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COMPARATIVE PERFORMANCE OF MANUAL VS. AUTOMATED RT-PCR AT MALAGASY MEDICAL ANALYSIS LABORATORY FOR SARS-COV-2 DETECTION: A STUDY BETWEEN TWO THERMO CYCLERS AND THE QIASTAT® DX ANALYZER-DX RESPIRATORY SARS-COV-2 PANEL

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ABSTRACT

SARS-CoV-2, a single-stranded RNA virus with positive polarity belonging to the Betacoronavirus genus, is the etiological agent of the COVID-19 pandemic. Virological diagnosis primarily relies on the direct detection of the viral genome via real-time RT-PCR, which remains the gold standard due to its high sensitivity and specificity. This study aims to comparatively evaluate the analytical and clinical performance of two standard RT-PCR platforms (QuantStudio™ 7 Flex Real-Time PCR System and Fluorocycler® XT) with that of a molecular syndromic diagnostic system, the QIAstat® Dx Analyzer (SARS-CoV-2 Respiratory Panel). A retrospective study was conducted in 2021 on a total of 60 nasopharyngeal samples. The positivity rate was 43.3% for QuantStudio™, 35% for Fluorocycler®, and 48% for QIAstat®. The concordance analysis between QIAstat® Dx and QuantStudio™ revealed a Kappa coefficient of 0.657 (substantial agreement), with a sensitivity of 76.9%, a specificity of 88.2%, a positive predictive value (PPV) of 83.3%, and a negative predictive value (NPV) of 83.9%. The Fluorocycler® XT showed near-perfect concordance with the QuantStudio™ (Kappa = 0.885), with a sensitivity of 90.9%, a specificity of 97.0%, a PPV of 90.9%, and a NPV of 97.0%. No significant differences were observed in the Ct values between the tested systems. Despite its multiplexing capability and automation, the QIAstat® Dx shows lower sensitivity, which limits its use as a first-line standalone test in clinical settings.

Keywords: analytical performance, comparison, LA2M, Qiastat, RT PCR, SARS-CoV2.

INTRODUCTION

Identified in January 2020 through rapid genomic analysis, the virus responsible for COVID-19, named SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2), is a positive-sense single-stranded RNA virus belonging to the Coronaviridae family and the Betacoronavirus genus [1, 2]. This virus has a spherical, enveloped structure, with a genome of approximately 30 kb. It encodes several proteins, including four major ones: the S protein (spike), involved in cellular entry via the ACE2 receptor; the M protein (membrane); the E protein (envelope); and the N protein (nucleocapsid), which encapsulates the viral genome [3, 4]. The genes coding for the nucleocapsid (N) and the ORF1-ab region are mainly targeted by diagnostic RT-PCR tests.

This virus has caused a pandemic that has severely strained healthcare systems. SARS-CoV-2 belongs to the coronavirus family [5]. Due to its specific genetic characteristics, the development of molecular diagnostic tests, through real-time PCR, has contributed to the implementation of early detection strategies and infection control [1]. This RT-PCR technique is considered the gold standard due to its high sensitivity and specificity [6, 7]. However, this technique can be time-consuming and requires an equipped laboratory, which has encouraged the development of automated platforms such as the QIAstat® Dx Analyzer-Dx Respiratory SARS-CoV-2 Panel. The latter is a multiplex molecular diagnostic system offering rapid and simultaneous detection and identification of multiple respiratory pathogens, including SARS-CoV-2, from a single clinical sample [8]. Although both real-time PCR and the automated method on the QIAstat® Dx Analyzer-Dx Respiratory SARS-CoV-2 Panel are based on similar principles, they have notable differences in terms of methodology, performance, and clinical applicability. A comprehensive comparison of these two techniques provides a better understanding of their use in managing the pandemic and their role in SARS-CoV-2 screening strategies [9, 10].

The World Health Organization (WHO) played a key role in coordinating global efforts to develop and validate these tests during the early phases of the epidemic [11]. In Madagascar, in response to this pandemic, the Malagasy Medical Analysis Laboratory (LA2M) was established in 2020 to support the efforts of the Ministry of Public Health. It is the first national public health laboratory in Madagascar. Its primary mission is the prevention, control, and biological surveillance of diseases with epidemic potential and public health importance. It contributes to the development of laboratory-related initiatives and supports emergency interventions. The objective of this study is to compare the performance of real-time PCR diagnostic methods and the QIAstat® Dx Analyzer-Dx Respiratory SARS-CoV-2 Panel for the detection of SARS-CoV-2.

