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Comparison of in-house and commercial RT-PCR assays for the diagnosis of 2019-novel Coronavirus (SARS-CoV-2) infection

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Background: in December 2019 an outbreak of pneumonia of unknown cause spread from Wuhan (Hubei) to China: it rapidly became a global concern due to the risk of worldwide diffusion. The etiologic agent has been promptly identified as a novel coronavirus, recently named SARS-CoV-2, closely related to SARS-CoV (about 79% sequence identity). L.Sacco University Hospital in Milan is one of the two Italian reference centers for bioemergencies diagnosis and treatment.

Materials/methods: between February 10th and 14th 2020 a total of 10 nasopharyngeal swabs, collected from COVID-19 (2019 novel coronavirus disease) suspected patients, were assayed using different in-house and commercial kits, based on the market availability. Viral RNA was extracted by NucliSENS® easyMag® (bioMérieux) eluting 500 µL of sample in 50 µL. The following assays were used according to manufacturers' instructions: Novel Coronavirus (2019-nCoV) Real Time Multiplex RT-PCR Kit (targets: E gene, N gene, RdRP gene), Liferiver; TaqMan™ 2019 nCoV Assay Kit v1 (targets: N gene, Orf-1ab gene, S gene), Applied Biosystem; Allplex™ 2019-nCoV Assay (targets: E gene, N gene, RdRP gene), Seegene Inc; in-house protocol from Hong Kong University (targets: N gene, Orf-1b gene), available at WHO website (01/23/2020). The 7500 RT-PCR System (Applied Biosystem) was used for all but Seegene assay, running on CFX96 (BioRad). Viral RNA extracted from frozen cell-culture supernatant, obtained from a SARS-CoV diagnosed patient at L. Sacco University Hospital in 2003, was included as positive control due to the high sequence identity.

Results: All samples resulted negative for SARS-CoV-2 with any assays. Viral RNA from SARS-CoV showed a positive signal in RT-PCR for the following gene targets: two out of three genes for Liferiver assay (E and N); E gene for Seegene assay; both N and Orf1b genes with Hong Kong protocol, whereas none of the three targets was detected by Appllied Biosystem assay.

Conclusions: in-house Hong Kong protocol was able to detect SARS-CoV RNA for both gene targets. All commercial assays provided a good specificity for SARS-CoV-2: SARS-CoV RNA showed amplification profile negative for RdRP gene, or a pattern of positive results for E and/or N genes classified as negative according to manufactures' interpretation.

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