Abstract

Background: SARS-CoV-2 is a coronavirus that causes a respiratory disease, COVID-19. For COVID-19 testing, real-time PCR is considered the gold standard and therefore many commercial SARS-CoV-2 detection kits are available. Objective: The aim of the study is to determine diagnostic values of 10 different commercially available SARS-CoV-2 detection kits, based on their Ct value. Methods: For this study, thirty clinical nasopharyngeal samples were collected at ALEA Genetic Center. Twenty-four of them were positive, while six were negative and used as a negative control. Positive samples were selected based on the day when first symptoms appeared. RNA was extracted using the same extraction method for all samples. For amplification and comparison of detection kits, the same RT-PCR instrument was used. Results: Accuracy, sensitivity, specificity and Cohen’s kappa coefficient were estimated to evaluate diagnostic values of the tested kits. This study showed that all kits showed 100% specificity. Accuracy, sensitivity and kappa coefficient varied among examined assays. Based on clinical features, LabGun™ COVID-19 Assay by LabGenomics proved to be the most sensitive, the most accurate and most specific. Therefore, this assay was used as a reference kit. Conclusion: If things from practice are taken into account, accuracy and reliability of the tested commercial kits can vary compared to those obtained in this study where results were based on ideal functioning of the kits. When choosing the convenient commercial SARS-CoV-2 detection kit using RT-PCR method, many parameters need to be considered.

Keywords: SARS-CoV-2, SARS-CoV-2 detection kits, Real-time PCR.

2. OBJECTIVE

The aim of the study is to determine diagnostic values of 10 different commercially available SARS-CoV-2 detection kits, based on their Ct value.

3. MATERIALS AND METHODS

Thirty clinical nasopharyngeal samples (24 positive and 6 negative) were collected in ALEA Genetic Center using the same viral transport medium for all SARS-CoV-2 samples. Samples were chosen according to the day when the symptoms first occurred (day 3). Each patient had typical COVID-19 symptoms: cough, fever, muscle pain and anosmia. Each patient was tested on the third day since the symptoms began. Negative samples were tested as a control and as a validation of the real-time PCR reactions. RNA was extracted using automated magnetic extraction with TianLong® GeneRotex 96 extractor. It uses magnetic bead method and the instrument can process serum, plasma, swab, feces, tissue and other sample types. It has integrated UV light disinfection that can greatly reduce the risk of contamination. RNA extraction was performed according to manufacturer’s instruction. For magnetic extraction, 200 µl of viral transport medium is needed for the final elution buffer of 100 µl. For all kits, Bio-Rad CFX96 real-time PCR was used. Cycling conditions are different for every used kit (Table 1).

The performance of kits was evaluated in comparison with LabGunTM COVID-19 Assay by LabGenomics. The samples were divided into three groups and six samples were used as a negative control. First group contains samples with Ct value < 24, second group contains samples with Ct value between 25-33 and third group contains samples with Ct value >34. Accuracy, sensitivity, specificity and Cohen’s kappa coefficient were calculated for each group and for every kit separately (Table 2).

4. RESULTS

As already mentioned, 24 samples were chosen according to patients symptoms and the day when the symptoms started. The samples were tested using 10 different kits. All samples tested positive only on LabGunTM COVID-19 Assay by LabGenomics. The same patients were tested after 3 days to confirm the diagnosis of COVID-19. In this paper LabGunTM COVID-19 Assay by LabGe-
Comparative Analysis of SARS-CoV-2 Detection Kits

Savvygen™ SARS-CoV-2 by Savyon diagnostics

<table>
<thead>
<tr>
<th>Stage Cycle</th>
<th>Temperature</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (reverse transcription)</td>
<td>1</td>
<td>45</td>
</tr>
<tr>
<td>2 (initial denaturation)</td>
<td>1</td>
<td>95</td>
</tr>
<tr>
<td>3 (denaturation)</td>
<td>45</td>
<td>95</td>
</tr>
<tr>
<td>4 (annealing)</td>
<td>60</td>
<td>95</td>
</tr>
</tbody>
</table>

Table 1. PCR cycling conditions for SARS-CoV-2 detection kits

Cohen’s kappa coefficient is a correlation statistic test, where kappa can range from -1 to +1. Values ≤ 0 indicate no agreement, while values between 0.81 and 1 indicate almost perfect agreement. Kappa coefficient test was calculated between LabGunTM COVID-19 Assay by LabGenomics and the nine other evaluated kits. Savvygen™ SARS-CoV-2 has the lowest value of 0.26 indicating lowest fair agreement, Bosphore Novel Coronavirus (2019-nCoV) Detection Kit v2 by Anatolia Geneworks, Bio-Speedy SARS-CoV-2 (2019-nCoV) qPCR Detection Kit by Bioeksen, COVID-19 SARS-Cov-2 Real-TM by Saccace Biotechnologies, LabGun COVID-19 ExoFast RT-PCR Kit by LabGenomics, DIAGNOVITAL® SARS-CoV-2 Real-Time PCR Kit by RTA Laboratories and UltraGene Combo2Screen SARS-CoV-2 Assay by ABL (Advanced Biological Laboratories) are classified as moderate agreement and PhoenixDx® SARS-CoV-2 Multiplex and STAT-NAT® COVID-19 MULTI show highest kappa value of 0.67 which represents as substantial agreement.