METHODS

This retrospective comparative study was conducted in 2021. A total of 60 nasopharyngeal samples were analyzed using two methods:

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real-time RT-PCR and the QIAstat® Dx Analyzer-Dx Respiratory SARS-CoV-2 Panel.

1. Sampling and storage of samples

Nasopharyngeal samples were collected according to the recommendations of the World Health Organization (WHO) and stored at -80°C in cryotubes until analysis. The samples were anonymized. Demographic data were properly maintained and remained confidential.

2. Extraction of nucleic acides and Real time RT-PCR

Nucleic acid extraction was performed using the MagMax kit. Viral RNA was extracted from 200 µL of nasopharyngeal sample. Real-time RT-PCR was carried out following the standard protocols of the Sansure Biotech kit, using primers targeting the N and ORF1ab genes of SARS-CoV-2. The Sansure Biotech SARS-CoV-2 kit is a real-time RT-PCR detection kit designed for the identification of SARS-CoV-2. It primarily targets the N (Nucleocapsid) and ORF1ab genes, thereby enabling sensitive and specific detection of the virus from various clinical samples, such as nasopharyngeal or saliva swabs. This kit is widely used in diagnostic laboratories for rapid virus detection, offering high performance even at low viral loads [12]. Amplification was performed on both a QuantStudio™ 7 Flex thermo cycler using the 96-well block and the Fluorocycler® XT. The thermo cyclers were programmed according to the conditions specified for this protocol. The cycle threshold (Ct) value was determined and used to estimate the SARS-CoV-2 viral load. A Ct > 35 was considered negative. The one-way workflow was strictly followed throughout the analysis.

3. Analysis Using the QIAstat® Dx Analyzer – Respiratory SARS-CoV-2 Panel

The analysis was performed using the QIAstat® Dx Analyzer and the Respiratory SARS-CoV-2 Panel (QIAGEN), in accordance with the manufacturer's instructions. This fully automated system employs a multiplex quantitative RT-PCR test and requires no prior sample preparation, including nucleic acid extraction. The analyzer conducts multiplex detection by combining several fluorescent probes specific to respiratory pathogens, including 21 respiratory viruses and bacteria, such as SARS-CoV-2. These pathogens include, among others, Mycoplasma pneumoniae, Chlamydophila pneumoniae, Bordetella pertussis, influenza viruses (A, H1N1, H3N2, B), coronaviruses (229E, HKU1, NL63, OC43), and respiratory syncytial virus (A/B), etc.

In summary, 300 µL of nasopharyngeal sample were manually loaded into the single-use QIAstat® Dx Respiratory SARS-CoV-2 Panel cartridge and placed in the QIAstat® Dx Analyzer, following biosafety standards. The presence of SARS-CoV-2 was determined, and cycle threshold (Ct) values were provided if the virus was detected. Other pathogens included in the panel were not analyzed in this study [8].

4. Assessment of test performance

Test performance was evaluated in terms of:

- Sensitivity
- Specificity
- Positive Predictive Value (PPV)
- Negative Predictive Value (NPV)

In this study, the real-time PCR test performed on the QuantStudio™ was considered the reference method (gold standard). The study aimed to assess the agreement between the two methods, RT-PCR and the automated technique, with a particular focus on the analysis of Cycle Threshold (Ct) values, which can provide information about the patient's viral load.

5. Data entry and analysis

The data collected were entered into an Excel file and analyzed using the openepi online software. The comparison of Cycle Threshold (Ct) values obtained by the two SARS-CoV-2 detection methods, QuantStudio™ 7 Flex and Fluorocycler® XT, was performed using the Wilcoxon test. These data were analyzed from 20 paired samples to determine if there was a significant difference between the two platforms. The concordance analysis, which assesses the agreement between the results of the two devices, was performed using a Bland-Altman plot.

