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How should a positive PCR test result for COVID-19 in an asymptomatic individual be interpreted and managed?\textsuperscript{\dag}\textsuperscript{*}

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1. Introduction

In December 2019 in Wuhan, China an unexplained outbreak of respiratory distress syndrome occurred. The causal agent was identified as SARS-CoV-2, a new beta coronavirus responsible for COVID-19, which brought about not only severe infections in approximately 20% of cases, but also asymptomatic infections in a still insufficiently known proportion of patients. The emerging disease rapidly evolved into a worldwide pandemic, and Europe was chronologically the second most affected continent. Thanks to public health measures involving not only confinement of the French population but also the ever-widening organization of diagnostic testing, by June 2020 COVID incidence had markedly decreased.

Diagnosis during the acute phase of COVID is based on real-time quantitative PCR nasopharyngeal swab testing. Widened access to diagnosis by RT-PCR has entailed validation by the national center of reference of several commercial or in-house kits focusing on multiple targets and exhibiting variable performance. In this context, molecular biology tests facilitate identification of viral RNA, which does not necessarily foreshadow the presence of potentially infectious viruses. Moreover, in some health care establishments PCR-based testing has been extended to systematic screening on admission in view of counteracting viral spread and of monitoring viral excretions prior to the lifting of additional precautionary measures. Given the large number of PCR tests involving more and more asymptomatic patients in a fluctuating epidemic context and given relatively low incidence in present-day France, numerous questions have been put forward on how to interpret molecular biology tests, particularly in asymptomatic persons and with regard to risk of transmission/infectiveness.

We have consequently carried out a review of the literature at our disposal on 10 August 2020 so as to address the different questions underlying interpretation of positive PCR-based SARS-CoV-2 test results in an asymptomatic individual, the objective being to propose an algorithm suited to management of these persons and in accordance with the relevant scientific data. In the absence of official recommendations, the review was initiated before publication on 8 July 2020 of the opinion of the Haut Conseil de la Santé Publique (HCSP) (French public health council)\textsuperscript{[1]} pertaining to the course of action to be taken according to SARS-CoV-2 virological status in the framework of screening or contact tracing. The review is aimed at providing supplementary elements, especially as regards the interest of semi-quantitative PCR assay and the limited interest of serology when estimating the risk of contagiousness in this type of evolving situation.

2. Can PCR-based SARS-CoV-2 testing lead to false positives?

While the issue of false negatives has been widely taken up in the scientific literature, data on the possibility of false positives are very scarce. That said, a German study has highlighted false positives associated with commercial reagents used for PCR (probes or oligonucleotides), with intensity ranging from low to high\textsuperscript{[2]}. Similar incidents have occurred in several European laboratories\textsuperscript{[3]}. In comparison with an in-house PCR test of reference, the conclusion following assessment of a commercial Australian PCR test was that false positives were indeed possible\textsuperscript{[4]}. Even though their overall likelihood appears low, a multitude of tests during a period of relatively low incidence could lead to a situation in which half of the tests are false positives, particularly in the event of flawed
internal and external quality controls [2], as was recently illustrated by an upsurge of false positives in a French department (Meurthe et Moselle) that was associated with contamination of one of the reagents by SARS-CoV-2 RNA [5].

### Proposal

While the eventuality of a false positive cannot be totally excluded, no routinely applicable biological modality is presently able to pragmatically sort matters out in the face of positive PCR-based SARS-CoV-2 test results. That is the reason why any positive PCR-based SARS-CoV-2 test result must be managed as through it were a true and proven positive.

### 3. For how long do the results of PCR-based SARS-CoV-2 testing remain positive?

Out of 155 American patients having undergone a PCR-based control subsequent to an initial positive finding, 88% remained positive 1 to 5 days later, and 56% out of the 105 patients tested afterwards remained positive at 21–25 days [6]. Median PCR negativity time in the 56 patients of the Chinese series by Xiao et al. was 24 days [7]. In Wuhan, 36/378 (9.5%) of patients having tested positive remained positive 30 days later, and in some cases their positivity lasted as long as 83 days [8]. The more severe the clinical presentation, the longer the duration of positivity, with a median of 31 days in a Chinese study involving 41 serious cases [9]. In a Singaporean study, 32% out of 766 COVID19 patients still remained positive at D21, 22% at D28 and 5% at D33 [10].

Some of the factors reported as being associated with persistently positive tests were delayed hospitalization, a severe form, mechanical ventilation or corticosteroid use, comorbidities such as diabetes and hypertension, male gender and advanced age [7,11]. The role of advanced age may have to do with the fact that more often than not, additional utilization of the PCR method constitutes a control preceding transfer to another medicalized structure [6].

