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Research article

Performance characteristics of the boson rapid SARS–cov–2 antigen test card vs RT–PCR: Cross–reactivity and emerging variants

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ABSTRACT

Background: SARS–CoV–2 virus has undergone several mutations on its genome, since the onset of the pandemic. Multiple variants of concern (VOC) have emerged including Alpha, Beta, Gamma, and Delta with the more recent one being the Omicron (B.1.1.529). Specific rapid antigen tests (RADs) have been used for the detection of SARS–CoV–2. However, since the emergence of new VOCs, the performance characteristics of these RADs needs to be re–evaluated.

Objectives: The main purposes of this clinical study were to determine the diagnostic sensitivity and specificity of the BOSON Rapid Antigen Test compared to the gold standard real time RT–PCR and to determine the ability of the RAD to accurately depict different VOC. Additionally, the cross reactivity to other viruses and pathogen, as well as, the possible interference of non Covid–19 hospitalized patients for various causes, were investigated.

Results: A total of 623 individuals (symptomatic) were tested. The sensitivity, specificity and accuracy of the BOSON RAD was 95.27%, 100% and 98.45% (n = 448), meeting the WHO recommended standards. Additionally, the Delta (83.33%, Ct < 34) and Omicron (100%, Ct < 26) VOC were determined with high sensitivity. Also, there was no interference from hospitalized, non–Covid 19 patients, and no cross–reactivity was detected.

Conclusions: The study showed that this RAD could rapidly identify individuals with SARS–CoV–2, including those with the new dominant Omicron VOC, with no cross reactivity from other pathogens.

1. Introduction

Since its declaration as a world pandemic on March 2020 [1–3], the SARS–CoV–2 has undergone several mutations, and multiple

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variants of concern (VOC) or interest (VOI) have emerged [4]. Such mutations can radically alter the virus capacity to escape the immune system, thus increasing its pathogenic strength [5] and its spreading ability [6]. Earlier VOC included the B.1.1.7 (Alpha) [7–9], the B.1.351 (Beta) [10,11] and P.1 (Gamma) [12], with more recent ones the B.1.617.2 (Delta) and B.1.1.529 (Omicron) [4]. All five have mutations in the receptor binding (RDB) and the N–terminal (NTD) domains, enhancing their binding affinity and enhancing the subsequent entry of the virus into the cells [13]. The Omicron VOC has >30 mutations identified in the spike (S) protein, has a 13–fold increase in vital infectivity and is 2.8 times more infectious than Delta [14–16].

The SARS–CoV–2 is classified to the beta (β) coronaviruses (βCoV) sub–group [17,18]. These are single–stranded, positive sense RNA (+ssRNA), crown–like, enveloped, polymorphic viruses, about 60–140 nm in size [19–21]. The virus contains two open reading frames, ORF1a and ORF1b, that encode four major structural proteins: the nucleocapsid (N), the membrane (M), the envelope (E) and the S [22–27].

Rapid antigen (Ag) detection tests, targeting specifically the N or the S (S1 and S2) proteins have been used for the detection of SARS–CoV–2. Although their sensitivity is lower than the gold standard reverse transcription real–time PCR (RT–rtPCR), these RAD tests are cheap and can be used fast [28–31]. However, with the emergence of new VOC, the performance characteristics of RADs needs to be re–evaluated, for potential impairment of their sensitivity and specificity. Only a few studies to date have addressed the issue [32, 33]. The RAD validated here is a LFA test designed to rapidly and qualitative test individuals suspected of COVID–19, for the presence of SARS–CoV–2 antigens.

