



Research article

Diagnostic evaluation of nCoV-QS, nCoV-QM-N, and nCoV-OM detection kits based on rRT-PCR for detection of SARS-CoV-2 in Ecuador

Marco Salinas^{a,*}, Diana Aguirre^a, Lucy Baldeón^{a,b}, Jorge Pérez-Galarza^{a,b}^a Research Institute of Biomedicine, Central University of Ecuador, Quito 170201, Ecuador^b Faculty of Medicine, Central University of Ecuador, Quito 170403, Ecuador

ARTICLE INFO

Keywords:
 COVID-19
 Coronavirus
 ORF3a gene
 rRT-PCR

ABSTRACT

Background: Ecuador was harshly impacted by COVID-19, in the region was the epicenter of the pandemic with the highest mortality rates and with the lowest rates of processed samples. Real-time reverse transcription PCR assays are essential to identify and manage the SARS-CoV-2 outbreak. Because of the global emergency, in Ecuador several commercial kits were introduced for use without clinical validation. In this manner, having the need to perform an evaluation with clinical samples before use for population screening.

Objective: This study aimed to evaluate the diagnostic performance of the nCoV-QS, nCoV-QM-N, nCoV-OM detection kits lately available in Ecuador, against the LightMix E/RdRp kit using nasopharyngeal swab (NPS) samples.

Materials and methods: 198 nasopharyngeal samples were used (66 fresh NPS and 132 RNA stored samples). All samples were analyzed for SARS-CoV-2 with nCoV-QS, nCoV-QM-N, nCoV-OM detection kits and compared the concordance (Cohen's Kappa index, positive percentage agreement and negative percentage agreement) to LightMix E/RdRp as reference detection kit.

Results: The 198 samples presented strong concordance (96% nCoV-QM-N, 100% nCoV-OM and 100% nCoV-QS). The individual performance of each gene showed that the nCoV-OM kit had a higher number of samples detected with the ORF3a (52.5%) and N (53.5%) genes. The combined genes demonstrated that ORF3a/N of nCoV-OM and nCoV-QS kits presented a higher percentage of detection with 52.5% and 48.5%, respectively. Finally, the detection rate and cycle threshold were not different between ORF3a, N, and E target genes.

Conclusion: The nCoV-QS, nCoV-QM-N, and nCoV-OM Detection kits have comparable diagnostic performance to the emergency approved LightMix E/RdRp kit for SARS-CoV-2 detection in suspected COVID-19 patients.

1. Introduction

The coronavirus disease 2019 (COVID-19) caused by the acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is a very infectious and highly transmissible from human to human disease [1, 2]. The World Health Organization (WHO) declared COVID-19 as a pandemic in March 2020 since the spread of the disease affected over 18 nations [3]. Currently, more than 600 million cases confirmed and 6 million deaths have been reported around the world [4]. In Latin America at the beginning of the pandemic, Brazil, Chile and Ecuador were the countries most affected by COVID-19, the increase in the number of infected and the inefficient response in managing the pandemic caused the collapse of their health systems [5]. As a result, Ecuador came to have the highest mortality and excess mortality rates in the region, estimating an excess of deaths of 171% of their expected value in a typical year [6]. Nowadays in

Ecuador, the WHO recorded more than 900 thousand confirmed infected people cases and over 35 thousand fatalities [7].

During the strongest stages of the pandemic, the strategies of the Ecuadorian Ministry of Health to control the spread of the virus were insufficient, revealing the lack of laboratories and reagents for the diagnosis of Covid-19. Between February–March 2020, Ecuador only had 21 laboratories authorized to process SARS-CoV-2 samples in 8 of the 24 provinces [8]. These problems contribute to underestimating the number of positive patient since the fact that there is a delay in the processing due to the excess of samples and also a delay in the reporting of results [9].

Real-time reverse transcription-polymerase chain reaction (rRT-PCR) is the gold standard technique for COVID-19 diagnosis, because of its ability to detect specifically viral genomes with high sensitivity [10, 11]. For the international healthy emergency, the U.S. Food and Drug Administration (FDA) has approved the uses of various rRT-PCR assays developed in

* Corresponding author.