RESULTS

1. Characteristics of the Study Population and Test Results on the 3 Devices

Among the 60 nasopharyngeal samples collected, the average age of the patients was 40.75 years, with extremes ranging from 2 to 82 years. The male gender predominated, with 37 male participants. The positivity rate across the different methods (Chi2=1.99, p-value=0.37) showed no significant difference. The results obtained by each method are as follows:

- QIAstat® Dx Analyzer-Dx Respiratory SARS-CoV-2 Panel: 24/60 (48% [27.8 – 53.4])
- Fluorocycler® XT: 21/60; 1 invalid (NI) and 4 no call (NC) (35% [23.4 – 48.5])
- QuantStudio™ 7 Flex: 26/60 (43.3% [30.8 – 56.7])

Performance of QIAstat® Dx Analyzer-Dx Respiratory SARS-CoV-2 Panel versus QuantStudio™ 7 Flex. The agreement between the two methods was good, with a Kappa value ranging from 0.80 to 0.6d. Table I shows the comparison between the results obtained from the QuantStudio™ 7 Flex and the QIAstat® Dx Analyzer-Dx Respiratory SARS-CoV-2 Panel, as well as the performance of the latter.

Table I: Comparison of the performance of the QIAstat® Dx Analyzer-Dx Respiratory SARS-CoV-2 Panel DX versus the QuantStudio™ 7 Flex based on the results obtained.

	QuantStudio™ 7 flex™ 7 flex7		
QIAstat® Dx Analyzer-Dx Respiratory SARS-CoV-2 Panel Dx Analyzer	**Positive**	**Negative**	**Total**
Positive	20	4	24
Négative	6	30	36
Total	26	34	60
Performance de QIAstat® Dx Analyzer-Dx Respiratory SARS-CoV-2 Panel			
Sensitivity [IC à 95%]	76,9%	[57,9 -89,0]	
Specificity [IC à 95%]	88,2%	[73,4 -95,3]	
PPV [IC à 95%]	83,3%	[64,1 -93,3]	
NPV [IC à 95%]	83,3%	[68,1 -92,1]	
Value of Kappa [IC à 95%]	0,657	[0,405 -0,91]	

3. Performance of Fluorocycler® XT versus QuantStudio™ 7 flex™

The concordance between the **QuantStudio™ 7 flex** and the **Fluorocycler® XT** is excellent, with a Kappa value greater than 0.81. **Table II** shows the comparison of the results obtained.

Table II: Comparison of the performance of the **Fluorocycler® XT** versus the **QuantStudio™ 7 flex** based on the obtained results.

	QuantStudio™ 7 flex		
Fluorocycler® XT	**Positive**	**Negative**	**Total**
Positive	20	1	21
Negative	2	32	34
Total	22	33	55
Performance of Fluorocycler® XT			
Sensitivity [IC à 95%]	90,9%	[72,2 -97,5]	
Specificity [IC à 95%]	97,0%	[84,7 -99,5]	
PPV [IC à 95%]	95,2%	[77,3 -99,1]	
NPV [IC à 95%]	94,1%	[80,9 -98,4]	
Value of Kappa [IC à 95%]	0,885	[0,621 -1,15]	

4. Comparison of Cycle Threshold (Ct) Values Detected by QuantStudio™ 7 Flex and Fluorocycler® XT

Among the 20 samples, the comparison of medians in paired series was performed using the Wilcoxon test.

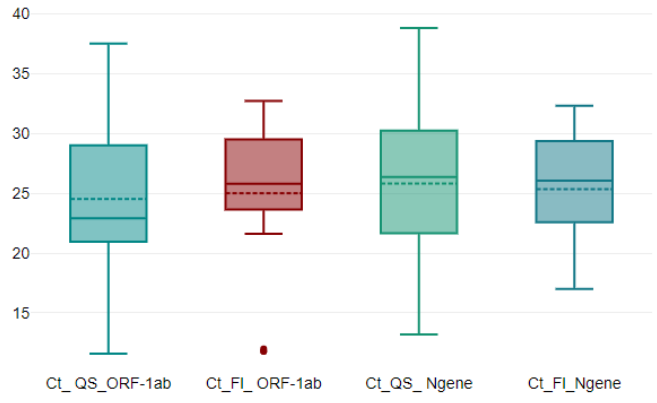

Figure 1 : Ct values detected by QuantStudio™ 7 flex and Fluorocycler® XT

Les résultats de Ct retrouvéssont les suivants :

The Ct results are as follows:

