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Research article

Assessing the performance of LumiraDx™ SARS-CoV-2 Ag test in detecting Omicron lineages: 2022–2023 study

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ABSTRACT

Background: The introduction of rapid antigen tests revolutionized the approach to SARS-CoV-2 diagnosis, offering prompt and accurate results with high sensitivity and specificity. Although it is more cost- and time-saving than the gold standard, real-time polymerase chain reaction (RT-PCR), the efficacy in general population screening in both hospital- and community-based settings remains unknown. Moreover, rapid antigen testing is limited by qualitative results. This study aims to evaluate the diagnostic reliability of the LumiraDx™ rapid antigen test during the Omicron era and to investigate its quantitative (analogue-to-digital converter (ADC)) results in comparison with RT-PCR Ct values.

Methods: This prospective study included all adult patients with mild-to-moderate SARS-CoV-2 symptoms who were not hospitalised and did not require oxygen supplementation, consented to participate, and attended the Infectious and Tropical Diseases Unit of Padua University Hospital from July 14th, 2022 to January 3rd, 2023. The patients underwent two different tests simultaneously: a nasal LumiraDx™ swab and a real-time RT-PCR assay performed on a nasopharyngeal swab. Sampling was repeated several times for a subset of subjects.

Results: We enrolled 266 consecutive participants and collected 601 pairs of LumiraDx™ and RT-PCR samples. The most prevalent variant was BA.4/BA.5 Omicron (60.2 %). The sensitivity and specificity of LumiraDx™ test when compared to real-time RT-PCR results as the reference standard were 93.1 % and 79.75 %, respectively. No significant differences in diagnostic reliability were found based on the available characteristics, age, sex, symptom status, or COVID-19 variant, except for the days from symptom onset. According to the multilevel logistic regression analysis, the only independent variable significantly associated with test concordance was the Ct value (adjusted odds ratio (OR) = 0.56, $p < 0.001$). Significant differences in quantitative ADC values were found between false negative (FN) *versus* true negative (TN), and false positive (FP) and true positive (TP) tests.

Conclusions: This study showed that LumiraDx™ test is reliable for SARS-CoV-2 diagnosis in patients with mild-to-moderate SARS-CoV-2 symptoms. This finding confirms the efficacy of rapid antigen tests in monitoring vulnerable individuals during the current post-vaccination era. When

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compared with the RT-PCR, LumiraDx™ test effectively quantitatively distinguishes between FN and TN cases, as well as FP and true TP tests, despite inaccuracies in qualitative results.

1. Introduction

Significant progress has been made since the first case of atypical pneumonia of unknown aetiology emerged in China in 2019 [1]. Over 750 million cases have been officially reported worldwide in approximately four years since the declaration of the COVID-19 pandemic [2]. Due to its high contagiousness and a global population naïve to Severe Acute Coronavirus Syndrome 2 (SARS-CoV-2), public health responses were among the strongest ever in all communities and health systems.

In addition to restrictions, service reorganisation, and mass vaccination implementation, the development of a testing strategy to promptly identify individuals with SARS-CoV-2 infection and reduce its circulation has been a top priority since the first phase of the pandemic.

Over time, international regulatory agencies have closely monitored real-time data and provided guidelines for the uniform and optimal management of pandemic emergencies across countries.

During the initial months of the SARS-CoV-2 pandemic, molecular assays were the only accessible testing method. However, due to the shortage of real-time RT-PCRs, these tests are recommended primarily for symptomatic individuals. With the realisation that asymptomatic individuals could also transmit the virus, the testing strategy shifted to universal testing, which introduced more rapid and affordable tests. Despite debates surrounding the reliability and optimal use of antigen tests, they have been adopted because of cost and time constraints, leading to a significant decline in the role of molecular tests over time [3].

As viral circulation persists, new variants emerge, prompting concerns regarding their unknown microbiological and immunological properties [4]. International regulatory agencies have emphasised the importance of the strict surveillance of Variants of Concern (VOCs) and Variants of Interest (VOIs) in sequencing. Consequently, the testing approach involves widespread antigen tests for the general population and targeted molecular tests for specific populations, enabling comprehensive monitoring of epidemiological trends.

In the autumn-winter season of 2022, the testing strategy will evolve further because of the co-circulation of other respiratory viruses, including influenza and respiratory syncytial viruses. The European Center for Disease Prevention and Control (ECDC) recommends the implementation of objective-driven and sustainable testing strategies for COVID-19 as a crucial component of the overall public health response to viral respiratory syndromes. This was facilitated by the robust response to the anti-SARS-CoV-2 vaccination campaign, which significantly reduced the risk of severe syndromes, especially among vulnerable populations [5].