Figure 1 shows used kits and Ct values for every sample. On X axis different kits are shown, while Ct values are shown on Y axis. Green dots represent true negative control samples and as seen, all tested kits showed 100% specificity. Red dots show false negative results of tested kits compared to LabGunTM COVID-19 Assay by LabGenomics; Savvygen™ SARS-CoV-2 shows the lowest sensitivity, while STAT-NAT® COVID-19 MULTI shows highest sensitivity. Black dots show the distribution of Ct values of true positive samples. Based on the Ct values of the positive samples on the LabGunTM COVID-19 Assay by LabGenomics, three groups of six samples were formed: group I includes samples with Ct values <24, group II includes samples with Ct values between 25-33 and group III includes samples with Ct values >34. All samples with Ct value over 40 are considered negative.

Considering the fact that first two groups contain samples with Ct value below 34, all used kits showed 100% sensitivity compared to LabGunTM COVID-19 Assay by LabGenomics, excluding Savvygen™ SARS-CoV-2 which showed 0% sensitivity in group I (Ct <24). Observing the results of the group III, it is shown that PhoenixDx® SARS-CoV-2 Multiplex and STAT-NAT® COVID-19 MULTI have highest sensitivity regarding Ct values over 34, while Bosphore Novel Coronavirus (2019-nCoV) Detection Kit v2 by Anatolia Geneworks and Bio-Speedy SARS-CoV-2 (2019-nCoV) qPCR Detection Kit by Bioeksen showed lowest sensitivity (Figure 2).

5. DISCUSSION

Statistical parameters show performance of used kits, but it should be noted that results of this study are based on flawless functioning of the kits. If things from practice are taken into account, such as probe instability, this can affect the period in which the results are reliable.
Using mentioned kits. For example, a kit that was at the top of the scales in statistical parameters (PhoenixDx® SARS-CoV-2 Multiplex) showed the lowest shelf life in terms of probe instability leading to false positive results, while other kits showed more stable results.

Main point of this study is to facilitate the choice in the detection of SARS-CoV-2 using RT-PCR method. During the pandemic in Bosnia and Herzegovina, we encountered several epidemic waves, so in those periods it was necessary to process as many samples as possible in a very short time. Therefore, the efficiency of kits is also reflected in cycling conditions, as well as in the ease of preparation of the mastermix. Although a total of 10 different kits on 30 samples were used in this study, the statistical results obtained show slight deviations from laboratory practice. Consequently, more samples should be processed in the future in order to bring the results of the study as close as possible to laboratory practice.

### 6. CONCLUSION

If things from practice are taken into account, accuracy and reliability of the tested commercial kits can vary compared to those obtained in this study where results were based on ideal functioning of the kits. When choosing the convenient commercial SARS-CoV-2 detection kit using RT-PCR method, many parameters need to be considered.

#### Table 2. Accuracy, sensitivity and specificity of used SARS-CoV-2 detection kits

<table>
<thead>
<tr>
<th>Name of the Kit</th>
<th>Accuracy %</th>
<th>Sensitivity %</th>
<th>Specificity %</th>
<th>Kappa coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>LabGun COVID-19 ExoFast RT-PCR Kit by Labgenomics</td>
<td>80</td>
<td>75</td>
<td>100</td>
<td>0,54</td>
</tr>
<tr>
<td>PhoenixDx® SARS-CoV-2 Multiplex</td>
<td>87</td>
<td>83</td>
<td>100</td>
<td>0,67</td>
</tr>
<tr>
<td>DIAGNOVITAL® SARS-CoV-2 Real-Time PCR Kit by RTA Laboratories</td>
<td>83</td>
<td>79</td>
<td>100</td>
<td>0,6</td>
</tr>
<tr>
<td>Bosphore Novel Coronavirus (2019-nCoV) Detection Kit v2 by Anatolia Geneworks</td>
<td>76</td>
<td>70</td>
<td>100</td>
<td>0,5</td>
</tr>
<tr>
<td>Bio-Speedy SARS-CoV-2 (2019-nCoV) qPCR Detection Kit by Bioeksen</td>
<td>76</td>
<td>70</td>
<td>100</td>
<td>0,5</td>
</tr>
<tr>
<td>UltraGene Combo2Screen SARS-CoV-2 Assay by ABL (Advanced Biological Laboratories)</td>
<td>83</td>
<td>79</td>
<td>100</td>
<td>0,6</td>
</tr>
<tr>
<td>COVID-19 SARS-CoV-2 Real-TM by Saccace Biotechnologies</td>
<td>80</td>
<td>75</td>
<td>100</td>
<td>0,54</td>
</tr>
<tr>
<td>Savygen™ SARS-CoV-2</td>
<td>56</td>
<td>46</td>
<td>100</td>
<td>0,26</td>
</tr>
<tr>
<td>STAT-NAT® COVID-19 MULTI</td>
<td>87</td>
<td>83</td>
<td>100</td>
<td>0,67</td>
</tr>
</tbody>
</table>

#### Acknowledgment:
All funding for this study was provided by ALEA Genetic Center from Sarajevo, Bosnia and Herzegovina.

*Author’s contribution: All authors were involved in preparation this article. Final proofreading was made by the first author.

*Conflicts of interest: There are no conflicts of interest.

*Financial support and sponsorship: None.

#### REFERENCES


13. Advanced Biological Laboratories (ABL) S.A. UltraGene Com-
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