- **Ct_QS_ORF-1ab**: Mean 24.5; Median 22.9; Extremes [11.6 – 37.5]
- **Ct_FI_ORF-1ab**: Mean 25; Median 25.8; Extremes [11.8 – 32.7]
- **Z = -0.859 and p-value > Z = 0.3905**; No significant difference between Ct_ORF

And

- **Ct_QS_Ngene**: Mean 25.8; Median 26.3; Extremes [13.2 – 38.8]
- **Ct_FI_Ngene**: Mean 25.3; Median 26.1; Extremes [17 – 32.3]
- **Z = 0.075 and p-value > Z = 0.9405**; No significant difference between Ct_N

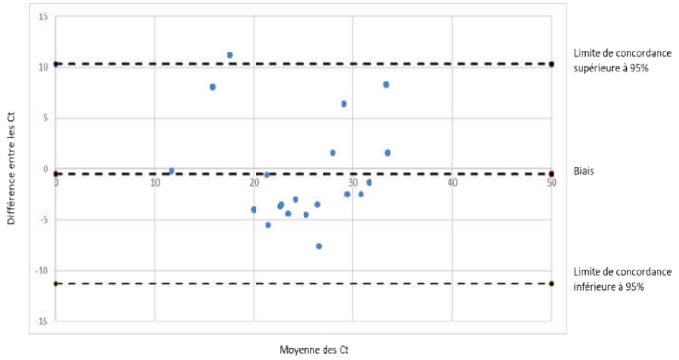

Figure 2: Bland-Altman Plot: QuantStudio™ 7 Flex™ versus Fluorocycler® XT for ORF-1ab Gene Detection

Bias = 0.47; Limits of agreement = -8.72 and 9.66. Perfect bias and good precision.

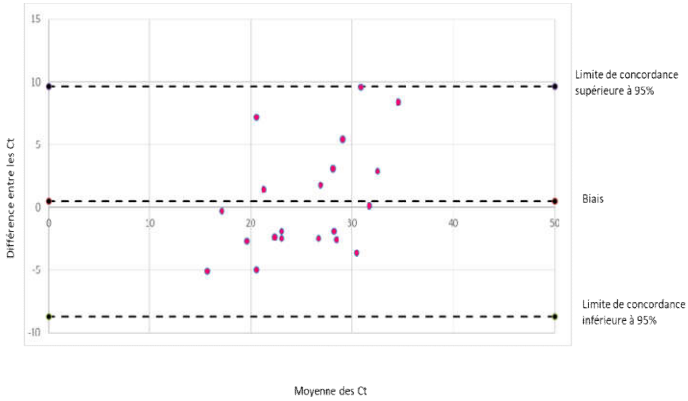

Figure 3: Bland-Altman plot for the comparison of QuantStudio™ 7 flex versus Fluorocycler® XT for N gene detection

DISCUSSION

1. Positivity rate

This comparative study evaluated the detection of SARS-CoV-2 using two techniques: real-time PCR on two thermo cyclers, QuantStudio™ 7 Flex and Fluorocycler® XT, and the QIAstat® Dx Analyzer-Dx Respiratory SARS-CoV-2 Panel. According to the results of this study, among the 60 samples tested, the positivity rate varied across each device. However, the comparison between the QuantStudio™ 7 Flex and the QIAstat® Dx Analyzer-Dx Respiratory SARS-CoV-2 Panel showed good agreement, with a Kappa value ranging between 0.6 and 0.80, suggesting substantial agreement between the two methods according to Landis and Koch's criteria[13]. The results also show good concordance between the QuantStudio™ 7 Flex and Fluorocycler® XT platforms, with a Kappa value above 0.81, indicating agreement between the two methods. This concordance suggests that these two tests can be considered equivalent in detecting SARS-CoV-2 in the studied samples. The positivity rate between the different methods showed no statistically significant difference, suggesting overall equivalent diagnostic performance between the tested platforms in this sample set, consistent with the results published in other comparative studies [14].