1.1. The aims of the clinical study were to determine

- the diagnostic sensitivity and specificity of the BOSON Rapid SARS–CoV–2 Ag Test Card (Xiamen Boson Biotech Co. Ltd., Fujian, P. R. China) for the rapid qualitative detection of SARS–CoV–2 Ag, when compared in parallel (blind) with a gold standard RT–rtPCR, the Sacace™ SARS–CoV–2 Test Kit (Sacace™ Biotechnologies Srl, Como, Italy).
- the ability of the RAD to accurately detect different VOC (Delta and Omicron).
- the possible interference of hospitalized, non Covid–19 patients to the clinical performance of the RAD, and the potential cross reactivity of other viruses, such as, *Influenza* A and B, *Respiratory Syncytial* virus (RSV), *Metapneumovirus* (hMpv), *Parainfluenza* virus 1–4, *Human Coronavirus*, *Rhinovirus, Adenovirus*, *Bocavirus, etc.,* and pathogens to the RAD results.

2. Materials and Methods

2.1. Study duration and locations

This prospective study was carried out from December 2021 to February 2022. Prevalence of SARS–CoV–2 in Greece on February 28, 2022 was 23.35% [34–37]. The location was the exterior facilities of the Locus Medicus S.A. diagnostic center, Athens, Greece. Specimen collection was performed in a specifically designed isobox, where two different testing areas were set for sampling, each one divided in such a way to ensure privacy.

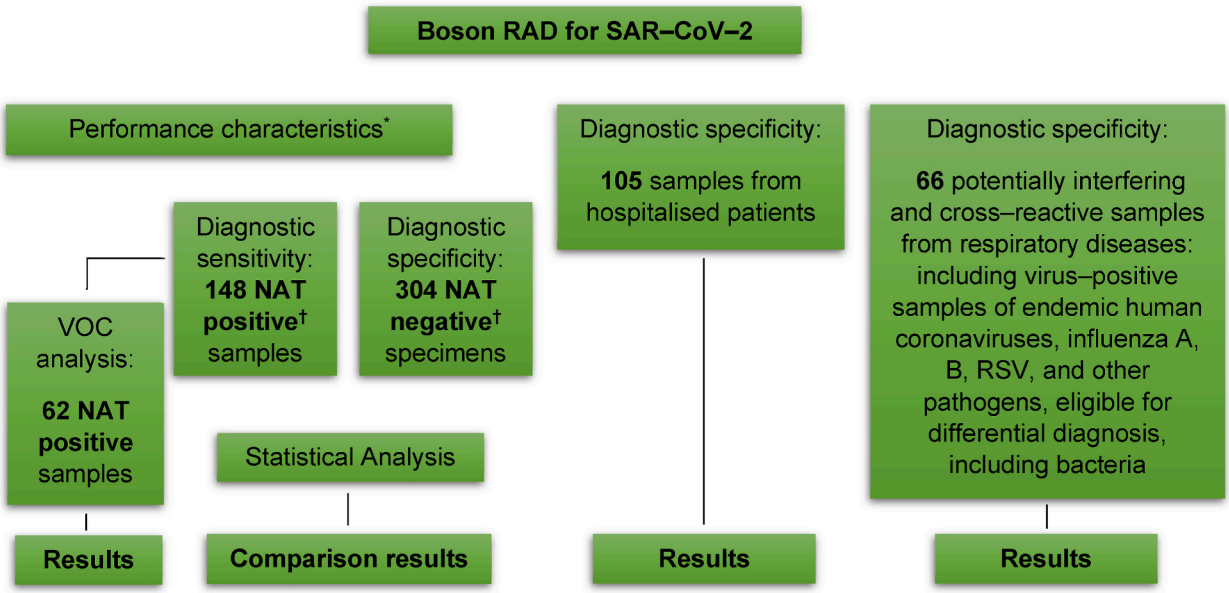

Fig. 1. Flowchart of the guidance points for the performance evaluation of SAR–CoV–2 in vitro diagnostic medical devices (IVD) showing the number of participants in each study. *Participant sample selection and test collection, as in Ref. [39]. †Matched samples from same individual.

2.2. Subject selection and sample size

A total of 623 individuals covering all age groups were included for all clinical trials (Supplementary Material S1) [38] (see Fig. 1).

