Qualitative evaluation of the new rapid point-of-care antigen AFIAS COVID-19/Flu Ag Combo assay

Mara Lorusso[2], Edmondo Adorisio[1], Giuseppe Gagliardi[2], Achille Meneghini[3], Domenico Lagravinese[4], Mario Antonio Lerario[5]

[1]Clinical Pathology and Microbiology Operative Unit, and [2]Microbiology Unit, Di Venere Hospital, Asl Bari; [3]Self-employed consultant; [4]Asl Bari Prevention Department, Bari; [5]Civil Protection Apulia Region, Italy

Summary

Background. An *interim* guidance document by the World Health Organization (WHO) suggests the use of rapid diagnostic tests based on antigen detection as an alternative to the polymerase chain reaction (PCR) test for the diagnosis of SARS-CoV2 infection, when the molecular real time (RT) - PCR test is not available, or the turnaround time is excessive, precluding its clinical and/or public health usefulness. Rapid antigenic tests are recommended when a minimum of 80% sensitivity and 97% specificity are ensured.

Methods. Here we employ a new assay for screening applications based on lateral-flow immunofluorescence assay, with microfluidic technology (Boditech AFIAS COVID-19/Flu Ag Combo, Boditech Med. Inc., Chuncheon, Gangwon, Republic of Korea) on a point-of-care analyzer AFIAS-6 (Boditech Med. Inc.) and compare it with a reference molecular method and an alternative screening immunochromatographic method (Boditech AFIAS COVID-19 Ag). Our study was carried out on universal transport medium (UTM)(COPAN, Brescia, Italy) for viruses (at -20°C) samples of patients admitted to Di Venere Hospital of Bari, Italy, that were already tested with molecular methods.

Results. The new AFIAS COVID-19 Flu/Ag Combo test reached a clinical sensitivity of 92% on positive samples with Ct<30, and a clinical specificity of 97.9% on negative samples. Sensitivity is higher than the reference immunochromatographic test AFIAS COVID-19/Ag (92.0% *vs* 88.0%) while specificity remains unchanged (97.9% *vs* 98.0%). In addition, the new AFIAS Combo test confirms the same negative predictive value (NPV, 95.9%) of the rapid reference test (AFIAS COVID 19-Ag) and an agreement with the molecular test of 95.9% (Cohen's K = 0.908).

Conclusions. Considering its qualitative improvement, rapidity and ease of use we suggests AFIAS Combo test as a valid alternative to the reference lateral flow test (AFIAS COVID-19 Ag) and an adequate screening test.

Correspondence: Mara Lorusso.
E-mail:maralorusso81@libero.it

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Key words: SARS-CoV2; COVID-19; POCT; diagnosis; screening; immunoassay; rapid antigen test.

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Introduction

The third zoonotic human coronavirus (CoV) of the century emerged in December 2019, with a cluster of patients connected to Wuhan, Hubei Province, China. This virus, the newly identified coronavirus 2019-nCOV, could cause risky pneumonia so that prevention and control of the infection has become highly required. The 2019-nCoV is a member of the beta-coronavirus genus, that also includes severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV) (27). Molecular testing on nasal and oropharyngeal swabs represents gold standard for the diagnosis of SarsCoV-2 infection. Accurate rapid diagnostic tests for SARS-CoV-2 infection could contribute to clinical and public health strategies to manage the COVID-19 pandemic. Since it is identified that symptoms

become rapidly severe without a proper treatment after onset of illness, early diagnosis of the virus infection is quite crucial. Currently, the spread of the viral transmission become fast so that the prevention of local transmission requires a point-of care test (POCT) (9). An interim guidance document by the World Health Organization (WHO) (26) suggests the qualitative criteria of a rapid antigenic system for SARS-CoV2 suitable in cases where the molecular system is not available or when the TAT (total turnaround time) is such as to preclude its clinical usefulness. These are recommended when a minimum of 80% sensitivity and 97% specificity are ensured.

The above-mentioned limits were then adopted by the Italian Ministry of Health (17) which, referring to the European Center for Disease Prevention and Control (ECDC) (10) recommendations in low-prevalence contexts, recommends a minimum of 90% for sensitivity and 97% for specificity. Furthermore, it identifies third-generation tests (lateral flow immunoassay with fluorescence readout and microfluidic technology) as the only ones with similar qualitative performance to real-time polymerase chain reaction (RT-PCR) tests (1) and therefore suitable to be used (2,15).

