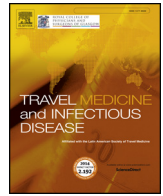




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Testing the repatriated for SARS-Cov2: Should laboratory-based quarantine replace traditional quarantine?

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ABSTRACT

Background: An ongoing epidemic of respiratory diseases caused by a novel coronavirus (COVID 2019, SARS-CoV2) started in Wuhan, Hubei, in China at the end of December 2019. The French government decided to repatriate the 337 French nationals living in Wuhan and place them in quarantine in their home country. We decided to test them all for SARS-Cov2 twice in order to reduce anxiety among the population and decision-makers.

Methods: We investigated the presence of SARS-CoV-19 in asymptomatic carriers by testing all repatriated patients within the first 24 h of their arrival in France and at day 5. Viral RNA was extracted from pooled nasal and oropharyngeal swab fluids or sputum in the absence of nasal/oropharyngeal swabs. Detection of SARS-CoV-2 RNA was then carried out using several real-time reverse transcription (RT)-PCR assays.

Results: We tested 337 passengers at day 0 and day 5. All the tests for SARS-CoV2 were negative. By optimising the sampling process, sending samples sequentially and reducing the time-scale for biological analysis, we were able to test the samples within 5 h (including sampling, shipment and biological tests).

Conclusion: Optimising our procedures reduces anxiety and reassures the population and decision makers.

1. Introduction

An ongoing epidemic of respiratory diseases caused by a novel coronavirus started in Wuhan in China at the end of December 2019 [1–3]. To date, more than 89,000 cases and more than 3000 deaths have been reported across China due to SARS-Cov2, mostly in the region of Hubei (WHO). To date, two hundred cases have been exported from China to 65 Countries [4]. COVID-19 has been notably extended to three countries outside China; Iran, Italy and South Korea. The concept of quarantine goes back to the plague outbreak in 1377, and was initially empirically set at 30 days for ships and 40 days for land travellers [5]. Quarantine times have recently been adjusted to reflect the known incubation period of the disease. Based upon preliminary reports on COVID-19 [6], the quarantine time was established at 14 days. At the end of January 2020, the French government decided to

repatriate French nationals living in Wuhan and place them in quarantine in their home country. In the context of European collaboration, all European nationals living in Wuhan who wanted to be repatriated to their home countries were invited to join the French group. The availability of the country's largest military airport, an empty holiday centre, and proximity to the largest referral centre for preparedness and care of highly infectious diseases in France (the Institut Hospitalo-Universitaire Méditerranée Infection, IHUMI) made the area of Marseille appropriate for this quarantine procedure (Fig. 1). In such epidemics the most important thing is to manage anxiety, as this in itself can have a significant effect. With this aim, we investigated the presence of SARS-CoV-19 asymptomatic carriers by testing all repatriated individuals for this virus within the first 24 h of their arrival in France and at day 5. Considering the duration of repatriation travel and the quarantine that began in China 2–7 days prior travelling back for all the