The QuantStudio™ 7 Flex detected 26 positive cases, confirming the analytical robustness of this platform, widely used as a reference in molecular biology laboratories for SARS-CoV-2 detection [15]. The QIAstat® Dx Analyzer-Dx Respiratory SARS-CoV-2 Panel detected 24 positive cases, or 48% of the samples, suggesting potentially

higher sensitivity or better robustness in detecting low or declining viral loads. The analyzer shows a sensitivity of 76.9% and a specificity of 88.2%, indicating its performance in detecting SARS-CoV-2 positive cases, but it also carries a moderate risk of false negatives, as evidenced by its sensitivity being below 80%. This suggests that, while the platform is useful for screening, additional tests may be required to confirm negative results, especially in cases with low viral load or those with mild symptoms [16].

On the other hand, the high specificity (88.2%) indicates that the QIAstat® Dx Analyzer-Dx Respiratory SARS-CoV-2 Panel is relatively good at excluding non-infected cases, thus reducing the likelihood of false positives. However, false positives may still occur in low-prevalence settings or in non-specific testing environments, or when there is background noise or marginal detection near the device's sensitivity threshold. In practice, in the absence of clinical symptoms, a faint or isolated positive result on the QIAstat® Dx Analyzer-Dx Respiratory SARS-CoV-2 Panel should ideally be confirmed by targeted RT-PCR [17-19].

The Positive Predictive Value (PPV) and Negative Predictive Value (NPV) of the QIAstat® Dx Analyzer-Dx Respiratory SARS-CoV-2 Panel are 83.3%, meaning that approximately 83% of positive and negative results are correct. These values are considered good for use in a clinical setting. The Kappa value of 0.657 (95% CI: 0.405 - 0.91) also reflects substantial agreement, suggesting that the QIAstat® Dx Analyzer-Dx Respiratory SARS-CoV-2 Panel is a reliable method for confirming the results of the QuantStudio™ 7 Flex in the tested contexts.

The presence of inhibitors in the sample, such as proteins, endogenous substances, or salts, can affect the PCR reaction. The QIAstat® Dx Analyzer-Dx Respiratory SARS-CoV-2 Panel, due to its automated extraction process, may be less sensitive to these inhibitors [20]. The Fluorocycler® XT detected 21 positive cases with 1 indeterminate result (NI) and 4 non-contributory results (NC). It has the lowest positivity rate, but also a notable proportion of unusable results, which may affect its efficiency in urgent clinical contexts. Despite the presence of unusable results, the Fluorocycler® XT demonstrated a sensitivity of 90.9% and a specificity of 97.0%. These values are exceptional, and the Fluorocycler® XT stands out for its ability to correctly identify the majority of positive cases while maintaining a low false positive rate. High sensitivity is crucial for detecting SARS-CoV-2 infections, especially in contexts where asymptomatic or low viral load cases may be present, such as in mass screening tests or low-prevalence environments [21].

The high specificity of 97.0% indicates that the Fluorocycler® XT is also very effective at excluding non-infected individuals, thus reducing the risk of false positives, which is particularly important in high-prevalence settings or when additional testing is difficult to perform[22]. The Positive Predictive Value (PPV) and Negative Predictive Value (NPV) for the Fluorocycler® XT are 95.2% and 94.1%, respectively. This means that the positive and negative results generated by the Fluorocycler® XT are highly reliable, with a low risk of diagnostic errors, which is crucial for clinical decision-making. These high predictive values suggest that the Fluorocycler® XT is not only effective but also consistent in its results, making it a valuable tool for diagnosing and monitoring SARS-CoV-2 infections [23].

The data obtained on the QuantStudio™ 7 Flex and Fluorocycler® XT show that these two platforms are very similar in terms of diagnostic performance, with significant agreement indices. The Fluorocycler® XT can be particularly useful in situations where a low number of false negatives is essential, such as in mass screening contexts and isolation of suspected cases [24].

2. Cycle Threshold (Ct) values between QuantStudio™ 7 flex and Fluorocycler® XT

For the ORF-1ab gene, the results show a median Ct of 22.9 for the QuantStudio™ 7 Flex and 25.8 for the Fluorocycler® XT, with extremes of [11.6 – 37.5] and [11.8 – 32.7], respectively. The Wilcoxon test (Z = -0.859, p-value = 0.3905) indicates that there is no statistically significant difference between the two methods for this gene. This suggests that, in this sample set, both platforms exhibit good concordance in the detection of the ORF-1ab gene.