E-mail address: masalinas@uce.edu.ec (M. Salinas).<https://doi.org/10.1016/j.heliyon.2022.e11137>

Received 8 June 2022; Received in revised form 13 September 2022; Accepted 13 October 2022

2405-8440/© 2022 The Author(s). Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

laboratories of several countries [12]. These commercial kits are targeting SARS-CoV-2 genes such as nucleocapsid (*N*), envelope (*E*), and RNA-dependent RNA-polymerase (*RdRp*) [13, 14]. However, given the increase in the number of infected people, the shortage of reagents and the difficulty of importing these kits, new rRT-PCR assays targeting genes of accessory proteins like open reading frame 3a (*ORF3a*) of the virus, had been introduced and currently are available [15]. In fact in Ecuador, of the 35 kits with authorization for diagnosis of SARS-CoV-2 by the Ecuadorian regulatory entity—ARCSA, only 10 have been tested and they are included in the list of emergency authorization use by the FDA [16].

In this way, it is necessary to verify the efficiency of any kit to avoid both false positive and negative results [17]. This study aimed to evaluate the diagnostic performance of the nCoV-QS, nCoV-QM-N, nCoV-OM detection kits lately available in Ecuador, against the LightMix *E/RdRp* kit (FDA approved) using nasopharyngeal swab (NPS) samples. Knowing that one of the best methods to minimize the transmission of the virus is the early diagnosis of the disease, this study will provide the necessary information on the efficiency of these kits in clinical samples.

2. Materials and Methods

2.1. Clinical samples

In this study, 198 viral RNA samples isolated from NPS were used. 66 fresh NPS samples from people with suspected COVID-19 were selected for SARS-CoV-2 RNA detection. The remaining 132 samples were stored at -80 °C and

consisted of 66 positives and 66 negatives previously diagnosed samples. These viral RNAs were obtained from the RNA biobank of the Research Institute in Biomedicine at the Central University of Ecuador (INBIOMED-UCE). For the nCoV-QM-N kit, 28 samples were discarded, nine RNA samples were invalid because amplification of internal control and viral target genes were not detected and 19 RNA samples were not carried out rRT-PCR due to insufficient volume of RNA and kit reagents. Therefore, only 170 RNA samples were included in the statistical analysis with nCoV-QM-N kit (Figure 1).

2.2. Nucleic acid extraction

The RNAs in the 66 NPS samples were extracted using the Nucleic Acid Extraction kit (Magnetic bead method) (Zybio, China) following the manufacturer's instructions. This kit works with a semi-automatic instrument where the magnetic beads in the kit have specific polymeric groups of adsorbed nucleic acid (DNA/RNA) on the surface. Briefly, 15 uL of proteinase K and 200 uL of the NPS sample were pipetted into each 16-well plate. The viral RNA is separated from the magnetic beads by changing the liquid phase conditions using 50 uL of elution buffer [18]. Finally, the samples were stored at -20 °C until use in the following 8 h at maximum. The 132 RNAs stored samples were extracted using the same methodology and extraction kit.

2.3. rRT-PCR for SARS-CoV-2 RNA detection

nCoV-QS, nCoV-QM-N, and nCoV-OM Detection Kits (MiCo BioMed, Korea) were used. All kits detect *ORF3a* and *N* viral target regions. The