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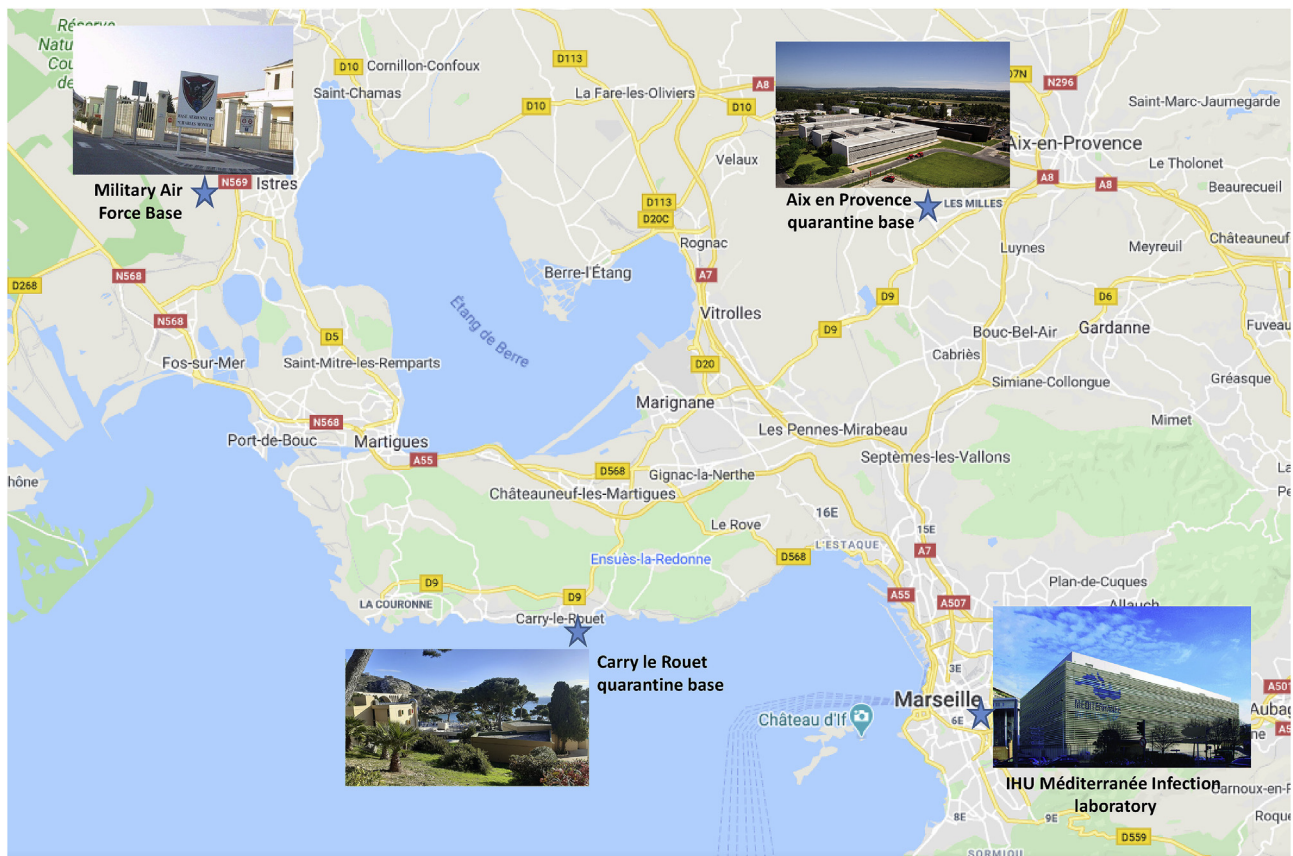


Fig. 1. Location of operational quarantine. Upper left the military airport, bottom left the holiday centre, upper right the firefighters' training school and bottom right, the Institute Méditerranée Infection.

individuals, this strategy seemed to be adapted to detect potential carriers.

2. Material and methods

All French nationals and all foreign nationals who were repatriated, including children of all ages, who were willing to be tested and provided their written consent after being orally informed were included in the study. We collected one nasal and one oropharyngeal swab and one sputum sample from each individual. For children under the age of three, a nasal rinse was performed. A team consisting of between two and seven infectious disease specialists was sent to the two quarantine bases to test asymptomatic persons. When an individual became symptomatic, they were transferred from the base to our Institute and cared for in the BSL3 ward while awaiting the results of the SARS-CoV-19 diagnosis. Once SARS-CoV-19 had been ruled out, the person was returned to the quarantine base. Individuals who presented respiratory symptoms during the flight were sampled at the airport, and were then referred to their quarantine base if testing for SARS-CoV-19 was negative.