Aim

The aim of this study is the qualitative evaluation of the new AFIAS COVID-19/Flu Ag Combo test as appropriate screening tool and its correlation to AFIAS COVID-19 antigenic test and with the molecular gold standard reference method. Both tests are distributed by Menarini Diagnostic, Firenze, Italy. The evaluation was performed at the COVID Laboratory of Di Venere Hospital of Bari, Italy.

Materials and Methods

Samples

The specimens collected for the study were from 108 patients admitted to hospital wards and emergency department from November the 3rd, 2020 to July the 27th, 2021. Nasopharyngeal (NP) swabs referred to patients under investigation were processed at the time of sample collection and later specimens obtained were stored at -20°C in universal transport medium (UTM)(COPAN, Brescia, Italy) for viruses. The antigenic tests, instead, were performed on stored UTM in July,2021.

Antigenic test

The Boditech AFIAS COVID-19 Ag is a semi-quantitative fluorescence immune-chromatographic point-of-care assay with microfluidic technology (3,25) for automatic (through signal intensity cut-off index (COI)) determination of SARS-CoV-2 antigens using monoclonal anti-SARS-CoV-2 antibody targeting the nucleocapsid protein (8) within 12 min on human NP swab specimens.

AFIAS-6 (automated fluorescence immuno-assay system) platform (3) is designed to perform a wide range of immunochromatographic diagnostic tests (36 tests/hour) based on the use of a dedicated all-in-one single sample (cartridge) device, which contains, in sealed wells, all the reagents required for the specific test. It is a small benchtop instrument that can run up to 6 cartridges at the same time (two sections of 3 cartridges each) for different tests. It is automated diagnostic device combination of high sensitivity (18) fluorescence technology based on time-resolved fluorescence (TRF) optical system which uses europium fluorescent chelate as a label (11,21). A fluorescence-labeled antibody conjugate in detection buffer binds the antigen in a specimen to form antibody-antigen complexes. The complexes migrate onto the nitrocellulose membrane and are captured by anti-SARS-CoV-2 antibody on the test line of the strip. The presence of more antigens in the specimen results in the formation of more antigen-antibody complexes and leads to a fluorescence signal of stronger intensity, which is processed to determine the relative concentrations of SARS-CoV-2 antigens in the specimen. Results were interpreted according to the COI, which was determined by using an equation based on the specimen-to-positive-control signal ratio. COI <1.0 was interpreted as "negative", and COI ≥1.0 was "positive" for the SARS-CoV-2 antigen. The AFIAS COVID-19 Ag extraction buffer was able to inactivate the SARS-CoV-2 virus after just a few minutes of incubation (18,24).

AFIAS COVID-19/Flu Ag Combo shows the same structural and methodological properties to the above-mentioned test (3,25,11,21,18,8,24). In addition, it can distinguish SARS-CoV2 N-antigen and influenza A and B virus specific antigens. It can provide three different semi-quantitative results using the same test cartridge. Data on flu-A and flu-B, although provided by the system, have not been considered as they are outside the purpose of this work. Both AFIAS tests are capable of detecting viral variants currently circulating, including the Delta variant (4). Results were interpreted according to the COI: COI <1.0 negative and COI ≥1.0 positive.

In AFIAS COVID-19 Ag, serial dilution analysis on a SARS-CoV2 strain reached an analytical sensitivity (LoD, limit of detection) of 0.47x102 TCID50/mL while in AFIAS COVID-19/Flu Ag Combo the limit of detection reached was 0,55x102 TCID50/mL.

In this paper, we will abbreviate the tests as AFIAS Combo Ag and AFIAS Ag.

Limits of the test

The test may yield false positive result(s) due to the cross-reactions and/or non-specific adhesion of certain sample components to the capture/detector antibodies. The test may yield false negative result(s) due to the non-responsiveness of the antigen to the antibodies which is most common if the epitope is masked by some unknown components, so therefore not being able to be detected or captured by the antibodies. The instability or degradation of the antigen with time and/or temperature may also cause false negative as it makes antigen unrecognizable by the antibodies. Other factors may interfere with the test and cause erroneous results, such as technical/procedural errors, degradation of the test components/reagents or presence of interfering substances in the test samples.