- 452 nasal (NS) and nasopharyngeal (NP) samples were collected from symptomatic (from 0 to 7 days of symptoms onset) and asymptomatic (without a known SARS–CoV–2 exposure) individuals.
- 105 NS and NP samples were collected from hospitalized patients (negative for COVID–19).
- 66 negative cases, all of which had other clinical manifestations (e.g., respiratory infections), for cross reactivity investigation.

62 out of 148 positive SARS–CoV–2 identified RT–rtPCR specimens were further investigated for the ability of the test to accurately detect different VOC, Delta (B.1.617.2) and Omicron (B.1.1.529).

2.2.1. Subjects – symptomatic and asymptomatic patients

The study population included individuals that had chosen Locus Medicus S.A. to perform PCR or other diagnostic tests. Inclusion and exclusion criteria were similar to the ones described by Leventopoulos et al., 2022 [39]. Prior to performing the Boson RAD test, each subject was asked to sign a written informed consent form, and immediately after, was given information about the clinical trial process, duration, potential risks and benefits. For children <18 years of age, informed consent of the parents or legal guardians was required. Symptomatic patients were identified as those that were still exhibiting symptoms on the sample collection day, like fever, chills, cough, fatigue, muscle or body aches, new loss of taste or smell, sore throat, runny nose, etc. [40,41]. On the other hand, patients with no relevant clinical symptoms (self–perceived or clinically recognizable) 24 h prior to and after sample collection were identified as asymptomatic [40,41].

2.2.2. Professionals

Included the specialized laboratory staff and operators who performed the RT–PCR and the RADs, and who were "blind" to each other's results in order to eliminate bias (and vice versa).

2.3. Hospitalized patients

After the Ethical Approval and Informed Consent was obtained, 105 NS and NP samples (duplicates) were taken from patients hospitalized for various reasons (not Covid–19 infected patients), in two major Athens Hospitals, the Leto General, Maternity and Gynecology Clinic S.A. and the Athens Hospital, by specialized health professionals, following instructions and supervision by Locus Medicus professional staff. One sample was immediately tested using the RAD and the other was placed in appropriate viral transport medium (VTM) and send to Locus Medicus S.A. for testing with RT–rtPCR within an hour.

2.4. Experimental methods

2.4.1. Viral RNA extraction and detection using RT–rtPCR

The viral RNA extraction and detection using RT–rtPCR was carried out as previously described [39]. Briefly, collected NP samples were immediately transferred to the laboratory (Biosafety level II) for viral RNA extraction, using the High Pure viral nucleic acid kit (Roche Diagnostics, Mannheim, Germany). Detection of SARS–CoV–2 was carried out according to manufacturer's instructions (Supplementary Material S2). Briefly, a Real Time thermal cycler (SaCycle–96, Sacace Biotechnologies Srl, Como, Italy) was used for the RT–rtPCR, targeting the E and N genes specific for SARS–CoV–2 and a region of E gene common for all SARS–like coronaviruses (SARS–CoV, SARS–CoV–2). Moreover, prior to extraction, an internal control (IC, synthetic RNA) was added to the reaction to confirm the validity of negative results (i.e., absence of PCR inhibitors and proper RNA extraction). A single, one step reaction of 50 cycles was used in RT and amplification, according to manufacturer's instructions. In addition, the Accuplex SARS–CoV–2 kit (reference material 0505–0126, 5000 copies/mL) was used in order to test the reproducibility, repeatability and Limit of Detection (LOD) of the method. The LOD was set to 300 copies/mL, approximately equal to 30–32 cycles (ELOT IEC 15189:2012 standards).