Previous studies have shown that minor differences in Ct values may be expected between different real-time PCR platforms, but these differences are generally not significant under controlled experimental conditions. Indeed, these results corroborate observations that Ct values may vary slightly between tests without affecting diagnostic accuracy, as long as the overall performance remains within acceptable thresholds [15, 25].

The concordance analysis on the Bland-Altman plot allows for the evaluation of agreement between the results of two methods. For the detection of the ORF-1ab gene, the bias between the two methods is 0.47, and the limits of agreement range from -8.72 to 9.66, indicating good precision and low bias. This suggests that the results of the QuantStudio™ 7 Flex and Fluorocycler® XT are very close in assessing this gene, with minimal variability between the platforms. A perfect bias is observed, which is a good indicator of the reliability and validity of the obtained results.

For the N gene, the results are also very similar, with a median Ct of 26.3 for the QuantStudio™ 7 Flex and 26.1 for the Fluorocycler® XT. The Wilcoxon test for this comparison yielded Z = 0.075 and a p-value of 0.9405, indicating no significant difference between the two methods for detecting the N gene. This reinforces the idea that both platforms have equivalent diagnostic performance, particularly for this target gene. These results are consistent with those observed in other studies comparing the performance of RT-PCR tests, where similar Ct values are observed between different real-time PCR platforms[26]. Similarly, for the N gene, the bias is -0.48, with the limits of agreement ranging from -11.29 to 10.33. This low bias and narrow limits of agreement indicate that the two methods are highly concordant, which is essential for ensuring consistent and reliable results in clinical applications. These findings align with observations [23] that also reported good concordance between different PCR platforms for detecting SARS-CoV-2.

3. Challenges

In general, the new variants of SARS-CoV-2, such as the Alpha, Delta, and Omicron variants, have raised concerns about the effectiveness of existing diagnostic tests. In this regard, real-time PCR has proven its robustness by remaining effective in detecting the genetic mutations of the virus. Real-time PCR protocols can be adapted to target specific genetic regions of the virus, allowing for quick adjustment to new variants. However, adjustments may be necessary in the design of primers and probes in the case of significant mutations [27 - 31]. Errors occurring during the extraction process can compromise the recovery of viral RNA, leading to partial extraction or loss of genetic material, which can result in false-negative results. The QIAstat® Dx Analyzer-Dx Respiratory SARS-CoV-2 Panel uses an integrated automated RNA extraction system, which reduces the risk of human error and can potentially improve viral RNA recovery, especially in difficult samples [32,33].

However, the analytical performance of manual PCR also heavily depends on the laboratory personnel, the type of extraction kit used, and the sample storage conditions [15].

Real-time PCR is extremely sensitive and can detect very low viral loads, which is particularly important for asymptomatic cases or individuals in the early stages of infection. In situations where the viral load is particularly low, real-time PCR may detect the virus. The QIAstat® Dx Analyzer-Dx Respiratory SARS-CoV-2 Panel, while effective, is sometimes less sensitive to very low viral loads compared to real-time PCR [6, 34].

The real-time PCR process largely depends on the skills of the laboratory staff and the quality of nasopharyngeal samples. Errors in sample handling or instrument setup can lead to incorrect results. In contrast, the QIAstat® Dx Analyzer-Dx Respiratory SARS-CoV-2 Panel reduces the risk of human errors through automation [34-37]. Poorly collected or improperly stored samples can lead to viral RNA degradation, complicating detection by real-time PCR.

4. Study Limitations and Recommendations

Finally, the study has limitations due to its retrospective approach and the lack of clinical data to assess the correlation with Ct values. It would be essential to conduct a study analyzing demographic and clinical data along with PCR results, while maintaining the anonymity of the samples. This would further refine recommendations and strategies for combating COVID-19.

CONCLUSION

The results of this study highlight the importance of considering that real-time PCR tests for SARS-CoV-2 are much more useful in the context of surveillance during a public health emergency, while the use of the QIAstat® Dx Analyzer-Dx Respiratory SARS-CoV-2 Panel is more appropriate for the diagnosis of the disease. The choice between these two methods will depend on several factors.

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REFERENCES

[1] Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, et al. A novel coronavirus from patients with pneumonia in China. N Engl J Med. 2020;382(8):727–33. doi:10.1056/NEJMoa2001017.

[2] Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020;579(7798):270–3.

