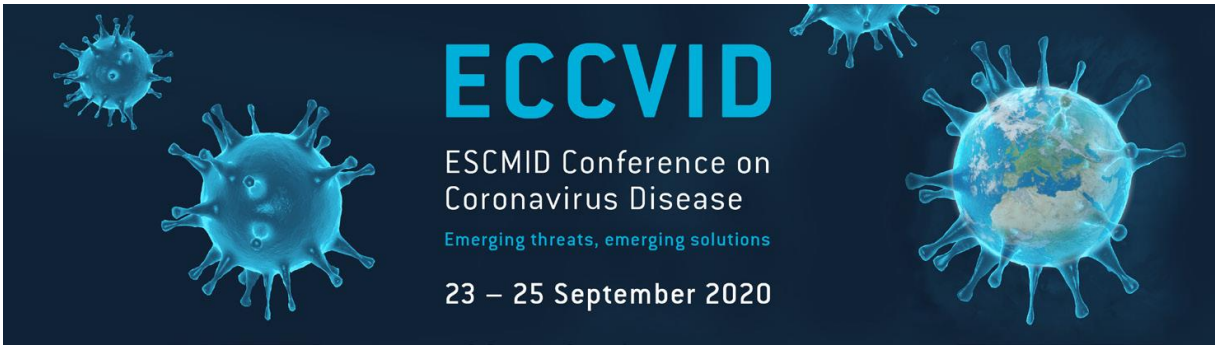

d. Diagnostics

00626 RAPID RNA EXTRACTION METHOD FOR SARS-COV-2 CORONAVIRUS (SARS-COV-2) DETECTION FROM NASOPHARYNGEAL SWABS.

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Background SARS-CoV-2 infection has had a global diffusion. Given the large demand, due to the massive increase in cases of COVID-19, diagnostics companies are struggling to meet the demand for reagents required for molecular diagnosis. In particular, the greatest difficulty is the supply of RNA extraction kits that represents the real "bottleneck" and limiting factor for carrying out the large number of necessary tests. The development and validation of rapid and easy-to-perform RNA extraction methods to apply on a large number of samples are of high priority. The use of a rapid RNA extraction method based on heat treatment of respiratory samples prior to amplification of SARS-CoV-2 was evaluated.

Methods A rapid RNA extraction method was compared to total nucleic acid extraction using Boom technology from nasopharyngeal swabs resuspended in UTM® medium (Copan). A 200µL aliquot of each sample was extracted using Nuclisens easyMag (standard procedure). Another 100µL aliquot was processed by adding 10µL Proteinase K, incubated for 15min at 55°C followed by 5min at 95°C and cooled at 4°C before amplification (rapid procedure). SARS-CoV-2 detection was performed on all samples using Allplex™2019 nCoV assay (Seegene). Rapid extracted samples were also evaluated at 2 further dilutions (1:5 and 1:10) to determine the presence of amplification inhibitors.

Results Preliminary results from 15 samples showed an overall positivity of 86.7% (13/15) using the standard extraction procedure combined with Allplex™2019 nCoV assay. In particular, 11/15 (73.3%) of samples showed positivity for all 3 SARS-CoV-2 assay targeted genes and 2/15 (13.3%) for 1 or 2 genes.Using the rapid extraction method, SARS-CoV-2 positivity was observed in 53.3% (8/15), 73.3% (11/15) and 73.3% (11/15) of undiluted, 1:5 and 1:10 diluted extracts, respectively.

Conclusions Comparing standard and rapid extraction, a good positivity agreement of results was obtained only from diluted rapid extracts (86.7% vs 73.3%). A loss of sensitivity was observed particularly in samples with low viral loads. Moreover, data obtained from the analysis of undiluted samples indicate that UTM® may contain inhibitors of RT-PCR. Alternative resuspension media should be considered for their use in combination with rapid RNA extraction for the molecular diagnosis of SARS-CoV-2.

Conflict of interest The authors have no conflict of interest.