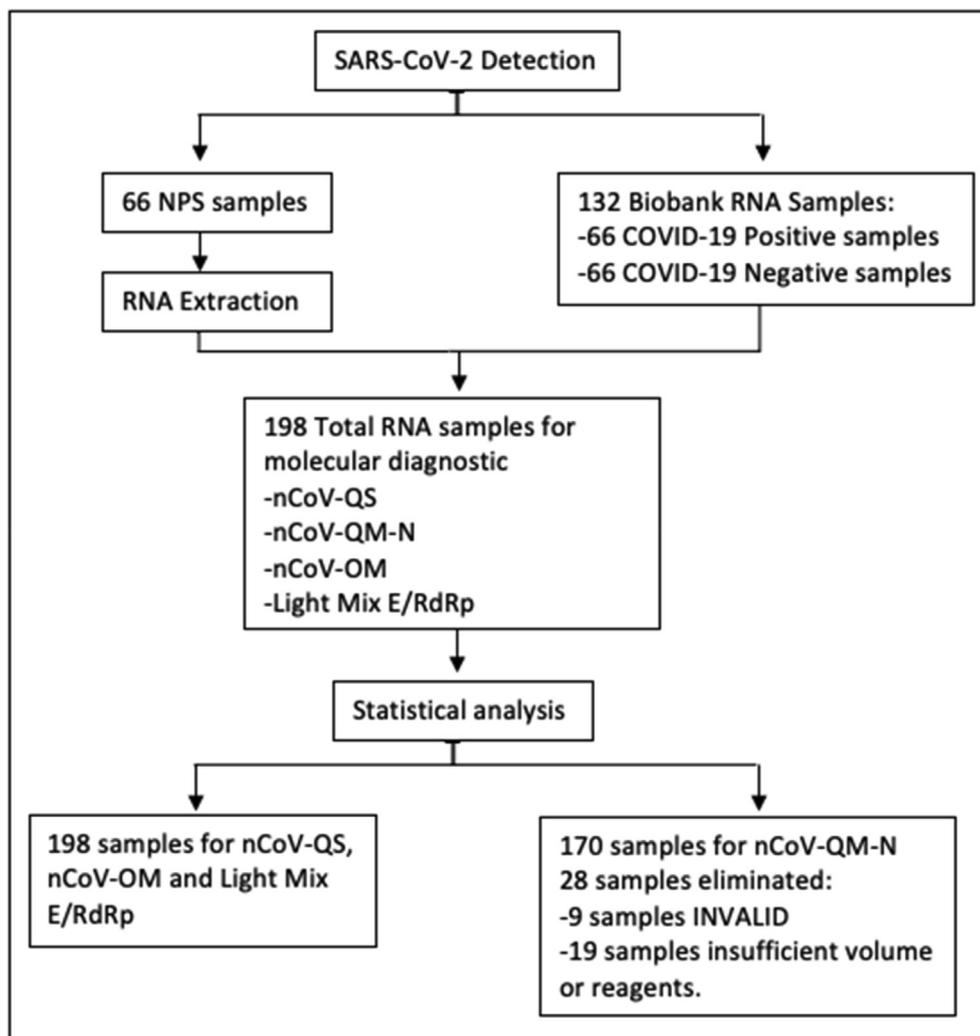


Figure 1. Summary of the methodology using in this study.

nCoV-QM-N and nCoV-OM detection kits were designed to perform reverse transcription and detection of target genes in a multiplex assay, while the nCoV-QS kit determines each gene separately. The kits were used following the manufacturer's instructions adding the exogenous control gene into the rRT-PCR master mix [15]. LightMix SarbecoV *E*-gene plus *EAV* control, LightMix Modular SARS-CoV-2 (COVID-19) *RdRp* with One-Step RT-PCR Polymerase Mix 5x Lyophilized (TIB Molbiol, Germany) were used as a reference kit. All reactions were carried out according to the manufacturer's instructions. For the *E* gene, the exogenous internal control was added to the rRT-PCR mix [19, 20]. The PCR specifications and conditions for each kit are detailed in Table 1.

The nCoV-QS and nCoV-QM-N kits are designed to load the sample onto a special PCR plate (LabChip) and specifically run with the Veri-Q PCR 316-QD-P100 device (MiCo BioMed, Korea) [21]. CFX-96 instrument (BioRad Laboratories, USA) was used for nCoV-OM and LightMix *E/RdRp*. For all kits, the samples were diagnosed as positive for SARS-CoV-2 when at least one viral gene had a valid Ct value according to the recommendations by WHO [22].

2.4. Statistical analysis

The Cohen's Kappa index, positive percentage agreement (PPA), and negative percentage agreement (NPA) were used to evaluate the concordance between nCoV-QS, nCoV-QM-N, nCoV-OM kits against the LightMix *E/RdRp* kit (Figure 1). Additionally, Pearson's chi-squared test and Kruskal-Wallis test with Dunn's multiple comparisons test were used to compare the performance of the assays and Cycle threshold (Ct) values. SPSS software version 23 (IBM) and Graph Pad Prism 9 (San Diego, CA) was used for the statistical analysis.

3. Results

Overall, 198 RNA samples were evaluated in this study. First, the global diagnostic of each sample was evaluated, that is, positive or negative result. Of all the samples tested, the LightMix *E/RdRp* kit diagnosed 99 samples as positive. The nCoV-OM and nCoV-QS kits detected the same number of positive samples showing a PPA of 100%. In addition, seven and five samples were detected as positive with nCoV-OM and nCoV-QS, respectively but negative with the reference kit. For this reason of the 99 samples diagnosed as negative with the reference kit, the percentage of concordance was 92.9% for nCoV-OM and 94.9% for nCoV-QS. For the nCoV-QM-N kit, the 170 RNA samples showed PPA = 96.3% and NPA = 94.3%. Three samples were detected as negative with nCoV-QM-N but positive with the reference kit. On the other hand, five samples were positive with nCoV-QM-N but negative with the reference kit. All kits presented Cohen's Kappa values >0.9 (Table 2).