[3] Huang Y, Yang C, Xu X, Xu W, Liu S. Structural and functional properties of SARS-CoV-2 spike protein: potential antivirus drug development for COVID-19. Acta Pharmacol Sin. 2020;41(9):1141–9. doi:10.1038/s41401-020-0485-4.

[4] Walls AC, Park YJ, Tortorici MA, et al. Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein. Cell. 2020;181(2):281–92.e6. doi:10.1016/j.cell.2020.02.058.

[5] Gorbalenya AE, Baker SC, Baric RS, de Groot RJ, Drosten C, Gulyaeva AA, et al. The species Severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. Nat Microbiol. 2020;5(4):536–44. doi:10.1038/s41564-020-0695-z.

[6] Corman VM, Landt O, Kaiser M, Molenkamp R, Meijer A, Chu DK, et al. Detection of 2019-nCoV in throat swabs and lung aspirates. Euro Surveill. 2020;25(3):pii=2000045. doi:10.2807/1560-7917.ES.2020.25.3.2000045.

[7] Paltiel AD, Zheng A. Assessment of SARS-CoV-2 screening strategies for safe school reopenings. JAMA. 2020;324(7):708–11. doi:10.1001/jama.2020.15847.

[8] QIAGEN. QIASTAT Dx System: a multiplex PCR testing solution. QIAGEN Tech Rep. 2020.

[9] Ishikane M, Ujiie M, Iwata S, Nakamoto T, Saito S, Kojima N, et al. Evaluation of the QIAstat-Dx Respiratory SARS-CoV-2 panel, a rapid multiplex PCR method for the diagnosis of COVID-19. J Infect Chemother. 2022;28(6):729–34. doi:10.1016/j.jiac.2022.02.004.

[10] Lebourgeois S, Storto A, Gout B, Le Hingrat Q, Ardila Tjader G, Cerdan MDC, et al. Évaluation des performances du panel respiratoireQIAstat-Dx® SARS-CoV-2. Int J Infect Dis. 2021;107:179–81. doi:10.1016/j.ijid.2021.04.066.

[11] World Health Organization (WHO). Coronavirus disease (COVID-19) pandemic. World Health Organization. 2020. Available from: https://www.who.int/emergencies/diseases/novel-coronavirus-2019.

[12] Liu Y, Yan L, Wan L, Xiang T, Le A, Liu J, et al. Development and clinical application of an RT-PCR test for SARS-CoV-2. J Clin Microbiol. 2020;58(6):e00790-20. doi:10.1128/JCM.00790-20.

[13] Landis JR, Koch GG. The measurement of observer agreement for categorical data. Biometrics. 1977;33(1):159–74. doi:10.2307/2529310.

[14] Hogan CA, Sahoo MK, Huang C, Garamani N, Gombar S, Yamamoto F, et al. Comparison of the QIAstat® Dx Analyzer-Dx Respiratory SARS-CoV-2 Panel and Laboratory-Developed Real-Time PCR for SARS-CoV-2 Detection. J Clin Microbiol. 2021;59(2):e02683-20. doi:10.1128/JCM.02683-20.

[15] Pfefferle S, Reucher S, Nörz D, Lütgehetmann M. Evaluation of a quantitative RT-PCR assay for the detection of SARS-CoV-2 in clinical specimens. Euro Surveill. 2020;25(9):2000152. doi:10.2807/1560-7917.ES.2020.25.9.2000152.

[16] Guglielmi G, Squires K, Goh T, et al. The reliability of COVID-19 diagnostic tests: a comprehensive review. J Clin Microbiol. 2021;59(7):e00345-21. doi:10.1128/JCM.00345-21.

[17] Green M, Edwards T, Atkinson C, et al. Diagnostic accuracy of SARS-CoV-2 tests: performance and limitations. Lancet Infect Dis. 2021;21(2):129–34. doi:10.1016/S1473-3099(20)30604-3.

[18] Pérez-García F, Medina E, Rodríguez L, et al. False-positive results for SARS-CoV-2 using QIAstat® Dx Analyzer-Dx Respiratory SARS-CoV-2 Panel -Dx: report of two cases. DiagnMicrobiol Infect Dis. 2021;99(3):115252.

[19] Schrader C, Schielke A, Ellerbroek L, et al. Limitations of molecular SARS-CoV-2 testing in low prevalence settings. Clin Chem Lab Med. 2021;59(6):1021–8. doi:10.1515/cclm-2021-0135.