In addition to vaccination, the health emergency has witnessed a surge in technological innovations in diagnostics. The introduction of new-generation rapid antigen testing has shifted the paradigm of rapid testing; in nearly the same amount of time as a lateral flow test, this technology yields qualitative results (positive or negative) with high sensitivity and specificity. Previous studies have identified this technology as a valuable solution for screening asymptomatic patients for acute SARS-CoV-2 infection, both in hospital settings and within the community [6]. Furthermore, a recent meta-analysis estimated pooled diagnostic sensitivity and specificity to be 0.86 (95 % confidence interval (CI), 0.84–0.88) and 0.99 (95 % CI, 0.98–0.99), respectively, for one of the first prototypes of microfluidic assays functioning as a decentralised testing device [7]. Although these data are suboptimal compared to the test performance originally reported by the manufacturer (97.6 % sensitivity and 96.6 % specificity), they still surpass the WHO requirements [8] (a minimum of 80 % sensitivity and 97 % specificity) and the more conservative ECDC proposal for the sensitivity parameter ($\geq$90 %) [9].

The performance of most modern rapid antigen tests is becoming increasingly close to that of the gold standard RT-PCR; however, they are limited to providing only qualitative results. Quantitative rapid antigen tests have not yet been reported.

Even if COVID-19 is no longer a public health emergency, further advancements in diagnostic technologies might offer not only more rapid and convenient alternatives to molecular testing, but also valuable quantitative results comparable to Ct values or virus cultures, informing clinicians about infectivity.

Therefore, this study aimed to assess the accuracy of the LumiraDx™ rapid antigen test for Omicron VOCs (BA.2, BA.4-5, BA.5, and BQ.1.1) and to investigate the quantitative (analogue-to-digital converter (ADC) results in relation to RT-PCR Ct values in a prospective cohort of fragile individuals with mild to moderate SARS-CoV-2 symptoms.

2. Materials and methods

2.1. Study settings

All consecutive adult patients referred to the Infectious and Tropical Diseases Unit COVID-19 outpatient clinic with SARS-COV-2 suspected infection between July 14th, 2022 to January 3rd, 2023 were included in the study. The study was conducted in accordance with the Declaration of Helsinki and the Principles of Good Clinical Practice. All the participants signed an informed consent form. The Padua Hospital Ethics Committee approved the study protocol (No. 02192–01/12/2023). During the first contact, all participants simultaneously underwent RT-PCR for SARS-CoV-2 and a rapid antigen nasal test LumiraDx™. The LumiraDx™ SARS-CoV2 Ag Test (LumiraDx UK Ltd, Dumyat Business Park, Alloa, FK10 2 PB, UK) is a rapid active microfluidic immunofluorescence assay intended for the qualitative detection of nucleocapsid protein antigen to SARS-CoV-2 in nasal or nasopharyngeal swab samples. LumiraDx claimed

that this test achieved a sensitivity of 97.6 % (95 % CI 91.6–99.3 %) and specificity of 96.6 % (95 % CI 92.7–98.4 %) up to 12 days after symptom onset [10]. LumiraDx also provided access to analogue-to-digital converter (ADC) values as part of the experiments conducted for this study. The ability to provide ADC values is not currently available for use with the LumiraDx™ SARS-CoV-2 Ag test, nor is it part of any product claim.

Nasopharyngeal swab sampling was performed in accordance with the ECDC guidelines for oropharyngeal/nasopharyngeal testing [11]. Nasal swabs for LumiraDx™ testing were obtained according to the manufacturer's instructions. Comprehensive information regarding the laboratory materials and methods employed for both RT-PCR and rapid antigen assays is available in our previous study [3]. Neither assay showed cross-reactivity of the endemic human coronaviruses HCoV-229E, HCoV-NL63, HCoV-OC43, and HCoV-HKU1 with MERS-CoV. The RT-PCR RdRp gene cycle threshold (Ct) values and SARS-CoV-2 variant typing were performed by the Microbiology and Virology Unit of Padova University Hospital, as described previously [12].

Individuals who tested positive for COVID-19 (for either the molecular or antigenic test) were prescribed an early antiviral treatment (such as remdesivir, molnupiravir, or nirmatrelvir/ritonavir) by an Infectious Disease specialist if they presented at least one of the following risk factor (according to Italian Drug Agency recommendations) for severe COVID-19 outcome: age equal or greater than 65 years, BMI over 35 kg or the 85th percentile, nephropathy, uncontrolled diabetes, chronic respiratory disease, cardiovascular disease, acquired or congenital immunodeficiency, addiction to technological device, neurologic disorders, and haemoglobinopathies [13–15]. Previously hospitalised or COVID-19 related oxygen therapy patients were excluded from the study. Symptom onset should not have occurred more than 7 days before the first day of therapy in at-risk patients, while immunocompromised patients must have a late worsening of the clinical status and a positivity duration of no more than 28 days.