Table 1. rRT-PCR parameters of the kits used for SARS-CoV-2 RNA detection.

Kit name, manufacturer	PCR Equipment	Target Genes	Final reaction volume(μL)	Volume of RNA added per test (μL)	Termocycler setup	Cut off, LoD
nCoV-QS, MiCo BioMed	Veri-Q PCR 316-QD-P100	<i>ORF3a</i> , <i>N</i>	10 (only 8μL was loaded in each LabChip channel)	3	50 °C × 5 min, 95 °C × 8 sec, 45 cycles of 95 °C × 9 sec, 56 °C × 13 sec	<40, <i>ORF3a</i> = 5.4 copies/Rx <i>N</i> = 12.7 copies/Rx
nCoV-QM-N, MiCo BioMed	Veri-Q PCR 316-QD-P100	<i>ORF3a</i> , <i>N</i>	10 (only 8μL was loaded in each LabChip channel)	5	50 °C × 10 min, 95 °C × 3 min, 45 cycles of 95 °C × 9 sec, 58 °C × 30 sec	<40, <i>ORF3a</i> = 11.5 copies/Rx <i>N</i> = 8.0 copies/Rx
nCoV-OM, MiCo BioMed	CFX-96	<i>ORF3a</i> , <i>N</i>	20	8	50 °C × 10 min, 95 °C × 3 min, 45 cycles of 95 °C × 9 sec, 58 °C × 30 sec	<40, <i>ORF3a</i> = 6.0 copies/Rx <i>N</i> = 10.1 copies/Rx
LightMix SarbecoV <i>E</i> -gene plus <i>EAV</i> control, TIB Molbiol	CFX-96	<i>E</i>	20	5	55 °C × 3 min, 95 °C × 30 sec, 45 cycles of 95 °C × 3 sec, 60 °C × 12 sec	<36, <i>E</i> = 5.2 copies/Rx
LightMix Modular SARS-CoV-2 (COVID-19) <i>RdRp</i> , TIB Molbiol	CFX-96	<i>RdRp</i>	20	5	55 °C × 5 min, 95 °C × 5 min, 45 cycles of 95 °C × 5 sec, 60 °C × 15 sec, 72 °C × 15 sec	<40, <i>RdRp</i> = 10.6 copies/Rx

Table 2. Diagnosis performance of SARS-CoV-2 rRT-PCR detection kits and Nucleic Acid Extraction kit (Magnetic bead method).

	PPA	NPA	Kappa
nCoV-OM	100.0(99/99)	92.9(92/99)	0.93
nCoV-QS	100.0(99/99)	94.9(94/99)	0.95
nCoV-QM-N	96.3(79/82)	94.3(83/88)	0.91

Value in parenthesis represents the number of positives/total number of true positives or the number of negatives/total number of true negatives. Abbreviations: PPA = Positive Percentage Agreement, NPA = Negative Percentage Agreement, Kappa = Cohen's Kappa Index.

The individual detection capacity for each gene was verified. The lowest detection percentage was observed in *E* (47%) and *RdRp* (49%) genes belonging to the reference kit. The nCoV-OM kit had a higher number of samples detected with the *ORF3a* (52.5%) and *N* (53.5%) genes. By analyzing the combined genes of each kit, *ORF3a/N* genes of the nCoV-OM and nCoV-QS kits presented a higher percentage of detection with 52.5% and 48.5%, respectively. The *E/RdRp* genes showed 46% detection only above the nCoV-QM-N kit genes with 45.3% (Table 3).

Regarding to the Ct values. In none of the three kits evaluated, there were no significant differences between median Ct values of *ORF3a*, *N*, and *E* target genes. Nevertheless, the median Ct value of LightMix *RdRp* kit (Ct = 27.57) was significantly higher than nCoV-QS *ORF3a* and *N* (Ct = 23.35, 22.17, respectively), nCoV-QM-N *ORF3a* and *N* (Ct = 23.08 and 22.35, respectively), nCoV-OM *ORF3a* and *N* (Ct = 23.67, 22.44, respectively) and LightMix *E* (Ct = 23.85) assays (Figure 2).