2.4.2. Multiple detection of respiratory viruses using RT–rtPCR

Extracted viral nucleic acid samples of patients with symptoms (fever, cough, fatigue etc) that tested negative for SARS–CoV–2, were subsequently tested for the presence of viruses that cause acute respiratory infections, as per manufacturer's instructions. Briefly, reverse transcription of the RNA and multiplex Real Time PCR amplification followed for specific nucleic acid fragments of viral pathogens: RSV, hMpv, *Parainfluenza* virus 1–4, *Human Coronavirus* OC43, E229, NL63, and HKU1, *Rhinovirus, Adenovirus* (B, C, E) and *Bocavirus* (Sacace™ ARVI Screen Real–TM PCR kit, Sacace Biotechnologies Srl, Como, Italy; Supplementary Material S3). For *Influenza A* and *B,* the same three major processes were followed. Isolation of viral RNA, reverse transcription and real time amplification of cDNA. Similarly, the detection was based on the amplification of the viral genome specific region using specific primers and detection using fluorescent dyes (Sacace™ SARS–CoV–2/Influenza A/B multiplex Real–TM, Sacace Biotechnologies Srl, Como, Italy; Supplementary Material S4). In each reaction, positive, negative samples and an IC were used as amplification controls for each individually processed specimen, that fluorescence's in different channel.

2.4.3. Delta/omicron VOC detection using RT–rtPCR

Extracted viral RNA tested positive for SARS CoV–2 was subsequently tested for the presence of Delta or Omicron VOC. A master mix using specific primers and probes for S protein mutations for Delta (B.1.617.2) and Omicron (B.1.1.529) VOC was used. A reverse transcription and a melting curve analysis in a LightCycler 2.0 (Roche Diagnostics, Mannheim, Germany) according to manufacturer's instructions followed (Immundiagnostik AG, MutaPLEX® CoV–2 MUT 3 Real–Time–RT–PCR–Kit Bensheim, Germany; Supplementary Material S5). In each reaction reference positive samples for Omicron (Tm ~58.5 °C) and Delta (Tm ~64 °C) VOC, and negative sample were used (Fig. 2).

2.4.4. Boson rapid SARS–CoV–2 antigen test card

Samples were collected, processed and added samples according to the IFU of Boson Rapid SARS–CoV–2 Antigen Test Card (Xiamen Boson Biotech Co. Ltd., Fujian, P.R. China) and as previously described [39]. The test is based on the LFA technique, which applies the principle of the double–antibody sandwich method (described in detail in Supplementary Material S6). A positive result was considered when both the control (C) and SARS–CoV–2 antigen (T) lines appeared within the first 15–20 min.

2.5. Statistical analysis

Data were analyzed with SPSS (IBM Corp., USA) using Cohen's kappa analysis, for the comparison of the performance characteristics of the Boson RAD versus the Sacace™ RT–rtPCR. K value interpretations were: $0.75 < k \leq 1.00$ good, $0.40 < k \leq 0.75$ general, $0 = k \leq 0.40$ poor consistency. Furthermore, sensitivity, specificity, and accuracy values were calculated as in Leventopoulos et al. [39], according to the formulas presented in Supplemental Material S7. The normal approximation formula: $\widehat{p} \pm z \times \sqrt{\frac{\widehat{p}(1-\widehat{p})}{n}}$, was used to calculate the 95% confidence intervals (CIs), where $\widehat{p}$ describes sensitivity, specificity and accuracy, and $z = 1.96$. The p value was considered significant and set at <0.05.

3. Results

Data analysis of the 452 samples showed that the participants' age ranged from 6 to 85 years, with 229 being males (50.66%) and 223 (49.34%) females. The mean ± SD of age was 40 (SD = 17.65; range = 6–85). RT–rtPCR identified 32,74% positive (148/452) individuals with SARS–CoV–2. The RAD, identified the virus in 141 cases, with 95.27% (141/148; 95% CI: 90.56%–97.69%) sensitivity and 100.00% (304/304; 95% CI: 98.75%–100.00%) specificity. Total accuracy was 98.45% (445/452; 95% CI: 96.84%–99.25%) (Table 1). There was a good agreement between the Boson Rapid SARS–CoV–2 Antigen Test Card and the RT–rtPCR ($k = 0.964$, $p < 0.001$). Table 2 provides the results of the RAD in relation to the Ct values.