### Proposal

It can be estimated that roughly 10 to 30% of persons with positive PCR test results will still have positive PCR results a month later, especially to the extent that their initial viral inoculum level was high, that they were severely diseased and that they were elderly or immunosuppressed.

In the event of persistently positive PCR more than 3 months after initially positive PCR, in the current state of knowledge re-contamination cannot be excluded as a possibility (see question 5).

### 4. For how long can the positive results of a PCR-based SARS-CoV-2 test be associated with actual risk of transmission/of infectiousness in a symptomatic individual?

During the first week of symptoms at the level of the throat there exists an active viral replication of SARS-CoV-2, with maximum viral concentrations being reached during the 4th or 5th day of COVID19 symptoms, and more rapidly than in SARS cases [12]. No viable virus in sputum or at the nasopharyngeal level was found after the 7th day of sickness in a German study [12], after the 9th day in two American studies [13,14] and after the 11th day according to data from Singapore [10].

The above virological data are consistent with epidemiological observations showing that most transmissions occur at a very early stage of the disease [15], with an attack rate among contacts exposed during the first 5 symptomatic days markedly higher than in persons in contact after 5 days (2.4% vs 0% in the event of exposure after the 6th day) [16]. Along with models synthesized in two reviews [17,18] and in accordance with recently voiced HCSP opinions [1,19], the above-mentioned virological and epidemiological data allow transmission to be considered as possibly occurring during 10 days following symptom onset.

One potential marker of contagiousness seems to be the cycle threshold value (Ct), which is inversely correlated with quantity of viral RNA; each increase by a factor of 3.3 corresponds to an RNA rate ten times lower [20], and Yu et al. demonstrated a linear relationship between Ct and RNA rate in upper respiratory tract samples with Ct<34 [21]. In another study, no cultivable virus was found when the value exceeded 30 [10] or when RNA quantity was lower than 100,000 copies/mL [12]. In fact, the cycle threshold value depends on the genomic target of PCR [22,23]. When PCR-based SARS-CoV-2 amplifies gene E, the likelihood of finding a viable and cultivable virus declines, with a 32% reduction of odds ratio by increment of Ct unit, and once Ct exceeds 24, it becomes nil [14]. In the Marseille-based study authored by La Scola et al. (PCR targeting gene E), it was due to the development of subcultures permitting detection in some patients of small quantities of replicating viruses that Ct value reached 34 [24]. In another study, according to whether genes nsp12, E and N were amplified, the minimum quantities needed to highlight a viable virus were 5.4, 6 and 5.7 log10 copies/mL (Ct of 31.47, 31.46 and 35.2, respectively) [25]. It is consequently more likely to have a cultivable virus (and, as a result, a risk of transmissibility) when the PCR(s) target the whole genome, and not just one of the genes [25]. Indeed, the notion of genome integrity, which would limit cross-reactions with other coronaviruses, underscores the interest of having several genomic targets, especially in questionable situations [26,27]. If it is possible to have on-site access to PCR with two genomic targets (and without a sensitivity deficit identified by the laboratory), only when the two targets are detected will the PCR be confirmed as positive.

To conclude, even though the gold standard allowing for prediction of viral viability varies from one study to the next, it seems important to be cognizant of the Ct (or estimated quantity of RNA) and the number and nature of the targeted gene(s). It is thereby possible to apprise the risk of contamination of an asymptomatic individual with positive PCR and without any notion of previous infection. Given that more often than not, only one gene (gene E) is amplified, cycle threshold value (Ct) exceeding 35 seems associated with contagiousness risk neighboring zero; when Ct is higher than 30, the risk may be considered exceedingly low.

### Proposal

One may estimate, in agreement with the opinion voiced by the HCSP, that infectiveness persists during the first week of symptoms, following which it decreases rapidly, disappearing by the 10th day at the latest. There is consequently no risk of contagion 10 days after the symptoms first appear, even in the event of persistently positive PCR SARS-CoV-2 [1,19].

Before 10 days have elapsed, cognizance of the Ct can help apprise the degree of risk, which is nil when Ct exceeds 35.

That much said, its practical interest with regard to a symptomatic person is decidedly low.
5. Is a newly positive PCR-based PCR SARS-CoV-2 test result following one or more negative test results to be associated with a risk of transmission?

This question concerned as many as 19.8% of the persons having been tested positive for COVID in a study by Brunei [28] and as many as 21.4% in a Wuhan-based study [29]. As regards to the “Diamond Princess” cruise ship, 35% of passengers who had been hospitalized in a Tokyo hospital center were newly PCR SARS-CoV-2 positive after having tested negative at least one time [30].