2.1. Laboratory testing

Viral RNA was extracted from 200 μ L of pooled nasal and oropharyngeal swab fluids or sputum in the absence of nasal/oropharyngeal swabs, using the QIAamp Viral RNA Mini Kit (Qiagen, Courtaboeuf, France) on the QIAcube automated nucleic acid purifier (QIAGEN). Detection of SARS-CoV-2 RNA was then performed by several real-time reverse transcription (RT)-PCR assays, as shown in Fig. 2 [7]. We used two different real-time RT-PCR systems with a hydrolysis probe and the LightCycler Multiplex RNA Virus Master kit (Roche

Diagnostics, Mannheim, Germany). The first system targets the envelope protein (E)-encoding gene and was recently described [7]; it was used with a synthetic RNA positive control corresponding to the target gene that was supplied by the Charité Virology Institute-Universitätsmedizin Berlin, Berlin, Germany (<https://www.european-virus-archive.com/>). The second system (SpikeP_ps80) targets the spike protein-encoding gene and was designed in-house based on the first SARS-CoV-2 genome available (GenBank Accession no. MN908947; sense primer: 5'-AAACTTGTGCCCTTTTGGTG-3'; antisense primer: 5'-TGCTGATTCTTCCCTGTTCC-3'; probe: 5'-CGCCACCAGATTGCATCTG-3'). It has been used since 6 February, with a synthetic RNA positive control (5'-ATCTATTGTTAGATTTCCTAATATTACAACTTGTGCCCTTTGGTGAAGTTTTAAAGCCACCAGATTGCATCTGTTATGATTATAGCGGCCGCTTACTTATGCTTGGAAACAGGAAGAGAATCAGCAACTGTGTTGCTGATTATTCTGT-3') ordered from Eurogentec (Seraing, Belgium). In addition, a real-time RT-PCR was concomitantly carried out with the QuantiNova SYBR Green RT-PCR kit (Qiagen) which targeted either the E gene with the same primers as above or with previously described primers targeting the RNA-dependent RNA polymerase (RdRp) encoding gene [7] with a positive control consisting in a synthetic RNA (5'-TGAGTGTGCTCAAGTATTGAGTAAATGGTCATGTGTGGCGTTCACTATATGTTAAACCAGGTGGAACCTCATCAGGAGATGCCACattatagcgccgcttattaAACTGCTTATGCTAATAGTGTTTTTAACATTTGTCAAGCTGTCACGGCCAATG-3') (Eurogentec). A phage RNA was used as internal control [8]. Tests were performed on LightCycler 480 instruments (Roche Diagnostics).

All these tests were performed by between three and six trained, qualified technicians who were available 24 h a day, seven days a week, either during routine working days or on an on-call basis. In the event of respiratory symptoms, a multiplex molecular assay was concomitantly carried out at our point-of-care laboratory [9] using the

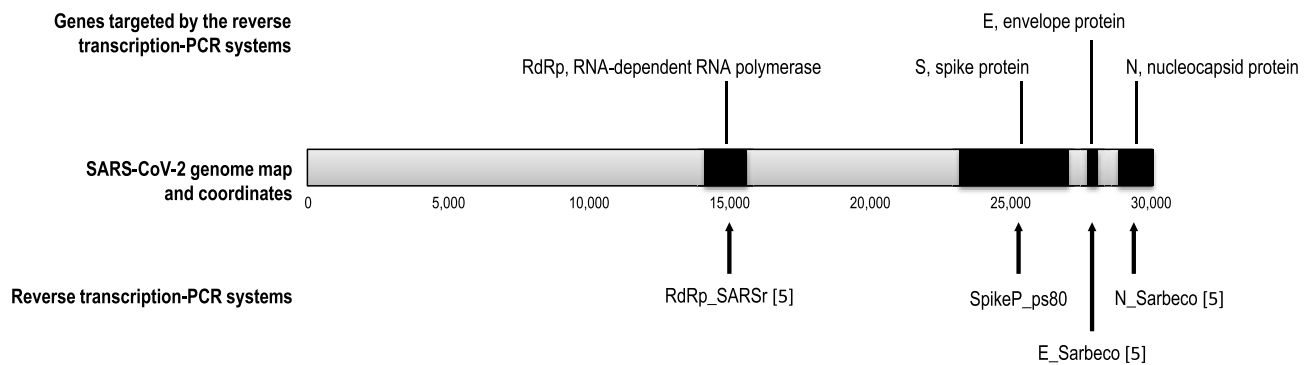


Fig. 2. Reverse transcription-PCR systems for the detection of SARS-CoV-2 RNA from respiratory samples.