Throughout the study, the participants were divided into symptomatic and paucisymptomatic groups. An individual was defined as symptomatic when they experienced fever, difficulty breathing, or at least two of the following symptoms: dysgeusia, anosmia, sore throat, myalgia, gastroenteritis, tachypnoea, cough, cold, asthenia, or headache. Conversely, we used the term "paucisymptomatic" to describe a subject who did not have fever or mild difficulty breathing, and had a maximum of two of the secondary symptoms mentioned above.

Multiple samples were available over time for a subset of subjects, and the double-testing procedure described above was repeated for each visit to the Infectious and Tropical Diseases Unit COVID-19 outpatient clinic during the study period.

2.2. Statistical analysis

Population characteristics and test results were summarised using frequencies, percentages, medians, and interquartile ranges (IQR), as appropriate. Chi-square and Student's *t*-tests were used to compare the percentages and mean values among the various subgroups.

To assess the diagnostic reliability of the LumiraDx™ rapid antigen test the following performance metrics were computed: sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy. Real-time RT-PCR results were assumed to be the ground truth. The mean RT-PCR Ct value for the first positive test was also obtained.

All parameters were stratified by age, sex, symptomatic status, number of days elapsed since symptom onset, testing time, and COVID-19 variant.

Both the LumiraDx™ test diagnostic performance and the mean real-time RT-PCR Ct were also evaluated considering the sampling temporal order (first, second, etc.).

The correlation between rapid antigen test reliability and both Ct values and the number of days from symptom onset were further investigated by i) plotting the bivariate distribution (via multivariate kernel density estimation) and the marginal distributions by the LumiraDx™ test–real-time RT-PCR concordance (true positive (TP) *versus* false negative (FN)) and ii) computing the sensitivity metric jointly stratified by these two variables. The correlation and association between Ct values and the number of days elapsed from symptom onset were tested using Spearman's rank test and a univariate linear regression model.

Univariate and multivariable multilevel logistic regressions were performed on real-time RT-PCR positive tests, considering the concordance between the LumiraDx™ test and real-time RT-PCR outcome as dependent variables, and sex, age, days from symptoms, Ct values, symptomatic status, and COVID-19 variants as independent covariates. Having undergone more than one test per person, the subject identifier was used as a grouping variable, and a separate random effect (intercept) was modelled for each patient to account for individual-level variability.

Finally, LumiraDx™ platform quantitative ADC statistics were obtained. Pairwise comparisons by RT-PCR outcome concordance group (FN *versus* true negative (TN), and false positive (FP) *versus* TP) and Ct values (<30.6 *versus* ≥ 30.6) were conducted using the Wilcoxon-signed rank test, while p-values were adjusted according to the Bonferroni method. The ADC and Ct values for bivariate distribution and linear association were analysed using the methodology described above.

Confidence intervals (CI) at the 95 % level for proportions and odds ratios (OR) were approximated using the Wald formula, while 95 % CI for the mean was obtained from the Student's *t*-distribution. Results were considered statistically significant at the p-value <0.05.

Data cleaning, visualisation, and analysis were performed using statistical packages in Python 3.8.8 (Python Software Foundation, Fredericksburg, TX, USA). A multilevel logistic regression analysis was conducted using R 4.2.2 (R Core Team, Vienna, Austria).

3. Results

3.1. Study population

During the study period, 266 individuals were consecutively referred to the Infectious and Tropical Diseases Unit for testing for SARS-CoV-2 infection. The median age was 67 years (IQR:19 years), and 159 patients (59.8 %) were males. We simultaneously performed RT-PCR and LumiraDx™ tests and collected 601 sample pairs. Each subject was tested, on average 2.26 ± 1.39 times.

There were 198 symptomatic (74.44 %) and 68 paucisymptomatic (25.56 %) patients.

The majority of tests were sampled within one week of symptom onset (382, 63.6 %), with the most prevalent variant being BA.4/BA.5 Omicron (362, 60.2 %). All sample demographics and other available characteristics at the time of testing are summarised in Table 1 and Supplementary Table S1.

3.2. LumiraDx™ tests diagnostic reliability

Among the 601 tests, the outcome of the LumiraDx™ test was false negative (FN) in 36 cases and false positive (FP) in 16 cases. As a whole, indeed, the LumiraDx™ test presents a sensitivity of 93.1 % (90.6, 94.98), a specificity of 79.75 % (69.6, 87.13), a positive predictive value (PPV) of 96.81 % (94.89, 98.03), a negative predictive value (NPV) of 63.64 % (53.82, 71.44), and an overall accuracy of 91.35 % (88.83, 93.34).

Table and Supplementary Tables S1 and S2 show the results of the LumiraDx™ test accuracy metrics stratified by available variables. No significant differences were found in test sensitivity according to age, sex, symptom status, or COVID-19 variant. Only the number of days from symptom onset was confirmed to affect test reliability: LumiraDx™ test sensitivity was statistically significantly reduced when performed after one (82.8, 95 % CI 76.0, 88.0) or two weeks (77.8, 95 % CI 45.3, 93.7) from the first symptoms than when performed within a maximum of 6 days (97.8, 95 % CI 95.7, 98.9).