4. Discussion

Ecuador is one of the South American countries most affected by the COVID-19 pandemic. The mathematical estimates projected the number of infected that the country could have if efficient government strategies were not carried out to stop the transmission of the disease [23]. International experience has shown that early diagnosis is the best approach to contain the spread of the virus. However in Ecuador the national average time for case completion was estimated in 3 days and 12.1% of samples took more than 10 days to complete [9].

Due to the health emergency, several kits have been approved for use but without validation with clinical samples. In this way, each laboratory has the obligation to evaluate each kit to ensure the diagnostic utility in the general screening of the population [24]. Thus, this study chose the nCoV-QS, nCoV-QM-N and nCoV-OM kits because they are recently available kits in Ecuador. Unlike other studies where evaluations are

Table 3. Comparative performance of samples that amplified with each gene.

	<i>E</i>	<i>RdRp</i>	<i>E, RdRp</i>		<i>ORF3a</i>	<i>N</i>	<i>ORF3a,N</i>
LightMix SarbecoV <i>E</i> -gene plus <i>EAV</i> control, LightMix Modular SARS-CoV-2 (COVID-19) <i>RdRp</i>	93/198 (47.0%)	97/198 (49.0%)	91/198 (46.0%)	nCoV-OM	104/198 (52.5%)	106/198 (53.5%)	104/198 (52.5%)
				nCoV-QS	100/198 (50.5%)	100/198 (50.5%)	96/198 (48.5%)
				nCoV-QM-N	80/170 (47.1%)	81/170 (47.6%)	77/170 (45.3%)

Values represent the percentage of No. of positive test results/No. of specimens (%).

performed with reference kits that have the same target genes [25]. The performance of these three kits was compared to the LightMix *E/RdRp* kit. The choice of this kit was due to the fact that the sequence of the primers of the *E* and *RdRp* target genes were the same provided by Corman, et al. These sequences of primers were widely used as the gold standard in the detection of SARS-CoV-2 [26, 27]. In this manner, our study prioritized and verified if the global diagnosis of a sample (positive or negative result) had the same results even when the target genes of the tested kits were *ORF3a* and *N*.

The results revealed a positive agreement of 100% (nCoV-OM and nCoV-QS) and 96.3% (nCoV-QM-N). The NPA results showed values of 92.9%, 94.9% and 94.3% with nCoV-OM, nCoV-QS and nCoV-QM-N, respectively. And the degree of concordance of the findings between the tested kits and the LightMix *E/RdRp* kit was strong (Kappa index = 0.93 nCoV-OM, 0.95 nCoV-QS, 0.91 nCoV-QM-N). The evaluations made with the LightMix *E/RdRp* kits has proven to have a high sensitivity to detect viral RNA in NPS samples [28, 29]. Our findings showed the detection rate of *ORF3a* and *N* in positive samples was equivalent to *E* and *RdRp*. Also, there were no differences in the median Ct values of *ORF3a*, *N*, and *E* genes suggesting high sensitivity.

Although the results revealed a diagnostic probability of a false positive lower than 8% with all the kits, it is necessary to carry out an evaluation of the kits using a greater number of samples. A limitation of this study was the lack of a three way of comparison using a reference

standard kit could be useful to clarify the discrepancies of the results [30, 31]. Nonetheless, the slightly differences in the count of positive cases detected by *ORF3a* and *N* genes in the nCoV-OM kit could be explained by the eluate volume of RNA and the difference of the limit of detection (LoD) of each kit.

Moreover, because the samples come from a population screening, the possible phase of infection of each person may vary. For this reason, the target abundance in the sample might be the explanation for the difference in the median Ct values between *ORF3a*, *N*, and *RdRp*. The expression rates of the *N* gene are slightly higher than *RdRp* since its function in viral assembly and its presence in subgenomic RNA [32, 33, 34, 35]. This assumption could be applied to the *ORF3a* gene given its functions in viral release through lysosomal trafficking [36].

5. Conclusion

The rapid spread of the disease around the world made the diagnosis a tool to stop the increase of COVID-19 cases. The imminent evolution of the pandemic forced the development and evaluation of performance of several rRT-PCR kit targeted to SARS-CoV-2. In this study, we determined that the nCoV-QS, nCoV-QM-N, and nCoV-OM detection kits have comparable SARS-CoV-2 diagnostic performance in patients with suspected COVID-19 to the emergency approved LightMix *E/RdRp* kit. In this way, these kits can be used for population screening that will allow timely management of new cases, improving epidemiological fences and avoiding the collapse of the health system.