In a Chinese study conducted in Hubei province, the 38 patients with newly positive PCR results were relatively young and had presented with a slightly or moderately severe form of COVID19 [31]. None of them presented new signs of COVID and none of their 21 close contacts had been contaminated. In a larger, Korean study [32], 285 “repositized” persons were successfully identified (following a mean time interval of 44.9 days), and 44.2% were symptomatic. Among these patients, 108 (with mean real-time PCR Ct values exceeding 30 in 89.5% of cases) underwent viral sample collection, without any viable virus being isolated. Moreover, only 3 out of the 790 identified contact persons presented with positive PCR, which was in all likelihood due to previous exposure, once again without any manifestation of a viable virus, and with neutralizing antibodies being found in 96% of the patients tested. Similar observations were put forward in the Brunei study, where the “repositized” persons presented with Ct superior to Ct at the time of diagnosis, and out of 111 tested contact persons, not a single one was contaminated, a finding underlining the non-contagiousness of individuals with “repositized” PCR [28].

Moreover, clinical and biological data from SARS-CoV-1 [33] and SARS-CoV-2 [34] infections and data from animal models [35] tend to demonstrate that there is a form of protective immunity subsequent to COVID-19, even if it is not presently possible to determine whether it develops in all infected persons and whether remains enduring. A recent French study highlighted the presence of neutralizing antibodies, 28 to 41 days after the appearance of symptoms, in 98% of the caregivers having suffered from moderate forms of the disease [36]. Along with the fact that very few cases of potential new infection have been reported in the literature so far, these findings tend to show that at least in the short and middle term, the potentiality of a new infection remains relatively remote.

However, the above-mentioned humoral immune response seems limited in time, given the diminution of IgG and neutralizing antibodies 2 to 3 months following infection, particularly in pauci-symptomatic or asymptomatic forms [37]. This was previously reported for other coronaviruses, with reinfection being deemed possible with regard to 3 out of 4 benign human coronaviruses [38]. Repositized PCR several months after an uneventful time interval consequently raises the possibility of recontamination, all the more so when it is associated with suggestive symptoms [1]. In that respect, a three-month time interval has been adopted by the Canadian authorities, who have decided, in relevant cases, to propose supplementary investigations (viral culture, phylogenetic analyses, search for neutralizing antibodies...). [39].

6. Is a positive PCR-based PCR SARS-CoV-2 test result to be associated with a genuine risk of transmission of infectiveness in a person never having previously presented with known COVID?

Setting aside the improbable issue of potential false positives, an asymptomatic individual can potentially be:

- a person who has been asymptomatic with an infection several days or weeks previous, which went unnoticed.

In the hypothetical event of a pre-symptomatic person, the median incubation period approximates 5 days (CI95%, 4.5 to 5.8 days), with 97.5% of the persons developing symptoms within fewer than 10 days [13,40]. The contagiousness period begins 2 to 4 days before the symptoms first appear, and is likely to peak 0.7 days before [17,41].

While a Singaporean study did not show transmission from persons remaining asymptomatic [42], it nonetheless remains highly probable that cases of secondary contamination originating in an asymptomatic individual (who has remained asymptomatic) have occurred in the USA [13], in China [43,44] and in Germany [45]. Asymptomatic patients may present a positive PCR SARS-CoV-2 dynamic comparable to that presented by other infected persons [46], with Ct not differing from Ct observed in pre-symptomatic or symptomatic patients [13,47–49], and even in some cases with earlier Ct reduction during asymptomatic infections [50]. On the other hand, immunological response appears less pronounced with lower IgG rates, lower neutralizing antibodies rates [47], and a lower seroconversion rate (1 patient out of 5) [46] than in symptomatic persons.

There exist no data on correlation between Ct and RNA quantity and likelihood of a viable and cultivable virus in asymptomatic individuals.

Proposal

In the absence of data on Ct value (or on estimated viral RNA quantity), any and every asymptomatic person with positive PCR and without preliminary notion of infection must be considered as being potentially at risk, a consideration leading to preventive measures equivalent to those taken with regard to a symptomatic person.

On the other hand, based on present-day knowledge of symptomatic persons, it would probably be of interest to be apprised of the Ct value of the PCR as well as the number of sought-out and positive genes. A value exceeding 35 and/or the positivity of only a single gene could lead to interpretation of the PCR result as probably corresponding to excretion of residual RNA at a time exterior to the period of contagiousness, which would mean that in this case, reinforced preventive measures need not be applied.

While Ct value is known in laboratories, it is not systematically indicated in the reported results. Ideally, however, it should appear in the latter, along with an indication of the targeted gene(s), in addition to the simple indication of positivity.