3.3. Impact of Ct values and days from symptoms' onset on LumiraDx™ test sensitivity

The Ct value for the first test was available for 246 of the 266 subjects. The mean value at first swab collection was 22.5 (95 % CI 21.9, 23.0). No significant variations in Ct distribution were found for different demographic classes, considering neither COVID-19 variants nor the presence of symptoms (Table 1, S1). As expected, the only feature for which statistically significant differences were found was, again, time since symptoms onset: the average Ct value was 22.1 (95 % CI 21.6, 22.6) for individuals who underwent their initial test within one week of symptom onset, and 27.2 (95 % CI 25.6, 28.9) for those who were tested within two weeks of symptom onset.

Investigating the behaviour of Ct values and the diagnostic responses of the LumiraDx™ test at different times during the course of infection (first, second, third, or fourth swab sampling) confirmed the same results. Ct value increased with time from 22.4 (95 % CI 21.9, 22.9) to 26.7 (95 % CI 25.6, 27.8), while sensitivity decreased from 97.6 % (95 % CI 94.9, 98.9) to 85.7 % (95 % CI 70.6, 93.7), with significant differences only between the first and subsequent tests (Supplementary Table S3).

Table 1
Descriptive statistics, outcomes (+= positive; - = negative), mean Ct values, and estimated sensitivity and specificity of the LumiraDx™ test compared with standard real-time RT-PCR stratified by characteristics presented at testing time.

	Testing n (%)	+ LumiraDx™ test n (%)	+ RT-PCR n (%)	Mean Ct (95 % CI)	FN n (%)	FP n (%)	Sensitivity (95 % CI)	Specificity (95 % CI)
Days elapsed from symptoms onset at testing time								
0–6	382 (63.6)	361 (71.9)	361 (69.2)	22.1 (21.6, 22.6)	8 (22.2)	8 (50.0)	97.8 (95.7, 98.9)	61.9 (40.9, 79.3)
7–13	199 (33.1)	131 (26.1)	151 (28.9)	27.2 (25.6, 28.9)	26 (72.2)	6 (36.5)	82.8 (76.0, 88.0)	87.5 (75.3, 94.1)
14–20	18 (3.0)	8 (1.6)	9 (1.7)	25.0 (-, -)	2 (5.6)	1 (6.3)	77.8 (45.3, 93.7)	88.9 (56.5, 98.0)
21–27	2 (0.3)	2 (0.4)	1 (0.2)	– (-, -)	0 (0.0)	1 (6.3)	100.0 (20.7, 100.0)	0.0 (0.0, 79.5)
COVID-19 variant								
BA.4/BA.5	362 (60.2)	326 (64.9)	334 (64.0)	21.2 (20.6, 21.7)	15 (41.7)	6 (37.5)	95.5 (92.7, 97.3)	77.8 (59.2, 89.4)
BQ1.1	54 (9.0)	50 (10.0)	53 (10.2)	20.4 (19.0, 21.7)	3 (8.3)	0 (0.0)	94.3 (84.6, 98.1)	100.0 (20.7100.0)
BA.2	15 (2.5)	12 (2.4)	13 (2.5)	20.9 (17.0, 24.8)	1 (2.8)	0 (0.0)	92.3 (66.7,98.6)	100.0 (34.2, 100.0)
B.1.1.529	1 (16.6)	1 (0.2)	1 (0.2)	17.0 (-, -)	0 (0.0)	0 (0.0)	100.0 (20.7, 100.0)	– (-, -)
Low viral load	169 (28.1)	113 (22.5)	120 (22.9)	27.3 (26.5, 28.1)	17 (47.2)	10 (62.5)	85.8 (78.5,91.0)	79.6 (66.4, 88.5)
Overall	601 (100.0)	502 (100.0)	522 (100.0)	22.5 (21.9, 23.0)	36 (100.0)	16 (100.0)	93.1 (90.6, 95.0)	79.8 (69.6, 87.1)

The number of days elapsed from symptom onset was positively associated with the Ct value (linear regression coefficient = 0.56 (95 % CI 0.45, 0.66), $p < 0.001$, Spearman correlation = 0.47, $p < 0.001$). The estimated linear relationship is shown in Fig. 1. The image shows the bivariate kernel density estimation and marginal distributions of Ct values and days after symptoms in the TP and FN cases (red and blue, respectively). False-negative tests were characterised by a higher number of days (first quartile = 7, median = 9, third quartile = 10 *versus* first quartile = 3, median = 4, third quartile = 7) and higher Ct values (first quartile = 29, median = 31, third quartile = 32 *versus* first quartile = 21, median = 23, third quartile = 27) than TP tests ($p < 0.001$ in both cases). In the multivariate density plot, the probability density related to FN was concentrated on much higher Ct values and days elapsed from symptom onset than those assumed by TP.

