the final publication of the study results.
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25 305 **Authors' contribution**

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27 306 ST-A, AB, J-AN, PF, AP, AM-P, CA, NG, VP, ML, AB, MD, CS, MR, MAT, FG and J-BF were

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29 307 involved in trial design and ST-A, CA-V, AB and J-BF were involved in the drafting of the

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31 308 manuscript. All authors were involved in critical revision of the article for important intellectual

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33 309 content and approved the final version.

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35 310 **Competing interests**

36
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39 312 work and research not related to this manuscript. The other authors declare no conflict of interest.

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316 TABLES

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

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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 COVID-19 IgG/IgM (RT)	LFIA	Qualitative
BIOSYNEX	none	BIOSYNEX COVID-19 BSS (IgG/IgM)	LFIA	Qualitative
SD BIOSENSOR	none	STANDARD Q COVID-19 IgM/IgG combo	LFIA	Qualitative

319 CMIA: Chemiluminescence Microparticule luminescence ImmunoAssay

320 CLIA: Chemiluminescence Luminescence ImmunoAssay

321 ELFA: Enzyme Linked Fluorescence Assay

322 LFIA: Lateral Flow Immunochromatographic Assay

323 RLU: Relative Light Unit

324 RFV: Relative Fluorescence Value

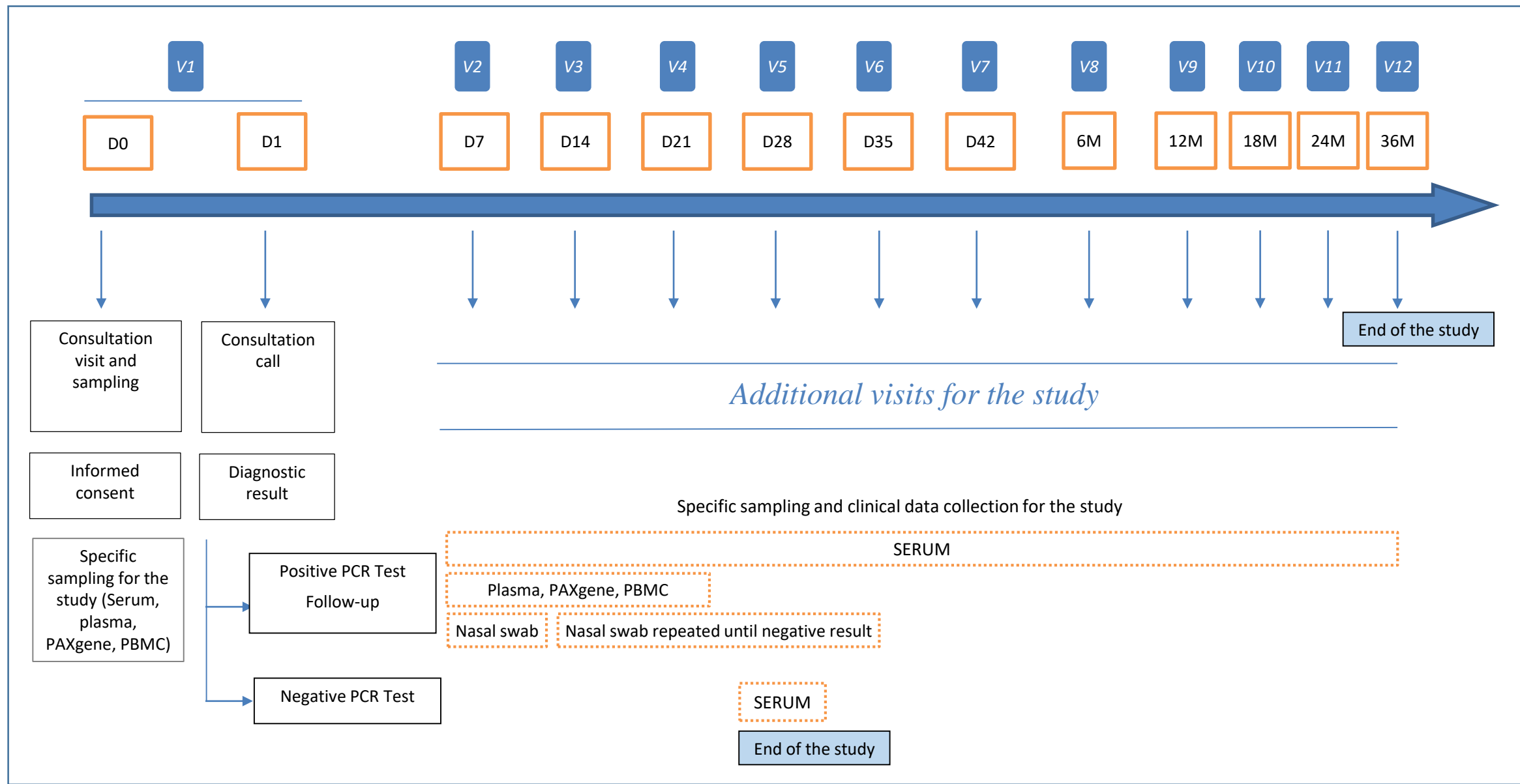
325 OD: Optical density

326 AU: Arbitrary Unit

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3 327 **FIGURE LEGEND**
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5 328 **Figure 1.** Schematic design of the COVID-SER project illustrating the various time-points of the
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7 329 study and the type of collected sample at each visit.
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