Biofire FilmArray Respiratory panel 2 test that detects 17 viral and bacterial respiratory pathogens (Biomérieux, Marcy-l'Etoile, France).

2.2. Ethics

This research was approved by the ethics board of the Committee for the Protection of Persons (CPP Île de France VI dated 06-02-2020). The names of repatriated individuals were anonymised.

3. Results

Three flights from Wuhan landed at the Istres military air force base carrying a total of 337 passengers, including 178 on the first flight, 124 (31 of which were other European nationals) on the second flight, and 35 on the third flight. Among 337 repatriates, 170 were male (50.4%), and their mean age was 31 years (ranging 0–75 years). Unfortunately, because of time constraint, we were not able to document duration of expatriation and professional status of participants. Among the passengers from the first flight, two became symptomatic in the following days. One was diagnosed with rhinitis with a rhinovirus, and the other had consolidated pneumonia without microbiological documentation. Once discharged from the BSL3 unit, these patients were returned to their quarantine base. Of the 178 passengers on the first flight, 172 asymptomatic repatriated individuals were tested at day 1 (D1) and all were negative; two refused sampling and four were not found. Of the 178 passengers present in the quarantine base on day 5, 173 tested negative for SARS-CoV-2; one refused sampling, four were not found but had tested negative on D1. Of the 254 passengers on the second flight, 124 were transferred from the Wuhan airplane to airplanes chartered by European countries, including Belgium, one national of which was later diagnosed in Belgium as being positive. These passengers were not tested for SARS-CoV-2 at the airport but we retrospectively tested people who had been in close contact with the Belgian patient, who were all negative. For 20 symptomatic passengers who were sampled at the airport, all SARS-CoV-2 tests were negative. Finally, 35 passengers landed with the third flight. No symptomatic cases were detected during the flight and 35 and 33 of the tested repatriates were negative at D1 and D5 respectively (two refused the test at D5). Finally, of the total number of 337 repatriated individuals, 23 were symptomatic, 336 were tested at least once, one refused to be tested, 328 were tested twice at D1 and D5, and all were negative for SARS-CoV-2.

The time between the arrival of respiratory samples at the laboratory to the results of SARS-CoV-2 PCR tests varied between the first batch and later batches of tests. For the first flight, samples for all 124 repatriated individuals were received at 8pm, and PCR results were communicated at 12.50am and 2.40am for the first batch of 54 samples and the second batch of 69 samples, respectively. Time-to-results was therefore 4 h and 50 min (290 min) and 6 h and forty (400 min), respectively. For the second flight, samples were received in two different

series, which made them easier to manage. In addition, we optimised the testing strategy by prioritising the extraction of RNA from the samples, rather than performing a complete registration of all samples in our laboratory computer system then preparing all aliquots from the samples including for preparation of the biobank. Thus, a first series of 20 samples was received at 6.20pm and the results were communicated at 9.10pm, 2 h and 50 min later (170 min), while a second series of 75 samples was received at 8.25pm and the results were available à 11.30pm, 2 h and 55 min later (175 min). The timescale for obtaining the PCR results was similar for the third flight and for the retests at day 5, as results were available approximately 3 h after samples were received at our laboratory. The timetable is presented in Fig. 3.

4. Discussion

Our study presents some limitations mostly because of the shortness of time to organize the sampling. The arrival time of the flights was only known a few hours before landing. The experience of our team regarding point-of care testing helped in designing this study [9]. Quarantine of expatriates was decided by the French government as other European country governments. However, although sampling was not invasive (swabbing), our team proposed to give individuals the choice to get tested or not. Interestingly, the acceptability rate was excellent and the vast majority of individuals accepted to be tested (336 on the 337 individuals were tested at least once).

