matologic examination is necessary to treat the carcinomas in a timely manner.

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Comparison of two RT PCR methods with two different genes for detection of severe acute respiratory syndrome coronavirus 2 (SARS-COV2)

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Background-aim

World Health Organization (WHO) announced that diagnostic testing for Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-COV2) should be performed by real-time reverse transcriptase-polymerase chain reaction (RT-PCR). Most of these methods use different gene props and therefore the sensitivity and specificity of each method may different. In this study, we have compared two RT-PCR methods using two different genes for detection SARS-COV2.

Methods

A total of random 40 nasopharyngeal swab samples were collected, transported and received in iced box shipment. All samples were performed on two separate semi-automated PCR systems (Qiagen and Abbott m2000). For Qiagen method, 200uL from each sample were added in 96-well QiAcube plate which loaded in QiAcube HT (SN:019658; Qiagen, Germany) to extract RNA. Following extraction, master mix prepared using SARS-COV2-RT-PCR kit 1.0 (REF: 821005; Altona, Germany) for 44 samples including 40 patient samples, two negative controls using nuclease-free water (with and without internal control), and two positive controls (with and without internal control). Extraction elute of each sample (20uL) added to master mix (10uL) to have a total volume of 30uL which uploaded into Rotor-Gene Q (SN: R0219307; Qiagen, Germany). The primer pair used to amplify S gene and E gene in SARS-COV2. Amplifications were done as follow: reverse transcriptase (20 minutes at 55oC); initial denaturation (2 minutes at 95oC); 45 cycles of denaturation (15 seconds at 95oC), annealing for (45 seconds at 55oC), and extension (15 seconds at 72oC). Results reported as valid for internal control less than 35 cycle threshold (CT).

The Abbott m2000 System uses SARS-CoV-2 assay was a dual target assay for the RdRp and N genes. All 40 samples were extracted using m2000sp (Abbott, United States) as recommend by manufacture using 100uL. An RNA sequence that was unrelated to the SARS-CoV-2 target sequence was introduced into each specimen at the beginning of sample preparation. This unrelated RNA sequence was simultaneously amplified by RT-PCR and serves as an internal control (IC) to demonstrate that the process has proceeded correctly for each sample. Following extraction, master mix (20uL) added to extraction elute of each sample (30uL) to have a total volume of 50uL which uploaded into m2000rp (United States). Amplification were done as follow: reverse transcriptase (25 minutes at 55oC); initial denaturation (5 minutes at 94oC); 40 cycles of denaturation (20 seconds at 94oC), annealing for (55 seconds at 55oC), and extension (15 seconds at 72oC).

Result

All samples had valid extraction process with a CT value of internal control between 26.97 to 28.89. A total of 30 samples displayed positive results and 10 samples exhibited negative results with 100% agreement for both methods. This has resulted with a 100% accuracy between both methods.

Conclusions

Both semi-automated methods from Qiagen and Abbott are comparable and accurate despite different technology and different primer genes.

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Interpretative cut-off for alpha-1-antitrypsin concentration in detection of alpha-1-antitrypsin deficiency among adults – a pilot study in the Republic of Serbia

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Background-aim

Alpha-1-antitrypsin deficiency (AATD) is a genetic risk factor for lung diseases in adults. In AATD, deficiency alleles of the AAT-encoding gene are present in homozygous or compound heterozygous combination and a heterozygous carrier is a subject with one deficient and one functional allele. Reduced AAT level in blood is the first-line laboratory hallmark of AATD. Since a number of acquired factors can influence AAT level in blood, we assessed the interpretative cut-off for AAT concentration – the level below which the presence of AATD should be investigated.

Methods

We retrospectively analyzed the group of 121 subjects (70 men and 51 women, age 45 (26-76) years), tested for AATD between 2007 and 2015. Laboratory methods included immunonephelometry, PCR-reverse allele specific hybridization, isoelectric focusing and DNA sequencing. Kruskal-Wallis test and ROC analysis were used in statistical evaluation.

Result

In total, there were 14 cases of AATD and 35 heterozygous carriers. Level of AAT in blood [median (min-max)] was significantly different (P < 0.001) between AATD cases [0.23 (0.11–0.53) g/L], heterozygous carriers [0.86 (0.55–2.30) g/L] and patients with no AATD [1.40 (0.88–2.35) g/L]. Seventeen carriers had AAT concentration in the reference range (0.9–2.0 g/L), while in four subjects with no AATD the AAT level was below the reference range. The level of 1.25 g/L was identified as the cut-off to distinguish group comprising both AATD and