105 samples were collected from hospitalized patients (25 males, 23.81%; 80 females, 76.19%), tested with the RAD (NS) and tested for SARS–CoV–2 with RT–rtPCR (NP). All samples were found negative for Covid–19, with no interference from other conditions, suggesting good specificity of the RAD. The hospitalized conditions of the patients tested is provided in Table 3.

In order to investigate potential cross reactivity, samples (NS and NP) were taken from 66 patients (26 males, 39.4%; 40 females, 60.6%) with other respiratory symptoms. These samples were tested (Sacace™ ARVI Screen RT–PCR kit) for the multiplex detection of potentially interfering and cross–reactive respiratory viruses and pathogens, for differential diagnosis. Table 4 shows the distribution of pathogens identified. All samples were confirmed to be ***negative*** for SARS–CoV–2 with the RAD and with RT–rtPCR, revealing a consistency rate of 100%.

The ability of the RAD to accurately detect different VOC was based on melting curve analysis. From the 148 RT–rtPCR positive

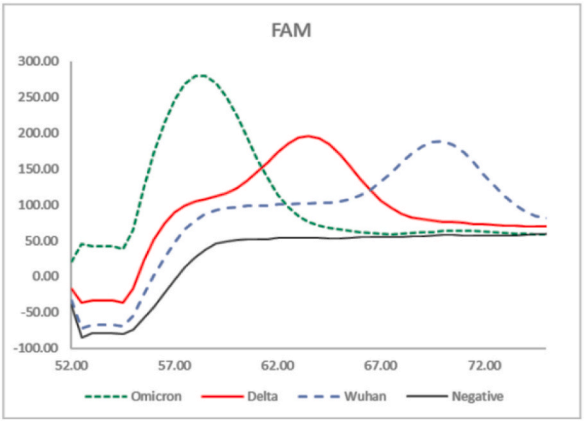

Fig. 2. Example of a real time RT–PCR melting curve analysis of the kits reference samples used that are positive for Delta VOC (red line, peak at ~64 °C), Omicron VOC (green dashed line, peak at ~58.5 °C), and Wuhan VOC (blue dashed line, peak at ~70 °C). Negative sample indicated by black line.

Table 1
Performance characteristics of the Boson Rapid SARS–CoV–2 Antigen Test Card versus the Sacace™ RT–rtPCR.

	RT–rtPCR		
Boson SARS–CoV–2 Ag Test	Positive	Negative	TOTAL
Positive	*141*	*0*	*141*
Negative	*7*	*304*	*311*
Total	*148*	*304*	*452*
Sensitivity	***95.27%*** *(90.56%–97.69%)* [a]		
Specificity	***100.00%*** *(98.75%–100.00%)*		
Total Coincidence rate	***98.45%*** *(96.84%–99.25%)*		

[a] 95% confidence intervals shown in parentheses.

Table 2
Rapid Test results for Different Ct values.

Ct value	Number of Samples	Detected by rapid test	Detection Rate
$Ct \leq 25$	129	129	100% (97.11%–100%)[a]
$25 < Ct < 30$	10	10	100% (72.25%–100%)
$Ct \geq 30$	9	2	22.22% (6.32%–54.74%)

LOD: 300 copies/mL approximately equal to 30–32 C t cycles.
[a] 95% confidence intervals shown in parentheses; Ct = Cycle threshold.

Table 3
Conditions of the hospitalized patients. In parentheses are the results for the Boson Rapid SARS–CoV–2 Antigen Test Card and RT–rtPCR. All results were negative.