Molecular test

The NeoPlex COVID-19 detection kit assay is a qualitative in vitro test for the simultaneous detection and confirmation of RdRp and N genes in SARS-CoV-2 virus causing COVID-19 from upper respiratory specimens (such as nasopharyngeal, oropharyngeal, mid-turbinate and nasal swab) and lower respiratory specimens (such as sputum, bronchoalveolar lavage (BAL), and tracheal aspirate) from individuals suspected of COVID-19 by their healthcare provider. This assay is real-time reverse transcription polymerase chain reaction (RT-PCR) assay (5,20), which requires a small sample volume and short hands-on time with results available in approximately 3 hours. It is based on two major processes: i) Isolation of nucleic acid from patient specimens: nucleic acids are extracted from specimens using the QIAamp DSP viral RNA mini kit; ii) Multiplex real-time PCR: nucleic acid isolated from specimens is reverse transcribed to cDNA and subsequently amplified in the Applied Biosystems 7500 Fast Dx Real-Time PCR Instrument with SDS version 1.4 software. The primer and probe system is based on the standard TaqMan® Technology. The SARS-

CoV-2 specific probes are labelled with the FAM fluorophore and JOE fluorophore to target COVID-19 RdRp and N genes, respectively and after amplification emit two kinds of fluorescence, letting users to identify SARS-CoV-2.

Real-time PCR results are visualized in an amplification plot with a curve for each detector. Since the level of fluorescence signal is variable depending on the dye, a threshold value will be independently set for each curve. The amplification signal for each dye will be considered positive whenever the detector curve crosses its threshold value. Therefore, samples will be considered positive whenever they display a FAM positive signal. By contrast, samples will be considered negative only when the FAM signal is negative but the JOE signal is positive. The results are interpreted according to the two below interpretation tables (Figure 1), first which describes the individual target gene Ct thresholds, and the second which outlines the patient specimen result interpretation algorithm.

The Internal Control (IC*), labelled with the Cy5 fluorophore, is to monitor the nucleic acid isolation procedure and the possibility of PCR inhibition. A positive template control is needed to monitor if the instrument and device work properly and is used through the entire sample processing procedure.

The Aptima SARS-CoV-2 assay (Hologic, Inc., San Diego, CA, USA) is a nucleic acid amplification testing (NAAT) that uses target capture and transcription-mediated amplification (TMA) technologies for the isolation and amplification of SARS-CoV-2 RNA (12). This assay targets two unique regions of the ORF1ab section of the SARS-CoV-2 viral genome and is performed on the Panther instrument. All testing was performed according to the manufacturer's instructions and is briefly described. A 500-µL aliquot of the primary NP swab specimen is transferred into a specimen lysis tube containing 710 µL of lysis buffer, and this tube is then loaded onto the Panther instrument. From the specimen lysis tube, 360 µL is taken for each reaction. Each specimen is processed with an IC, which is added via the working target capture reagent. Nucleic acid is purified using capture oligonucleotides and a magnetic field, and the purified nucleic acid is used as the template for the TMA reaction. After amplification, chemiluminescent probes hybridize to amplicons and emit light measured by a luminometer in relative light units (RLUs). The IC signal and SARS-CoV-2-specific signal are differentiated by kinetic profiles of the labeled probes (rapid versus slow). Assay results are determined by a cut-off based on the total number of RLU and the kinetic curve type.

Test procedure for UTM stored specimens:

- place all specimens at room temperature for at least 30 minutes before running assay;
- prepare the UTM-specimens by inverting the specimen tube;
- collect, with a manual pipette, of 450 µL of UTM from the specimen tube and transfer to the tube with the extraction buffer of the kit and closure with drip cap;
- invert the buffer tube 5-7 times to ensure complete mixing of the sample, taking particular care to avoid foaming and bubbling;
- transfer 9-10 drops of extraction buffer from the tube into the Sample well of the AFIAS reaction cartridge;
- start test.

In order to correlate Ct (cycle threshold) with COI values of antigenic tests, this study used Ct values related to the N region, because they overlap with those obtained from the RdRp gene on the same specimens. The correlation value was 0.956 (CI 95% 0.897-1.018; intercept: 1.0012; slope: 0.4731). The cohort recruited for this evaluation consisted of 108 patients group aged 2 to 95 years of 2-95 years (51 male and 57 female).

