

**Correlation between 3790 qPCR positives samples and positive cell cultures including 1941 SARS-CoV-2 isolates**

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Dear Editor -

Since the beginning of the outbreak of the emerging epidemic (Covid-19) due to SARS-CoV-2, declared as a pandemic on March 12<sup>th</sup> 2020 by the WHO [1], a major issue has been to correlate viral RNA load obtained after RT-PCR and expressed in cycle threshold (Ct) with contagiousness and therefore duration of eviction from contacts and discharge from specialized infectious disease wards. Several works published recently and based on more than 100 studies attempt to propose such cut off for Ct value and duration of eviction with a consensus at approximately Ct > 30 and at least 10 days, respectively [2–5]. However, in an article published in this journal, a group reported that patients could be not be contagious above 25 Ct as the virus was not detected in culture above this Ct [6]. This limit was then evoked in the French media during the interview with the member of the French Scientific Council Covid-19 as a possible value above which patients are no longer contagious [7]. At the beginning of the outbreak, we correlated the Ct values obtained by our PCR technique based on the amplification of the E gene and the results of the culture [8]. Since the beginning of the epidemic, we have performed in our institute 250,566 SARS-CoV-2 RT-PCR for 179,151 patients, of which 13,161 (7.3%) tested positive. Up to the end of May, 3 790 of these samples reported positives on naso-pharyngeal samples were inoculated and managed for culture as previously described [8]. Of these 3 790 inoculated samples, 1941 SARS-Cov-2 isolates could be obtained after the first inoculation or up to 2 blind subcultures. The correlation between the scanner values and the positivity of the culture allows us to observe that the image obtained with ten times more isolates than our preliminary work (1941 versus 129) does not change significantly (Figure 1). It can be observed that at Ct=25, up to 70% of patients remain positive in culture and that at Ct=30 this value drops to 20%. At Ct=35, the value we used to report positive result for PCR, less than 3% of culture are negative. Our Ct value of 35 initially based on the results obtained by RT-PCR on control negative samples in

our laboratory and initial results of cultures [8] is validated by the present work and is in correlation with what was proposed i.e. in Korea [9] or Taiwan [10]. We could observe that subcultures, especially the first one, allow increasing percentage of viral isolation on high Ct samples, confirming that these high Ct are mostly correlated with low viral loads. From our cohort, we now need to try to understand and define the duration and frequency of live virus shedding in patients on a case-by-case basis, in the rare cases where the PCR is positive beyond 10 days, often at a Ct above 30. In any cases, these rare cases should not impact public health decisions.

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## **Acknowledgments.**

**Ethical approval :** The protocol was approved by the ethical committee of the University Hospital Institute Méditerranée Infection (N°: 2020-01). All subjects provided informed consent in accordance with the Declaration of Helsinki.

**Funding:** This research was funded by the French Government under the “Investissements d’avenir” (Investments for the Future) program managed by the Agence Nationale de la Recherche (ANR, French National Agency for Research), (reference: Méditerranée Infection 10-IAHU-03), by Région Provence-Alpes-Côte d’Azur and European funding FEDER PRIM1.

**Conflict of Interest:** D.R. reports grants from Hitachi High-Tech Corporation, outside the submitted work. The others authors declare no conflict of interest.

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## References

1. WHO Director-General's opening remarks at the media briefing on COVID-19 - 11 March 2020. Available at: <https://www.who.int/dg/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19---11-march-2020>. Accessed 20 September 2020.
2. Jefferson T, Spencer E, Brassey J, Heneghan C. Viral cultures for COVID-19 infectivity assessment. Systematic review. medRxiv **2020**; :2020.08.04.20167932.
3. Rhee C, Kanjilal S, Baker M, Klompas M. Duration of SARS-CoV-2 Infectivity: When is it Safe to Discontinue Isolation? Clin Infect Dis **2020**; :ciaa1249.
4. Singanayagam A, Patel M, Charlett A, et al. Duration of infectiousness and correlation with RT-PCR cycle threshold values in cases of COVID-19, England, January to May 2020. Eurosurveillance **2020**; 25. Available at: <https://www.eurosurveillance.org/content/10.2807/1560-7917.ES.2020.25.32.2001483>. Accessed 20 September 2020.
5. Cevik M, Tate M, Lloyd O, Maraolo AE, Schafers J, Ho A. SARS-CoV-2, SARS-CoV-1 and MERS-CoV viral load dynamics, duration of viral shedding and infectiousness: a living systematic review and meta-analysis. Infectious Diseases (except HIV/AIDS), 2020. Available at: <http://medrxiv.org/lookup/doi/10.1101/2020.07.25.20162107>. Accessed 20 September 2020.
6. Bullard J, Dust K, Funk D, et al. Predicting Infectious Severe Acute Respiratory Syndrome Coronavirus 2 From Diagnostic Samples. Clin Infect Dis **2020**; :ciaa638.
7. Covid-19 : l'hypersensibilité des tests PCR, entre intox et vrai débat. Le Monde.fr. 2020; Available at: <https://www.lemonde.fr/les-decodeurs/article/2020/09/09/covid-19-1->

hypersensibilite-des-tests-pcr-entre-intox-et-vrai-debat\_6051528\_4355770.html. Accessed 20 September 2020.

8. La Scola B, Le Bideau M, Andreani J, et al. Viral RNA load as determined by cell culture as a management tool for discharge of SARS-CoV-2 patients from infectious disease wards. *Eur J Clin Microbiol Infect Dis* **2020**; 39:1059–1061.
9. Chang MC, Hur J, Park D. Interpreting the COVID-19 Test Results: a Guide for Physiatrists. *Am J Phys Med Rehabil* **2020**; Publish Ahead of Print. Available at: <https://journals.lww.com/10.1097/PHM.0000000000001471>. Accessed 20 September 2020.
10. Chen C-J, Hsieh L-L, Lin S-K, et al. Optimization of the CDC Protocol of Molecular Diagnosis of COVID-19 for Timely Diagnosis. *Diagnostics* **2020**; 10:333.

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**Figure 1.** Percentage of positive viral culture of SARS-CoV-2 PCR-positive nasopharyngeal samples from Covid-19 patients, according to Ct value (plain line). The dashed curve indicates the polynomial regression curve.

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Figure 1