Fig. 1 also shows the sensitivity heat map of the LumiraDx™ test, depending on the thresholds of both variables. According to the colour gradient, the higher the Ct values (lower the viral load) and the number of days elapsed from symptom onset, the lower the sensitivity. When the Ct value was less than 25, the sensitivity was always 100 %, whereas for swabs sampled no more than 5 days after the first symptoms, the sensitivity of the test was always above 98.3 % (95 % CI 96.1, 99.5). In contrast, considering all tests in which the Ct value is less than or equal to 30, the sensitivity remains above 96.8 % (95 % CI 94.7, 98.2). For higher thresholds of days or Ct values, the LumiraDx™ test diagnostic reliability drops until a sensitivity minimum of 93.9 % (95 % CI 91.4, 95.9).

Finally, according to the results of the multilevel logistic regression analysis (Table 2), the only independent variable significantly associated with concordance was the Ct value ($p < 0.001$). The estimated odd ratio is 0.56 (95 % CI 0.43, 0.72), indicating that every time the Ct value increases by one unit, the probability of obtaining the same outcome from the LumiraDx™ test and real-time RT-PCR decreases by 44 %.

3.4. Experimental quantitative detection

The mean analogue-to-digital converter (ADC) values measured by the quantitative platforms among (RT-PCR defined) positive and negative cases were 9374.3 ADC (std 1789.7, median 10,000), and 4207.7 ADC (std 2730.1, median 2933.3), respectively (Table 3). It is noteworthy that 5000 ADC are the threshold values set by the manufacturer to discriminate the presence of SARS-CoV-2, whereas 10,000 ADC represent the full scale of the software used.

Fig. 2A illustrates the distribution of the quantitative values obtained from the platform based on the qualitative results and concordance with the molecular test. Statistically significant differences were observed when comparing values for tests with the same

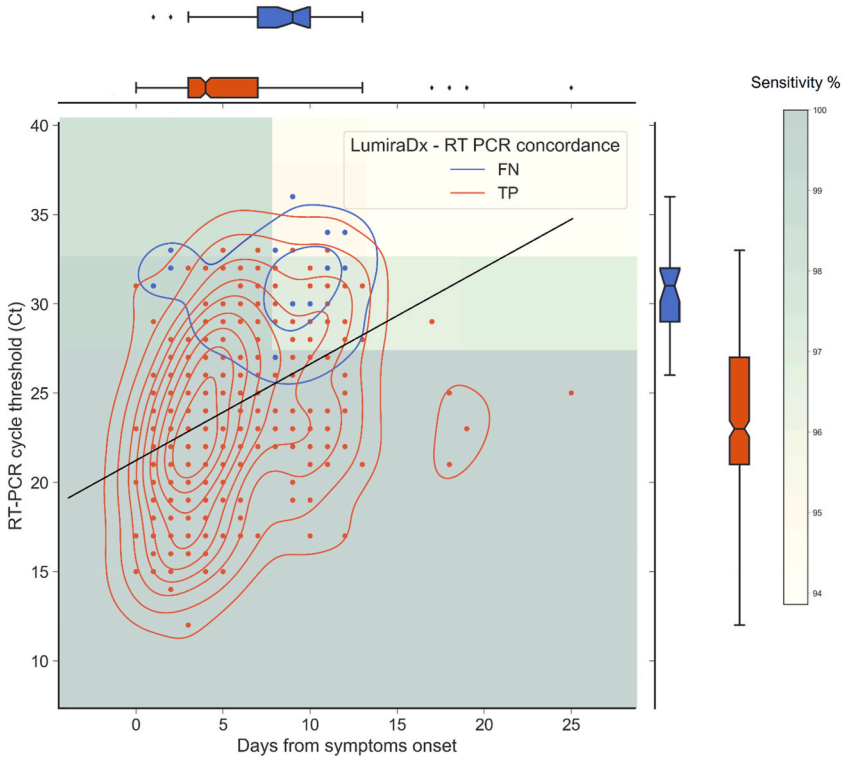

Fig. 1. Bivariate kernel density estimation and marginal distributions of Ct values and days after symptoms among true positive (TP) and false negative (FN) cases. The black oblique line represents the estimated linear association (regression coefficient = 0.56 (95 % CI 0.45, 0.66), p-value <0.001, Spearman correlation = 0.47, p-value<0.001). The background heat-map represents the sensitivity variation with respect to both Ct values and days cumulative threshold. The darker the colour, the higher the level of sensitivity of the LumiraDx™ test compared to the standard real time RT-PCR.

Table 2
Multilevel logistic regression models estimates and p-values on real time RT-PCR positive records. The dependent variable is the concordance between the LumiraDx™ test and real time RT-PCR (positive class = Yes, TP; negative class = No, FN).