No significant differences emerged from the age distribution between males and females (p=0.0622) represented as follows: female group aged 3 to 60 years (35; 61.4%), over 60 male group (30; 58.8%), male group aged 3 to 60 years (21; 41.2%) and over 60 female group (Table 1).

Item	RdRp, N gene (FAM, HEX or JOE) Result	* IC (Cy5) Result	Interpretation
Specimen	Ct ≤ 38		Positive (+)
	Ct >38 or N/A (Undetermined)		Negative (-)
Positive Control	Ct ≤ 30		Valid
Negative Control	N/A (Undetermined)		

Case	FAM	HEX or JOE	Cy5	Positive Control	Negative Control	Interpretation
	RdRp gene	N gene	IC *			
1	+	+	+	+	-	COVID-19
2	+	+	-			COVID-19
3	-	-	+			Negative
4	+/-	+/-	+			Negative or Re-test
**5	-	-	-			Re-test
6	+/-	+/-	+/-	+/-	+	Invalid
7	+/-	+/-	+/-	-	+/-	
8	+/-	+/-	+/-	-	-	

* IC is not necessary for the interpretation of positive or negative results and high load of pathogen's nucleic acid results in the low signal or negative signal of IC.

* Recommend re-extraction nucleic acid in specimens.

Figure 1. Interpretation table.

RLU values analysis on negative specimens taken as a parameter to evaluate the specimens stability (Figure 2). This gave mean value of 339.46 (range = 296-365):

The distribution of these values showed the substantial stability of the UTM specimens over time.

Statistics

Linear regression and Pearson's correlation coefficient (19) were calculated. Both linear and logarithmic graphs were plotted using M.S. Office Excel worksheet (ver. 2019). Confidence indices and Cohen's K (14,7) were calculated using GraphPam Prism 8 (13). Sensibility, specificity, VPN (negative predicted value), VPP (positive predicted value) (16) were based on:

True positive (TP) = a diseased person who is correctly identified as having a disease by the test

False positive (FP) = a healthy person that is incorrectly identified as having the disease by the test

True negative (TN) = a healthy person who is correctly identified as healthy by the test

False negative (FN) = a diseased person who is incorrectly identified healthy by the test

Table 1. Age and gender distribution of the evaluated cohort.

	n	Age	≤2	3-60	≥61	Average age
Male	51	6-89	0	21	30	60,35
Female	57	2-95	1	35	21	51,86

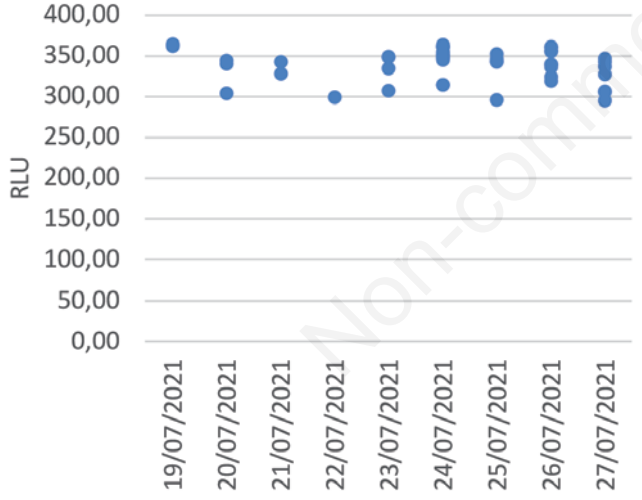

Figure 2. Trend of relative light units (RLU) values from negative specimens collected and stored in universal transport system (UTM) for viruses samples.

Results

Overall results are shown in Table 2.

For 3 specimens only the results of the molecular test and the AFIAS Ag test were available, the data for the AFIAS Combo Ag test were missing.

The semi-logarithmic plot is more suitable to display the distribution of points around the cut-off value of AFIAS tests (Figure 2, Figure 3). It can be distinguished in two-points distribution areas: one around cut-off values and the other with COI values lower than 1.0. The former matches Ct values ≤30. Three points (1*, 2*, 3*) marked in the graph illustrate specimens with COI >1.0 to AFIAS Combo Ag test (positive result), but negative to AFIAS Ag test.