Declarations

Author contribution statement

Marco Salinas: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Diana Aguirre: Performed the experiments.

Jorge Pérez-Galarza: Conceived and designed the experiments; Analyzed and interpreted the data.

Lucy Baldeón: Conceived and designed the experiments; Wrote the paper.

Funding statement

This work was supported by Central University of Ecuador [EP-001-001-2020].

Data availability statement

Data included in article/supp. material/referenced in article.

Declaration of interest's statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

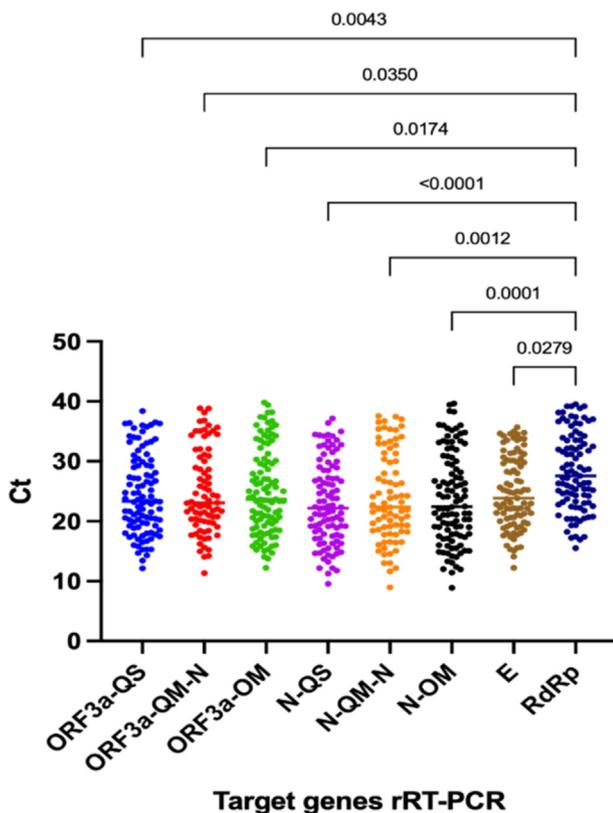


Figure 2. Comparison of the Ct values of the three SARS-CoV-2 rRT-PCR kits in this study. The numbers above the lines indicate the P values.

Acknowledgements

We are extremely grateful to Central University of Ecuador, for his support in managing the needed funds for this work that would have a great positive impact in our society.