Independent variable	Univariate model			Multivariable model		
	OR	95 % CI	p-value	Adj OR	95 % CI	p-value
Gender (reference Female)						
Male	1.48	(0.70, 3.16)	0.308	1.75	(0.58, 5.23)	0.319
Age						
	0.99	(0.97, 1.02)	0.624	0.99	(0.95, 1.03)	0.646
Ct value						
	0.57	(0.44, 0.73)	<0.001	0.56	(0.43, 0.72)	<0.001
Days from symptoms onset						
	0.56	(0.41, 0.75)	<0.001	0.88	(0.73, 1.05)	0.161
Symptoms (reference Paucisymptomatic)						
Symptomatic	0.814	(0.32, 2.05)	0.662	0.83	(0.24, 2.93)	0.775
COVID-19 variant (reference BQ1.1)						
BA.2	0.77	(0.07, 8.03)	0.824	0.17	(0.006, 5.10)	0.310
BA.4/BA.5	1.44	(0.396, 5.26)	0.578	1.12	(0.15, 8.19)	0.914
B.1.1.529	–	–	1.00	1.07	(-, -)	0.949
Low viral load	0.51	(0.14, 1.88)	0.308	1.01	(0.13, 7.80)	0.994

Table 3
RT-PCR Ct and quantitative platform ADC values distribution by SARS-CoV-2 positivity and tests concordance.

RT-PCR		n	RT-PCR Ct value			ADC value		
			mean	median	std	mean	median	std
-		79	–	–	–	4207.7	2933.3	2730.1
	LumiraDx™							
	FP	16	–	–	–	9305.8	10,000.0	1402.3
	TN	63	–	–	–	2912.9	2718.7	700.2
+		522	24.1	24.0	4.5	9374.3	1,0000.0	1789.7
	LumiraDx™							
	FN	36	30.6	31.0	2.4	3479.4	3421.8	872.0
	TP	486	23.6	23.0	4.3	9810.9	10,000.0	784.6
	Ct							
	<30.6	438	23.3	23.0	4.0	9709.2	10,000.0	1202.6
	≥30.6	40	32.1	32.0	1.1	6587.5	6889.8	3081.7

qualitative LumiraDx™ test outcome but different RT-PCR reports (FN *versus* TN and FP *versus* TP). Specifically, FN tests exhibited significantly higher ADC than TN (3479.4 *versus* 2912.9, p adj. = 0.002, Supplementary Table 3), and vice versa for FP and TP tests (9305.8 *versus* 9810.9, p adj. = 0.002). Therefore, the platform, even when qualitatively returning an incorrect outcome, quantitatively succeeds in discriminating doubtful cases on average. This result is confirmed even when stratifying ADC by Ct values (<30.6 *versus* ≥30.6) and grouping by LumiraDx™ concordance (Table 3 and Supplementary Fig. S1): LumiraDx™ quantitative values are statistically significantly higher when Ct values are below 30.6.

Testing the relationship between ADC and Ct values (among only rapid molecular swab pairs with positive PCR results), a significant negative association was estimated (linear regression coefficient = −0.0012, p-value <0.0001; see Fig. 2B). Therefore, low values on the quantitative platforms were associated with higher Ct values and hence lower viral loads. It is also observed that for $Ct \leq 22$, the quantitative platform always reported the maximum possible value, 10,000 ADC, when $22 < Ct \leq 25$ the average was instead lowered to 9873.2 ADC, but 95.3 % of the quantitative values were exactly equal to 10,000.

Consistent with the results described previously, for $25 < Ct \leq 33$, some FNs occurred (in about 13.9 % tests pairs ADC was below the threshold of 5000), however, in more than 70 % of cases the ADC remained equal to 10,000. Finally, for Ct values greater than 33, the platforms always returned negative responses.

4. Discussion

The aim of this study was to evaluate the diagnostic reliability of the LumiraDx™ test in comparison with the gold standard Real-Time PCR testing. The focus was on the Omicron VOCs of SARS-COV-2, which spread globally following the delta variant. The main Omicron lineages sequenced were BA.2, BA.4-5 (an undetermined tract among BA.4 and BA.5), BA.5, and BQ.1. These VOCs were not equally represented in absolute numbers and time; in the late summer season of 2022, the dominant variant was BA.4-5, which had been over classed by BA.5. During the last weeks of the study, variant BQ.1.1, was sequenced for the first time in Italy and quickly replaced the previously prevalent BA.5 variant.