In such situations, anxiety, often enhanced by the media, distorts how we think about new diseases, and when it comes to making decisions that involve risks, humans can be irrational in quite systematic ways [10]. Consequently, reducing anxiety through rapid diagnosis should improve decision making and crisis management. For example, since 5 February, more than 700 confirmed cases have been reported from the 3700 passengers on board the Diamond Princess cruise ship [11]. The anxiety on board and the difficulties in managing the quarantine are easy to imagine. Testing all passengers and crew after having diagnosed the index case and releasing SARS-Cov2 negative carriers from quarantine would likely have avoided supplementary cases. In outbreaks of disease, diagnosis and the time it takes is critical for patient management and establishing quarantine. Returning negative results to the individuals in question, the physician in charge, and decision makers is obviously important. Reducing the time scale to a matter of hours, which is now possible with molecular diagnosis, will provide us with a much easier way of managing crises.

The remaining question is whether repeated negative test results are sufficient to shorten the quarantine period. In the future, such strategies could usefully applied to other viruses or bacteria for patients returning from overseas travel for symptomatic or asymptomatic individuals [12].

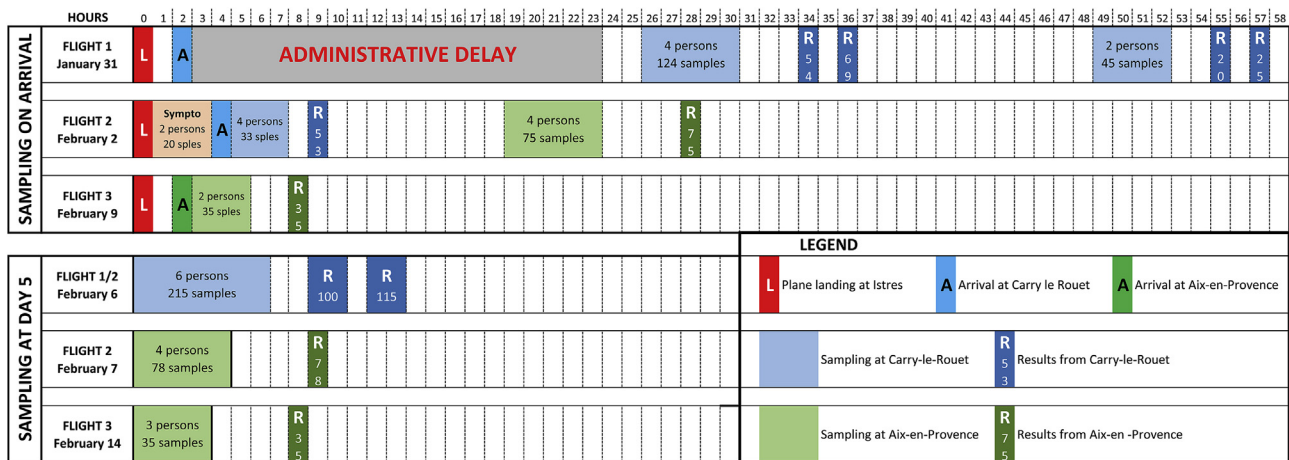


Fig. 3. The timetable from the aircraft landing (L) to analysis reports (R) for sample D1 and D5. In pink, the symptomatic people. Administrative timescale refers to the time spent writing the protocol and obtaining an exemption from the national health authorities to begin the study. Persons refer to infectious disease doctors for examinations and sampling. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

CRedit authorship contribution statement

Jean Christophe Lagier: Investigation, Methodology, Writing - original draft. **Philippe Colson:** Investigation, Methodology, Writing - original draft. **Hervé Tissot Dupont:** Investigation. **Jérôme Salomon:** Writing - review & editing. **Barbara Doudier:** Investigation. **Camille Aubry:** Investigation. **Frédérique Gouriet:** Investigation. **Sophie Baron:** Investigation. **Pierre Dudouet:** Investigation. **Rémi Flores:** Investigation. **Lucie Ailhaud:** Investigation. **Philippe Gautret:** Writing - review & editing. **Philippe Parola:** Writing - review & editing. **Bernard La Scola:** Investigation. **Didier Raoult:** Conceptualization, Methodology, Writing - review & editing. **Philippe Brouqui:** Conceptualization, Methodology, Investigation, Writing - original draft.

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manuscript.

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