In some cases, the above considerations should lead to shortening or lightening of reinforced confinement measures, especially when, in addition to the collective elements to be taken into account, the patient’s risk/benefit balance legitimates the shortening or lightening.
7. Does the presence of antibodies detected by serology attest to control of the infection and consequently to non-contagiousness, even in the event of positive PCR-based SARS-CoV-2 test results?

The virus is not suddenly eliminated at the time of seroconversion [12], which at the outset of the 2nd week corresponds to a slow but sustained reduction of the viral load contained in sputum [12]. Viable viruses have in fact been observed following detection of anti-SARS-CoV-2 antibodies [12,51].

Contrary to what might seem to be suggested in the 8 July 2020 opinion [19], serology has no impact on either PCR interpretation or short-term management of the persons concerned. And yet, even though a distinction is drawn in the text between asymptomatic positive PCR cases according to whether or not serological test results are positive, in agreement with our proposal this does not lead in clinical practice to any difference in management (see technical sheet #1 of the opinion). As discussed in point 5, it is the existence of proven past COVID-19 infection that conditions the need (or the absence of need) for a patient to be isolated.

8. Synthesis of the practical proposals

Synthesis of the practical proposals:

- while the eventualty of a false positive cannot be totally excluded, no routinely applicable biological modality is presently able to pragmatically sort matters out in the face of a positive PCR-based SARS-CoV-2 test result. That is the reason why any and every positive PCR-based SARS-CoV-2 test result must be managed as though it were a true and proven positive;

- it can be estimated that roughly 10 to 30% of persons with positive PCR test results will still have positive PCR results a month later, especially to the extent that their initial viral inoculum level was high, that they were severely diseased, and/or that they were elderly or immunosuppressed;

- one may estimate that infectiveness persists during the first week of symptoms, following which it decreases rapidly, disappearing by the 10th day at the latest, even in the event of persistently positive PCR-based SARS-CoV-2. Before 10 days have elapsed, cognizance of the Ct can help to apprise the degree of risk, which is nil when Ct exceeds 35; that much said, its practical interest with regard to a symptomatic person is decidedly low;

- detection of positive PCR following a period of transitory negativation is a frequently encountered situation that should lead to application of the same recommendations as those implemented for symptomatic persons maintaining positive PCR in the middle term, knowing that there is no risk of contagion 10 days after the initial appearance of symptoms;

- that said, once 3 months have elapsed since initially positive PCR, in the present-day state of knowledge the possibility of recontamination in the event of newly positive PCR cannot be completely excluded;

- any and every asymptomatic person with positive PCR and without preliminary notion of infection must be considered as being potentially at risk, a consideration leading to preventive measures equivalent to those taken with regard to a symptomatic person. On the other hand, based on present-day knowledge of symptomatic persons, it would probably be of interest to be apprised of the Ct value of the PCR as well as the number of sought-out and positive genes. A value exceeding 35 and/or the positivity of only a single gene could lead to interpretation of the PCR result as probably corresponding to excretion of residual RNA at a time exterior to the period of contagiousness, which would mean that in this case, reinforced preventive measures need not be applied;

- while the Ct value is known by laboratories, it is not systematically indicated in the reported results. Ideally, however, it should appear in the latter, along with an indication of the targeted gene(s), in addition to the simple indication of positivity;

- on the other hand, in the event of positive PCR devoid of any notion of previous or associated symptoms, a serological test will have no impact on either PCR interpretation or short-term management of the persons concerned, regardless
of whether the serology is negative, possibly corresponding to a pre-symptomatic phase, or positive, that is to say excluding the potential presence of a viable virus at the time of the seroconversion;
• in some cases, the above considerations should lead to shortening or lightening of reinforced confinement measures, especially when, in addition to the collective elements to be taken into account, the patient’s risk/benefit balance justifies the shortening or lightening.

The above responses to key questions have led to our proposing an algorithm for management of asymptomatic persons presenting with positive PCR-based SARS-CoV-2 (Fig. 1). Our proposals will obviously be called upon to evolve in accordance with the advancement of knowledge and the data of the literature. They will also be liable to evolve according to the epidemiological context, and their evolution during a largely inactive epidemic will differ from that to be imagined in the event of renewed and dynamic circulation amidst a possibly insufficiently immunized population.

Human and animal rights

The authors declare that the work described has been carried out in accordance with the Declaration of Helsinki of the World Medical Association revised in 2013 for experiments involving humans as well as in accordance with the EU Directive 2010/63/EU for animal experiments.

Informed consent and patient details

The authors declare that this report does not contain any personal information that could lead to the identification of the patient(s) and/or volunteers.

Disclosure of interest

The authors declare that they have no competing interest.

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