LETTER TO THE EDITOR

SARS-CoV2 virions in PM$_{10}$ pollutants. May further research reject this thesis?

Dear Editor,

The animated debate about the possibility that infecting SARS-CoV2 are widespread in open-air via particulate matter (PM), should need a thorough reappraisal. So far, purported SARS-CoV2 genomes were retrieved in PM$_{10}$ airborne pollutants in Italy, despite the consideration that these particles were never cell-cultured to ascertain an in vitro viral growth. Actually, some authors are describing the detection of possible SARS-CoV2 RNAs in PM$_{10}$, rather than complete infectious viral particles. If confirmed, the evidence will raise great concerns for COVID-19 epidemiology.

However, from a technical point of view, the ability to be particularly ensured about the existence of SARS-CoV2 RNAs, should account on the contemporary positive detection of three separated gene markers in a quantitative reverse-transcription polymerase chain reaction (RT-qPCR), as recommended by the World Health Organization (WHO) guidelines for the many challenged countries from pandemic, that is, genes E, N, and RdRp, as reported. Positive outcomes on one single gene marker, such as the E gene, usually linked to the lipidic envelope, which is frail in nonbiological microenvironments, may be biased, particularly if RT-PCR Co$_{0}$ occurs beyond the 34th amplification cycle, suggesting a false-positive result. The direct association of SARS-CoV2 with PM$_{10}$, that is, viral particles borne from PM$_{10}$ pollutants in urban areas, is a real concern for public health, therefore any researcher engaged with this study issue must be particularly ensured about its reliability.

Particulate matter, as airborne pollutants from urban and industrial wastes, may contain a huge deal of micro-organisms, including viruses and bacteria. Actually, a huge amount of unclassified bacterial, viral, and further micro-organisms can be found in industrialized urbanized areas, usually highly polluted by PM$_{10}$, when pollutant material is collected on quartz fiber filters. The majority of these organisms are still unidentified, so, their RNAs, are possible contaminants in a poorly stringent RT-qPCR approach. Table 1 summarizes some of the major microbical species found in particulate matter PM$_{10}$, here exposed only as a few exemplificative cohort of airborne pollutant micro-organisms, matching a sequence similarity with E primers sequences used in RT-qPCR for SARS-CoV2. The choice focused onto a ±70% sequence similarity to detect a minimal, biased primer/probe aspecific cycling. Highly specific forward primers for detecting and amplifying the SARS-CoV2 E gene and endowing researchers in diagnosing the presence of SARS-CoV2 viral particles, should give amplified products in a RT-qPCR around the 15–20th cycle, a way to be ensured that the sequence similarity is particularly high and close to 100%. Table 1 shows that many further coronaviruses can share a great sequence homology, in some genome regions, with primers usually employed in diagnostics. The same SARS-CoV1, for example, the strain Frankfurt 1 (GeneBank: AY291315.1), shares 100% similarity ($p = 2.2 \times 10^{-8}$), with maximum likelihood estimation (MLE statistics) giving $\lambda = 0.2558$, $K = 7.855$ (with 500 sequences) and a Waterman-Eggert score $= 130$, showing complete overlap (26 nt) at 26141–26166. This level is perfectly comparable with the Italian isolate SARS-CoV2 (INMI1/2020): similarity 100% ($p = 4 \times 10^{-9}$), MLE $\lambda = 0.2505$, $K = 7.235$, Waterman-Eggert score $= 130$, complete overlap $= 26213$–26238. The stringency in primer-template matching is only one of the crucial steps to warrant a correct identification and to claim the existence of a specified coronavirus strain in a sampled matrix. A high overlap, as occurring for some RNA molecule in Kineococcus radiotolerans SRS30216, for example, the 23S rRNA (Table 1), does not mean high genetic similarity but a significant percentage of aspecific PCR products carry over. Due to the wide diffusion of this bacterial species in PM$_{10}$ pollutants, it cannot be excluded that a Co$_{0}$ positivity over the 34th cycle using some recommended SARS-CoV2 E gene forward primers, may hide a false positivity bias. In this specific case, the use of three markers, that is, E, RdRp, and N, notably reduces similarity (from 68% with 25 nt overlap to 58.2% with 62 nt overlap).

Despite these issues could be highly questionable, on the technical perspective and from a clinical point of view, Table 1 exemplifies the possibility that further micro-organisms’ derived RNAs may be biased for SARS-CoV2 genomes, due to a similarity higher than 95% and close to 100% with long strings of primer sequences. As indicated, one possible “cut off” for the achievement of a proper decision-making is to cultivate PM$_{10}$-associated biological matrices onto living substrates, such as Vero or LLC-MK2 cell lines.