SARS-CoV-2 remains far from being eradicated, and the resolution of the emergency status of the pandemic does not mean the end of testing strategies. Currently, prioritised objectives focus on vulnerable patients; however, implementing syndromic monitoring of

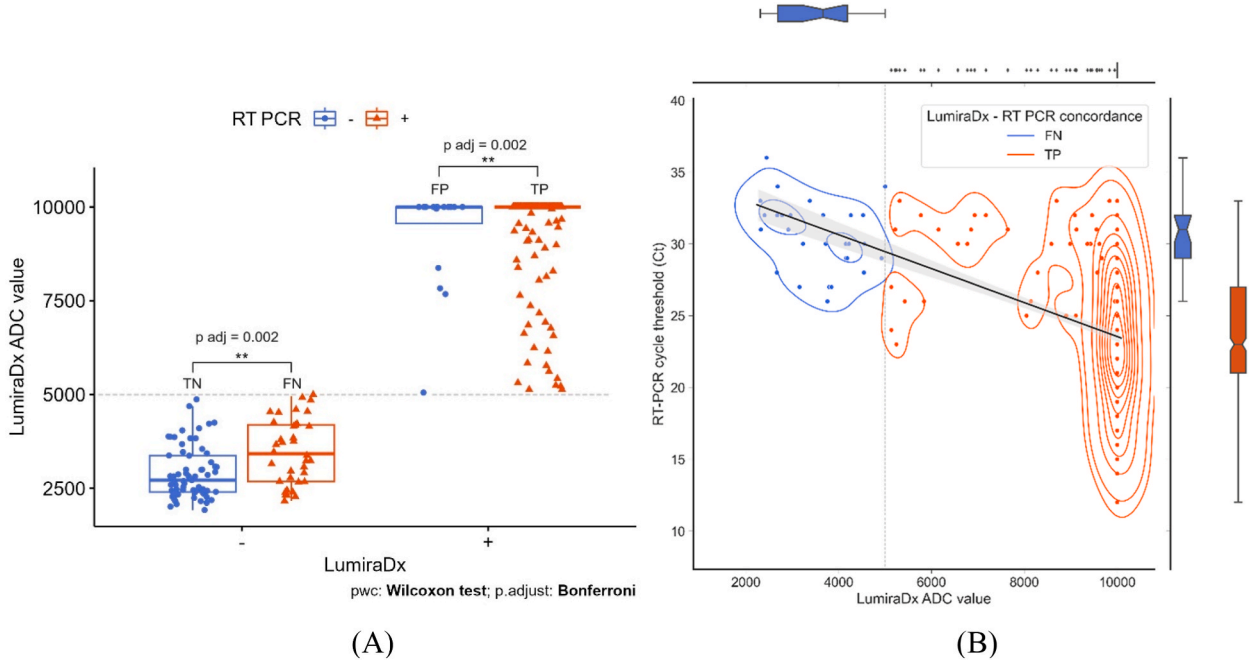

Fig. 2. ADC (Analog to Digital Converter) quantitative value distribution. (A) Grouped boxplots comparing ADC distribution between TN, FN, FP and TP results. Statistical significance was assessed conducting pairwise Wilcoxon-signed rank test, p-values were adjusted according to Bonferroni method. (B) Bivariate kernel density estimation and marginal distributions of Ct and ADC values. The black oblique line represents the estimated linear association (regression coefficient = −0.0012, p-value <0.0001), the grey shade area represents the 95 % confidence bands. Note that 5000 ADC is the threshold value discriminating SARS-CoV-2 presence (dashed Grey line).

respiratory viruses across the entire community remains challenging.

In this study, we specifically evaluated the diagnostic reliability of the LumiraDx™ test in a cohort of fragile individuals with mild-to-moderate SARS-CoV-2 symptoms who were at risk of developing severe COVID-19 due to pre-existing morbidities or predisposing clinical conditions. In this setting, we estimated slightly lower sensitivity (93.1 %) and specificity (79.75 %) values than those declared by the manufacturer; however, the results were acceptable according to the minimum requirements of the WHO [8] and ECDC [9]. Similar performances were measured by Gresh et al. [6], who systematically tested patients suspected of having SARS-CoV-2 infection in two different urgent care centres located in MA, USA (n = 2241, sensitivity = 96.2 %). Instead, a study conducted by Ota et al. [16] on 84 patients with mild COVID-19 estimated greater sensitivity (100 %) but lower specificity (27.6 %) than virus cultures. In general, the measured performance agreed with the pooled diagnostic accuracy obtained in a meta-analysis of 11 studies (n = 8527 samples) assessing LumiraDx™ testing effectiveness [7].

Our findings, along with those from recent studies [17], confirmed the high reliability of rapid antigen tests when dealing with the Omicron variant (sensitivity above 90 %) and demonstrated good performance even at low viral loads (sensitivity surpassing 80 % for Ct values between 25 and 30). Under these conditions, the LumiraDx™ test outperformed the other rapid antigenic tests [17,18]. This suggests that the test currently in use may demonstrate efficacy against future variants of concern (VOCs).