Hospitalized Condition	No of cases (N = 105)	Percentage Accuracy
Abdominal Pelvic Pain	3	100% (3/3)
Cardiac Failure	6	100% (6/6)
Caesarean section	15	100% (15/15)
Chronic Kidney/Kidney/Renal Failure	12	100% (12/12)
Dementia	12	100% (12/12)
Fracture Spinal Cord	3	100% (3/3)
Gastric Hemorrhage	1	100% (1/1)
Generalized malignancy unknown origin	1	100% (1/1)
Heart Failure	1	100% (1/1)
Hepatic Fibrosis Kirrhosis	1	100% (1/1)
Hypotension	3	100% (3/3)
Hysterectomy	1	100% (1/1)
Labor	8	100% (8/8)
Lower Respiratory Infection	2	100% (2/2)
Lung Cancer	2	100% (2/2)
Mobility Difficulty/Mobility Malfunction	10	100% (10/10)
Ovarian Cyst Endometriosis	1	100% (1/1)
Quadriplegia	1	100% (1/1)
Renal Failure Final Stage	6	100% (6/6)
Stress Urinary Incontinence	1	100% (1/1)
Stroke	7	100% (7/7)
Tetraplegia	1	100% (1/1)
Ventricular Fibrillation	1	100% (1/1)
Weakness	6	100% (6/6)

identified SARS–CoV–2 specimens, 62 were further investigated for VOC, of which, 42 were Delta and 20 were Omicron, respectively. The RAD was able to identify 35/42 of the Delta VOC showing sensitivity of 83.33% at $Ct < 34$ (35/42; 95% CI: 69.40%–91.69%), whereas it was able to detect all 20 Omicron VOC samples at $Ct < 26$ (100%; 95% CI: 83.89%–100%).

4. Discussion

The present clinical study re–evaluated the performance characteristics of the BOSON Rapid SARS–CoV–2 Ag Test Card against RT–rtPCR [39], along with its ability to detect new emerging VOC, and the possible cross reactivities. The performance characteristics of the RAD were sensitivity at 95.27%, specificity at 100% and accuracy at 98.45%, meeting the recommended performance profile cutoffs established by the WHO [42,43]. In addition, these results confirmed previous observations made by our laboratory, where we have identified sensitivity, specificity and overall accuracy of the RAD at 98.18%, 100.00%, and 99.28%, respectively [39]. Moreover, our results are qualitatively similar to previously published results from other groups on performance characteristics of RADs [44–46].

Table 4
Cross–reactivity test results for the Boson Rapid SARS–CoV–2 Antigen Test Card and RT–rtPCR. All results were negative.

Viruses	SacaceTM SARS–CoV–2/Influenza A/B multiplex Real–TM	Boson Rapid SARS–CoV–2 Ag Test Card	Sacace™ SARS–CoV–2 RT–rtPCR
Influenza A virus	5	–	–
Influenza B virus	5	–	–
	Sacace™ ARVI Screen Real–TM PCR kit		
Adenovirus	10	–	–
Adenovirus and Parainfluenza virus–3 coinfection	2	–	–
Adenovirus and Coronavirus (NL-63, 229 E) coinfection	2	–	–
Respiratory syncytial virus, RSV	14	–	–
RSV and Parainfluenza virus–3 coinfection	2	–	–
Metapneumovirus	2	–	–
Bocavirus	16	–	–
Coronavirus (NL–63, 229 E)	4	–	–
Bacteria[a]	**Bacterial Cultures**		
Streptococcus pneumoniae	2	–	–
Streptococcus group A	2	–	–

[a] Identification of *Streptococci* was carried out using Gram stain and common bacterial cultures.