References

- H. Amawi, G.I. Abu Deiah, A.A. Aljabali, K. Dua, M.M. Tambuwala, COVID-19 pandemic: an overview of epidemiology, pathogenesis, diagnostics and potential vaccines and therapeutics, *Ther. Deliv.* 11 (4) (2020) 245–268.
- K.G. Andersen, A. Rambaut, W. Ian Lipkin, E.C. Holmes, R.F. Garry, The proximal origin of SARS-CoV-2 [Internet], *Nat. Med.* 26 (2020) 450.
- World Health Organization (WHO), WHO Director-General's Opening Remarks at the media Briefing on COVID-19, 2020 [Internet]. 2020. Available from: <https://www.who.int/director-general/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19-11-march-2020>.
- World Health Organization (WHO), COVID-19 weekly epidemiological update [Internet], *World Heal Organ* (58) (2022) 1–23. Available from: <https://www.who.int/publications/m/item/covid-19-weekly-epidemiological-update>.
- R.P. Alvarez, P.R. Harris, Covid-19 in Latin america: challenges and opportunities, *Rev. Chil. Pediatr.* 91 (2) (2020 Mar 1) 179–182.
- L. Cuéllar, I. Torres, E. Romero-Severson, R. Mahesh, N. Ortega, S. Pungitore, et al., Excess deaths reveal the true spatial, temporal and demographic impact of COVID-19 on mortality in Ecuador, *Int. J. Epidemiol.* 51 (1) (2022 Feb 1) 54–62.
- World Health Organization (WHO), Geo-Hub COVID-19—Information System for the Region of the Americas, 2021. Available from: <https://paho-covid19-respo-nse-who.hub.arcgis.com/pages/paho-south-america-covid-19-response>.
- El Comercio, 21 Laboratorios Públicos Y Privados Ya Pueden Diagnosticar Covid-19—El Comercio [Internet], *El Comercio*, 2020 [cited 2022 Sep 11]. Available from: <https://www.elcomercio.com/tendencias/sociedad/laboratorios-publicos-privados-diagnostico-covid19.html>.
- I. Torres, R. Sippy, F. Sacoto, Assessing critical gaps in COVID-19 testing capacity: the case of delayed results in Ecuador, *BMC Publ. Health* (1) (2021 Dec 1) 21.
- K.R. Campos, C.T. Sacchi, C.R. Gonçalves, É.V.R.G. Pagnoca, A. dos S. Dias, L.O. Fukasawa, et al., COVID-19 Laboratory Diagnosis: Comparative Analysis of Different RNAextraction Methods for SARS-CoV-2 Detection by Two Amplificationprotocols, *Rev Inst Med Trop Sao Paulo*, 2021, p. 63.
- M. Yüce, E. Filiztekin, K. Gasia Özkaya, COVID-19 diagnosis—a review of current methods, *Biosens. Bioelectron. J* (2020) 172.
- U.S Food & Drug Administration (FDA), Emergency Use Authorization[FDA [Internet], FDA, 2022 [cited 2022 Sep 11]. Available from: <https://www.fda.gov/emergency-preparedness-and-response/mcm-legal-regulatory-and-policy-framework/emergency-use-authorization>.
- S.L. Mitchell, K. St George, D.D. Rhoads, S.M. Butler-Wu, V. Dharmarha, P. McNult, et al., Understanding, verifying, and implementing emergency use authorization molecular diagnostics for the detection of sars-cov-2 RNA, *J. Clin. Microbiol.* 58 (8) (2020) 1–8.
- K.H. Hong, S.W. Lee, T.S. Kim, H.J. Huh, J. Lee, S.Y. Kim, et al., Guidelines for laboratory diagnosis of coronavirus disease 2019 (COVID-19) in Korea, *Ann Lab Med* 40 (5) (2020) 351–360.
- MiCo BioMed. (주)미코바이오메드—COVID-19—Main [Internet]. [cited 2021 Aug 24]. Available from: <http://www.micobiomed.com/en/covid/immunodiagnosti cs.php>.
- Agencia Nacional de Regulación Control y Vigilancia Sanitaria, Pruebas y Reactivos PCR, aprobadas por Arca [Internet], 2020 [cited 2022 Sep 8]. Available from: <https://www.controlsanitario.gob.ec/pruebas-rapidas-y-reactivos-pcr-aprobadas-por-arca/>.
- C. Hernández, C. Florez, S. Castañeda, N. Ballesteros, D. Martínez, A. Castillo, et al., Evaluation of the diagnostic performance of nine commercial RT-PCR kits for the detection of SARS-CoV-2 in Colombia, *J. Med. Virol.* 93 (9) (2021) 5618–5622.
- Zybio, Nucleic Acid Extraction Kit (Magnetic Bead Method) Nucleic Acid Extraction Kit (Magnetic Bead Method), 2020, pp. 5–7. Available from: <https://m.zybio.com/en/Product/Molecule/>.
- TIB Molbiol, LightMix SarbecoV E-Gene Plus EAV Control [Internet], TIB Molbiol, 2022 [cited 2022 Sep 11]. Available from: [https://www.roch e-as.es/im/pdf/MDx/40-0776/96/Sarbeco-E-gene/V200204/09164154001% 20\(1\).pdf](https://www.roch e-as.es/im/pdf/MDx/40-0776/96/Sarbeco-E-gene/V200204/09164154001% 20(1).pdf).