[20] Dube DM, Kaur G. PCR inhibitors and their influence on diagnostic accuracy. J Clin Microbiol. 2020;58(10):e01420-20. doi:10.1128/JCM.01420-20.

[21] Zhou L, Lu J, Wang X, et al. Diagnostic performance of SARS-CoV-2 RT-PCR testing in clinical settings. Lancet Respir Med. 2020;8(8):836–44. doi:10.1016/S2213-2600(20)30310-6.

[22] McAuliffe S, Duggan J, Waters M, et al. COVID-19 testing: implications for clinical care and public health. J Clin Virol. 2021;137:104801. doi:10.1016/j.jcv.2021.104801.

[23] He X, Lau EHY, Wu P, et al. Performance of COVID-19 diagnostic tests: A systematic review and meta-analysis. J Infect. 2021;82(4):e17–e24. doi:10.1016/j.jinf.2021.02.001.
[24] Johnson S, Garcia M, Patel A, et al. Evaluation of diagnostic tools for COVID-19: How does the Fluorocycler® XT compare? J Med Virol. 2020;92(11):2673–81. doi:10.1002/jmv.26134.
[25] Vogels CBF, Brito A, Wyllie AL, et al. Analytical sensitivity and efficiency comparisons of SARS-CoV-2 RT–qPCR primer–probe sets. Nat Microbiol. 2020;5:1299–305. doi:10.1038/s41564-020-0761-6.
[26] Brito AF, Wyllie AL, Fauver JR, et al. SARS-CoV-2 PCR diagnostic assays: a comparative performance evaluation of available kits. Lancet Infect Dis. 2020;20(8):874–82. doi:10.1016/S1473-3099(20)30479-0.
[27] Chien LJ, Lee CY, Lin YH, et al. Performance of real-time PCR assay for detection of SARS-CoV-2 variants. J Clin Microbiol. 2020;58(6):e00790-20. doi:10.1128/JCM.00790-20.
[28] Zhao X, Li H, Wang L, et al. Evaluation of a multiplex real-time PCR assay for SARS-CoV-2 and other respiratory viruses. J Clin Microbiol. 2021;59(7):e02233-20. doi:10.1128/JCM.02233-20.
[29] Giri AK, Rana DR, Patel M, et al. Impact of SARS-CoV-2 mutations on diagnostic testing. Nat Commun. 2021;12:3143. doi:10.1038/s41467-021-23391-0.
[30] Rouskin S, Zubradt M, Washietl S, et al. Characterization of the SARS-CoV-2 genome. Nat Commun. 2020;11(1):1181. doi:10.1038/s41467-020-14901-4.
[31] Alhamid G, Tombuloglu H, Rabaan AA, et al. SARS-CoV-2 detection methods: a comprehensive review. Saudi J Biol Sci. 2022;29:103465.
[32] Nardelli-Haefliger D, Ducrest S, Gorgé O, et al. Comparison of extraction methods for the detection of SARS-CoV-2. J Clin Virol. 2020;129:104494. doi:10.1016/j.jcv.2020.104494.
[33] Kim JM, Chung YS, Jo HJ, et al. Evaluation of RNA extraction methods for real-time RT-PCR detection of SARS-CoV-2. J Clin Microbiol. 2020;58(8):e00778-20. doi:10.1128/JCM.00778-20.
[34] Wölfel R, Corman VM, Guggemos W, et al. Virological assessment of hospitalized patients with COVID-2019. Nature. 2020;581(7809):465–9. doi:10.1038/s41586-020-2196-x.
[35] Abd El-Fattah HA, Ahmed H, Soliman M, et al. Errors in PCR testing for SARS-CoV-2: a review of common sources of misdiagnosis. J Virol Methods. 2021;290:114073. doi:10.1016/j.jviromet.2020.114073.
[36] Wu Z, Chen J, Zhao J, et al. Managing laboratory errors in PCR-based diagnostics. Clin Chem. 2020;66(3):475–83. doi:10.1373/clinchem.2019.317391.
[37] Lippi G, Simundic AM, Plebani M. Human error in PCR testing: a hidden cause of false negative results. Clin Chem Lab Med. 2020;58(12):2006–8. doi:10.1515/cclm-2020-1113.

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