The evidence that PM$_{10}$ can bear SARS-CoV2 RNAs has been erroneously translated by nonscientific and popular information as the ability of infectious SARS-CoV2 particles to colonize PM$_{10}$ and to widespread wherever people lives. Naked RNAs from SARS-CoV2 are very difficult to be able to infect humans but, furthermore, more insightful clues are needed to be sure that PM$_{10}$ really bear COVID-10 causative agents.

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.
| Identification                                    | Genebank      | Similarity | Overlap       | Nucleotide sequence         | Score                                      |
|--------------------------------------------------|---------------|------------|---------------|-----------------------------|--------------------------------------------|
| **Viruses**                                      |               |            |               |                             |                                            |
| Bat SARS CoV Rs672/2006, complete genome         | FJ88686.1     | 100%       | 26 nt overlap | 1–26:25502–25527            | Waterman-Eggert score: 130; 44.8 bits; E(1) < 2.5 × 10⁻⁸ |
| Bat coronavirus 16B0133, complete genome         | KY38558.1     | 100%       | 26 nt overlap | 1–26:25493–25518            | Waterman-Eggert score: 130; 45.0 bits; E(1) < 2.2 × 10⁻⁸ |
| Bat coronavirus isolate Anlong-103 complete genome | KY770858.1   | 100%       | 26 nt overlap | 1–26:26065–26090            | Waterman-Eggert score: 130; 45.7 bits; E(1) < 1.4 × 10⁻⁸ |
| Bat SARS coronavirus Rm1, complete genome        | DQ412043.1    | 100%       | 26 nt overlap | 1–26:26114–26139            | Waterman-Eggert score: 130; 45.4 bits; E(1) < 1.6 × 10⁻⁸ |
| Sparrow coronavirus HKU17, complete genome       | NC_016992.1   | 87.5%      | 16 nt overlap | 4–19:5395–5410              | Waterman-Eggert score: 62; 20.3 bits; E(1) < 0.4 |
| Human adenovirus C, complete genome              | NC_001405.1   | 85%        | 20 nt overlap | 26–8:2276–2295              | Waterman-Eggert score: 63; 22.2 bits; E(1) < 0.17 |
| Pigeon coronavirus PSH050513 complete (S protein sequence) | DQ160004.1 | 77.8%      | 18 nt overlap | 9–26:1989–2006             | Waterman-Eggert score: 54; 16.5 bits; E(1) < 0.64 |
| Influenza A virus A/NewYork/PV01553/2018(H1N1) segment 2 | MH798340.1 | 76.5%      | 17 nt overlap | 2–18:491–507               | Waterman-Eggert score: 49; 15.5 bits; E(1) < 0.73 |
| Enterobacteria phage P1, complete genome         | NC_005856.1   | 76.2%      | 21 nt overlap | Waterman-Eggert score: 60; 19.9 bits; E(1) < 0.92 | 6–26:24135–24155 |
| Pseudomonas phage F116 complete genome           | NC_006552.1   | 69.2%      | 26 nt overlap | 25–2:6852–6877             | Waterman-Eggert score: 50; 18.9 bits; E(1) < 0.97 |
| **Bacteria and other micro-organisms**           |               |            |               |                             |                                            |
| Mycobacterium gilvum (2)                        | CP000658.1    | 78.9%      | 19 nt overlap | 7–25:18679–18697           | Waterman-Eggert score: 59; 22.8 bits; E(1) < 0.087 |
| Geodermatophilus obscurus DSM 43160 23S rRNA     | NR_076134.1   | 78.6%      | 14 nt overlap | 15–2:1521–1534             | Waterman-Eggert score: 43; 15.5 bits; E(1) < 0.82 |
| Blactococcus saxobidens DD2 23S rRNA             | NR_102997.1   | 78.6%      | 14 nt overlap | 15–2:1525–1538             | Waterman-Eggert score: 43; 15.6 bits; E(1) < 0.81 |
| Kineococcus radiotolerans SRS30216 23S rRNA      | NR_076522.1   | 68%        | 25 nt overlap | 2–26:1621–1643             | Waterman-Eggert score: 45; 16.1 bits; E(1) < 0.7 |
| Modestobacter marinus 42H12-1 16S rRNA (partial) | NR_116228.1  | 66.7%      | 18 nt overlap | 2–19:492–509               | Waterman-Eggert score: 36; 13.4 bits; E(1) < 0.97 |
| Blactococcus saxobidens DMS 44509 T 16S rRNA      | FN600641.1    | 66.7%      | 18 nt overlap | 2–19:488–505               | Waterman-Eggert score: 36; 13.3 bits; E(1) < 0.98 |

(1) From Corman et al.⁶ forward primer 5′-ACAGGTACGTTAATAGTATAGCGT-3′ (2) PYR-GCK plasmid pMFLV02, complete sequence (Software Lalign Expasy version 2.10).
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