- TIB Molbiol, Covid-19—TIB MOLBIOL [Internet], TIB Molbiol, 2022 [cited 2022 Sep 11]. Available from: <https://www.tib-molbiol.de/covid-19>.
- MiCo BioMed, Veri-Q PCR 316 Coronavirus Disease 2019 (COVID-19) nCoV-QS Detection Kit, MiCo BioMed, 2021, pp. 1–18.
- World Health Organization (WHO), Laboratory Guidelines for the Detection and Diagnosis of COVID-19 Virus Infection [Internet], Paho, 2020 (Julio):1–7. Available from: <https://iris.paho.org/bitstream/handle/10665.2/52458/PAHOIMSP HECOVID-19200038/eng.pdf?sequence=1&isAllowed=y>.
- T. Tene, M. Guevara, J. Svozilík, R. Tene-Fernandez, C.V. Gomez, Analysis of covid-19 outbreak in Ecuador using the logistic model [Internet], *Emerg Sci J 5* (Special issue) (2021) 105, 18. Available from: <https://www.ijournalse.org/index.php/ESJ/issue/view/26>.
- K.H. Hur, K. Park, Y. Lim, Y.S. Jeong, H. Sung, M.N. Kim, Evaluation of Four Commercial Kits for SARS-CoV-2 Real-Time Reverse-Transcription Polymerase Chain Reaction Approved by Emergency-Use-Authorization in Korea, *Front Med 7* (August) (2020) 1–10.
- B. Wang, M. Hu, Y. Ren, X. Xu, Z. Wang, X. Lyu, et al., Evaluation of seven commercial SARS-CoV-2 RNA detection kits based on real-time polymerase chain reaction (PCR) in China, *Clin. Chem. Lab. Med.* 58 (9) (2020) E149–E153.
- V.M. Corman, O. Landt, M. Kaiser, R. Molenkamp, A. Meijer, D.K.W. Chu, et al., Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR, *Euro Surveill.* 25 (3) (2020).
- X. Lu, L. Wang, S.K. Sakhivel, B. Whitaker, J. Murray, S. Kamili, et al., US CDC real-time reverse transcription PCR panel for detection of severe acute respiratory syndrome Coronavirus 2, *Emerg. Infect. Dis.* 26 (8) (2020) 1654–1665.
- CENTOGENE US LLC, CentoFast-SARS-CoV-2 RT-PCR Assay (SARS-CoV-2 Detection Based on E and RdRp Genes) [Internet], 2021, pp. 1–10. Available from: <https://www.fda.gov/media/139725/download>.
- J. Alcoba-Florez, H. Gil-Campesino, D. García-Martínez De Artola, R. González-Montelongo, A. Valenzuela-Fernández, L. Ciuffreda, et al., Sensitivity of Different RT-qPCR Solutions for SARS-CoV-2 Detection 99, *Int J Infect Dis*, 2020.
- C.M.U. Chikere, K. Wilson, S. Graziadio, L. Vale, A.J. Allen, Diagnostic test evaluation methodology: a systematic review of methods employed to evaluate diagnostic tests in the absence of gold standard—an update, *PLoS One* 14 (10) (2019) 1–25.
- U.S Food & Drug Administration (FDA), Guidance for Industry and FDA Staff Statistical Guidance on Reporting Results from Studies Evaluating Diagnostic Tests [Internet], Fda, 2007, pp. 1–39. Available from: <http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm071148.htm>.
- D. Kim, J.Y. Lee, J.S. Yang, J.W. Kim, V.N. Kim, H. Chang, The architecture of SARS-CoV-2 transcriptome [Internet], *Cell* 181 (4) (2020) 914–921. e10.
- S.K. Lal, Molecular Biology of the SARS-Coronavirus [Internet], in: S.K. Lal (Ed.), *Molecular Biology of the SARS-Coronavirus*, Springer Berlin Heidelberg, Berlin, Heidelberg, 2010, pp. 1–328. Available from: <http://link.springer.com/10.1007/978-3-642-03683-5>.
- A.A.T. Naqvi, K. Fatima, T. Mohammad, U. Fatima, I.K. Singh, A. Singh, et al., Insights into SARS-CoV-2 genome, structure, evolution, pathogenesis and therapies: structural genomics approach [Internet], *Biochim. Biophys. Acta, Mol. Basis Dis.* 1866 (10) (2020 Oct), 165878. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S092544392030226X>.
- J. Reina, L. Suarez, Evaluation of different genes in the RT-PCR detection of SARS-CoV-2 in respiratory samples and its evolution in infection [Internet], *Rev. Española Quimioter.* 33 (4) (2020 Jul 20) 292–293. Available from: <https://seq.es/abstract/rev-esp-quimioter-2020-may-27/>.
- M. Bianchi, A. Borsetti, M. Ciccozzi, S. Pascarella, SARS-Cov-2 ORF3a: mutability and function [Internet], *Int. J. Biol. Macromol.* 170 (January) (2021 Feb) 820–826. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S0141813020353137>.