One very important aim was to investigate the accuracy of this RAD to detect emerging SARS–CoV–2 VOC, with few published data available so far on the analytical validation of RADs for new VOC [32,33]. The RAD detected Delta and Omicron with high sensitivity, respectively. The fact that the sensitivity rate of Omicron was higher than that of the Delta VOC is in contrast to the findings of other groups [47]. However, such an observation could be explained, because the seven false negative results of the Delta VOC had a mean cycle threshold (Ct) of 32.33 (SD = 1.08), which would be indicative of a low viral load, and as such potentially out of the detection limit of the RAD. On the other hand, all Omicron VOC had a mean Ct value of 16.87 (SD = 4.05) that would indicate a high viral load. Overall, these observations are in parallel with the results obtained from other studies which reported that RADs are capable to identify the Delta and Omicron VOC [48–52]. However, other studies have shown that the sensitivity of RADs was impaired when tested on Omicron VOC infected individuals [32,33]. As a result, for the successful identification, it is apparent that the time of testing during the epidemic curve is vital [53] and RADs must be used at a high frequency, during the initial stages of the infection to have a high sensitivity ratio. Since Omicron is considered more contagious than previous VOC, with less viral load capacity, it becomes apparent that the emergence of new, future VOC should be followed by the immediate reassessment of the performance characteristics of all RADs [32].

In relation to hospitalized patients, or potential cross–reactivity, the specificity of this rapid test card was found to be 100%, demonstrating no false–positive results. To our knowledge, the above cross reactivity comparison was one of the few that has been carried out on RADs, with similar results reported from other groups [30,54].

Our study has several strengths, such as the large number of human samples tested. Moreover, immediate use of the RAD, interpretation and analysis, limited the possibility of false positive or false negative results, that usually appears with extensive sampling duration and testing conditions. The implementation of such an approach was crucial for all methodological and experimental techniques carried out, since it has been known that the sensitivity of detection of any method/test depends largely on its duration and the conditions of the specimens [30]. On the other hand, the present study has limitations, such as, the low number of cases in each part of the study. Also, the vaccination status of the individuals could be a potential influencing factor on RAD sensitivity, however, most of the individuals (75.68%) in Greece have received at least one dose by the end of this study, with 72.57% fully vaccinated [55,56]. Moreover, we would like to acknowledge the fact that this study did not differentiate the performance characteristics of this RAD, between asymptomatic and symptomatic cases, since this analysis was carried out and evaluated in a recent publication by our laboratory [39]. In addition, due to the high viral loads of the participants in the study, the performance characteristics of the RAD during broad screening use, in lower poc incidence settings, could be limited. All the above limitations are important future considerations for the performance characteristics of all RADs [52]. Future considerations for the performance evaluation of this RAD would be to define the viral load distribution (i.e., performance characteristics at lower viral loads/higher Ct values) and kinetics, and its performance characteristics between unvaccinated and vaccinated individuals [52].

5. Conclusions

In conclusion, although RADs cannot be used as the sole basis to diagnose or exclude SARS–CoV–2 infection, however, this study demonstrated that the Boson Rapid Ag test could rapidly identify individuals with SARS–CoV–2, including the now dominant Omicron VOC, with high sensitivity and specificity, and with no direct cross reactivities. Given the importance of improved turn–around times and faster diagnosis of cases with transmittable virus, assessment of clinical sensitivity of RADs against new VOC is essential for optimal management of the pandemic.

Author contribution statement

MICHAIL LEVENTOPOULOS: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Vassiliki Michou: Conceived and designed the experiments; Performed the experiments; Wrote the paper.

Chrysoula Kyprianidou; Christos Meristoudis; Dimitris Nikolopoulos: Performed the experiments; Contributed reagents, materials, analysis tools or data.

Nikolaos George Manias: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Harilaos Panagiotis Kavvadas: Conceived and designed the experiments; Analyzed and interpreted the data.

Vassilis Tsilivakos: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Georgios Georgoulias: Conceived and designed the experiments; Performed the experiments; Contributed reagents, materials, analysis tools or data.

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Data availability statement

The authors do not have permission to share data.

Declaration of interest's statement

The authors declare no conflict of interest.

Additional information

Supplementary content related to this article has been published online at [URL].

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Appendix A. Supplementary data

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