1) In AFIAS Ag test the limit of detected positivity (COI >1.0) corresponds to Ct=27.5, while in AFIAS Combo Ag test two cases were still positive at Ct=35.2 and 37. The distribution of points on the y-axis (Figure 3, Figure 4) shows the proportionality between Ct and COI values (Van der Poort, 2020) (22,23).

Correlation AFIAS Combo Ag, AFIAS Ag by TMA on negative specimens

For negative specimens the reference method was TMA with results expressed in RLU. Semi-log representation of data correlation is illustrated in Figure 5 and Figure 6.

All molecular results ranged between 300-350 with COI <1. Only one specimen was positive with both AFIAS test: this may suggest a possible False negative with TMA method or the pres-

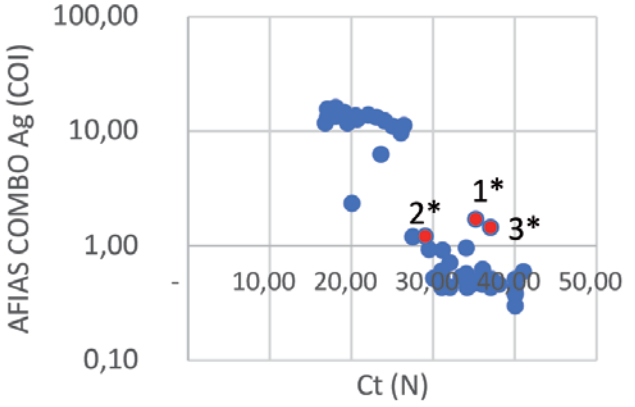

Figure 3. Correlation between Ct values of positive specimens and cut-off index (COI) values of AFIAS Combo Ag test.

Table 2. AFIAS Combo Ag and AFIAS Ag correlation with RT – polymerase chain reaction (PCR) method on positive specimens.

	RT-PCR		TMA		AFIAS Combo Ag		AFIAS Ag	
	pos	neg	pos	neg	pos	neg	pos	neg
Totali (n=108)	56	4	2	46	25	79	24	84
M (n=51)	28	2	2	19	14	34	13	38
F (n=57)	28	2	0	27	11	45	11	46

ence of secondary or tertiary molecular structures of the genome unable to replicate efficiently (6).

A bi-logarithmic graph was plotted on 105 antigen test data pairs available (Figure 7).

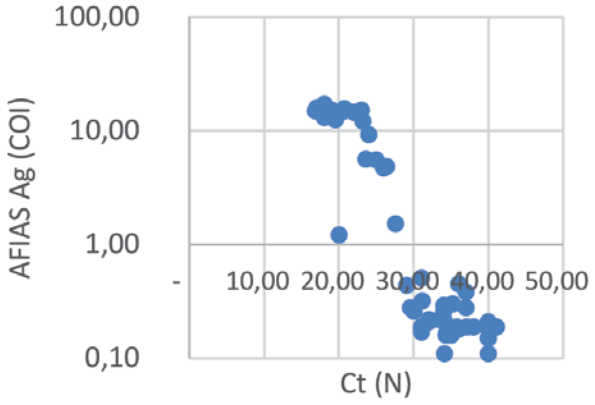

Figure 4. Correlation between Ct values of positive specimens and cut-off index (COI) values of AFIAS Ag test.

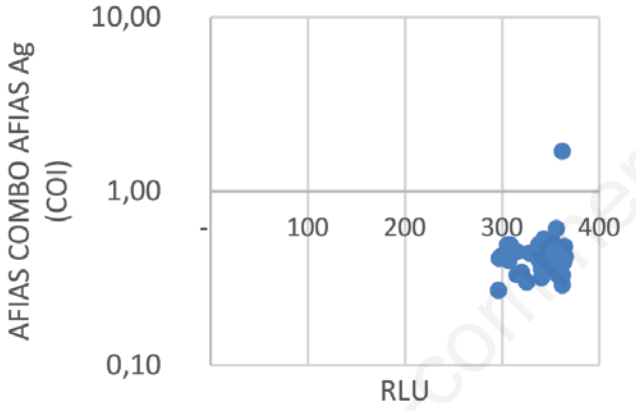

Figure 5. Correlation between Ct values of negative specimens and cut-off index (COI) values of AFIAS Combo Ag test.