No significant variations in performance were found for the main demographic characteristics, especially symptom severity, among the different lineages of Omicron. Conversely, we can conclude that FN tests were characterised by a higher number of days since the onset of symptoms (median = 9 *versus* median = 4) and a higher Ct value (median = 31 *versus* median = 23) than TP tests ($p < 0.001$ in both cases). Considering only patients who were tested for at least 10 days after symptom onset, the sensitivity increased to 94.7 %. In a real-world scenario, twice per month testing strategy for residents of long-term care facilities and nursing homes could be considered sustainable; hence, such sensitivity seems affordable. Domínguez Fernández et al. [19] tested a small sample (n = 24) to assess LumiraDx™ usefulness in deciding whether to end isolation in a nursing home and confirmed that despite a generally low sensitivity (52.63 %), the antigen test was negative when the RT-PCR showed elevated Ct values (>31), especially after >14 days of infection. Therefore, although a non-concordant negative result, LumiraDx™ correctly identified subjects with non-infectious viral loads in vulnerable groups in which access to molecular tests could be more difficult. Analogous results were also found in a cohort of asymptomatic adults and children (confirmed to be symptom-free for at least two weeks before testing) [20]. LumiraDx™ correctly identified 95.8 % of the samples that were confirmed positive in >33 RT-PCR Ct and 100 % in >30 RT-PCR Ct while maintaining a 100 % true-negative rate. Hence [20], confirmed the feasibility of LumiraDx™ testing for general population screening, particularly in asymptomatic subjects.

Finally, we evaluated the LumiraDx™ platform's quantitative results. We could not find an exact relationship between ADC and Ct values from the PCR results, but we demonstrated a statistically significant negative association. In addition, we reported that FN tests

were characterised by a significantly higher ADC than TNs, and vice versa for FP and TP tests. Therefore, even when qualitatively returning an incorrect outcome, the platform quantitatively succeeded in discriminating none or low viral-load cases from high viral-load cases. ADC values, although not as precise as Ct values, could provide an acceptable indication of the most infectious cases and guide clinicians in choosing isolation or the administration of antiviral therapy. We believe that integrating these technologies into platforms can offer a comprehensive clinical evaluation of patients from all angles. Providing a rapid and accessible means to estimate the viral load could introduce a new criterion for allocating antiviral drugs exclusively to specific vulnerable populations. Future innovation should not solely entail the creation of new technology but rather the feasible and smarter utilisation of existing technologies.

4.1. Study limitations

Our inclusion criteria focused on individuals with a high suspected likelihood of COVID-19, potentially introducing a selection bias and limiting the generalisability of our findings to individuals with asymptomatic viral presentations. Moreover, the performance of this test in patients with respiratory failure has not been specifically addressed. Finally, we did not investigate the potential effects of antiviral therapy on the diagnostic accuracy of the test, which is an important consideration in the context of the evolving treatment strategies for COVID-19. Future research should explore these aspects to provide a comprehensive understanding of the utility of rapid antigen testing in diverse clinical scenarios and treatment contexts.

5. Conclusion

The novel generation of rapid antigen tests is confirmed to be an effective diagnostic tool for detecting Omicron lineages, which were prevalent in Europe in 2022 and are still widely circulating. This study tested LumiraDx™ test in a cohort of vulnerable patients. These findings confirm the efficacy of the rapid antigen test in individuals who require timely diagnosis and careful monitoring.

While the COVID-19 pandemic has slowed down, one of the lessons we learned was the importance of a bio surveillance system capable of monitoring the circulation of multiple respiratory viruses (different lineages of SARS-CoV-2 and other respiratory viruses). Similarly, we need to improve the capability of identifying vulnerable patients to promptly provide them with appropriate treatment. Therefore, it is imperative to explore new evidence to classify patients, particularly those identified as vulnerable, into distinct risk levels for severe syndromes. From this perspective, we emphasise the importance of developing novel strategies to prevent and protect vulnerable groups. This approach is crucial not only for addressing COVID-19, but also for mitigating other respiratory syndromes during the approaching autumn and winter seasons.

Ethics statement

The study was conducted according to the Helsinki Declaration and Principles of Good Clinical Practice. All participants were required to sign informed consent. The study protocol was approved by Padua Hospital Ethic Committee (n. 02192 - December 01, 2023).

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Data availability statement

Data will be made available on request.
Researchers interested in accessing the data for academic and non-commercial purposes may contact the corresponding author.

CRediT authorship contribution statement

Silvia Cocchio: Writing – review & editing, Methodology, Investigation, Conceptualization. **Michele Nicoletti:** Writing – review & editing, Writing – original draft, Investigation, Data curation. **Claudia Cozzolino:** Writing – review & editing, Writing – original draft, Visualization, Software, Formal analysis. **Maria Mazzitelli:** Writing – review & editing, Validation, Resources. **Nicola Bonadiman:** Writing – review & editing, Investigation, Data curation. **Samuele Gardin:** Writing – review & editing, Investigation, Data curation. **Lolita Sasset:** Writing – review & editing, Resources. **Melissa Zucconi:** Writing – original draft, Software, Formal analysis. **Anna Maria Cattelan:** Writing – review & editing, Supervision, Resources, Investigation. **Vincenzo Baldo:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Not applicable.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e33229.

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