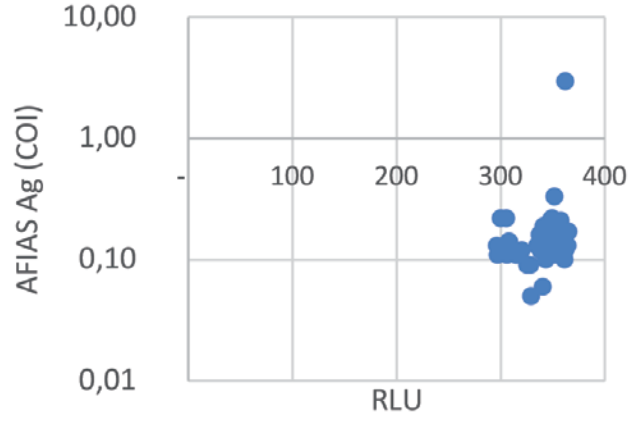

Figure 6. Correlation between Ct values of negative specimens and cut-off index (COI) values of AFIAS Ag test.

The distribution shows two areas for negative (COI <1.0 in both methods) and positive specimens. The correlating data are scattered in quadrants I and III (considering point 1;1 as the origin of the Cartesian axes). Three data which are discrepant between the two systems (1*, 2*, 3*, in quadrant IV, the same as above) are marked in red. The Ct values were 35.2, 29.0 and 37.9 respectively. Positivity was confirmed by the AFIAS Combo Ag but not by the AFIAS Ag test. The coefficient r=0.96 (CI 95%: 0.78-1.17) reveals a strong correlation.

There is a statistically significant difference (p=0.0074) between the mean COI values of the two tests, with a mean difference of 0.3458 (CI95%: 0.069-0.623) for the AFIAS Combo Ag test. This can be observed from the value of the intercept in the linear regression equation (-0.3349±0.1527).

Qualitative analysis

As AFIAS tests are semi-quantitative methods they were evaluated by correlating positivity and negativity values with reference methods. The data obtained can be used in two-by-two contingency tables to calculate qualitative parameters.

The obtained correlation on positive specimens provided these results (Table 3a, Table 3b).

The AFIAS Combo Ag test yielded 24 of 55 (43.6%; CI 95%: 31.4-56.7%) positive results (COI ≥1.0) with a mean Ct value of 22.50 (CI 95%: 19.6-25.6), whereas the negative (COI <1.0) had a mean Ct value of 34.92 (CI 95%: 33.78-36.09). The AFIAS Ag

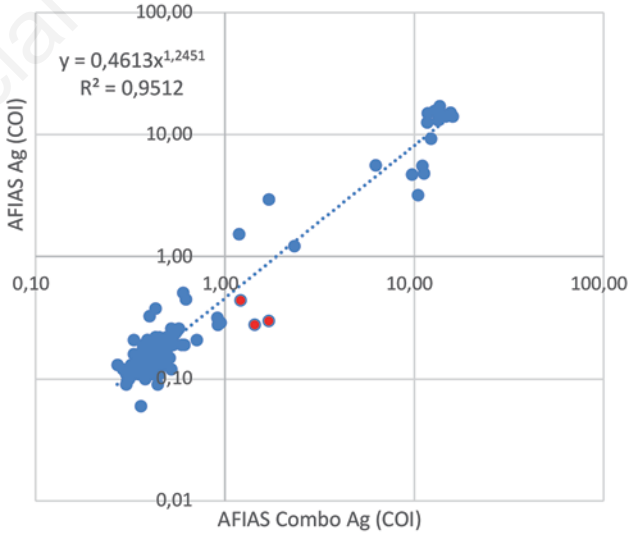

Figure 7. Correlation of cut-off index (COI) values obtained from all samples with the AFIAS Combo Ag and AFIAS Ag test.

Table 3. a, b) Qualitative correlation of positive specimens to molecular testing with the AFIAS Combo Ag and AFIAS Ag tests.

		Ct (N)	
		POS	NEG
AFIAS Combo Ag (COI)	POS	24	0
	NEG	31	4
		55	4
AFIAS Ag (COI)	POS	22	0
	NEG	34	4
		56	4

test, instead, yielded 22 of 56 (39.3%; CI 95%: 27.6-52.4%) positive results with a mean Ct value of 21.00 (CI 95%: 13.0-32.1), the negative (COI <1.0) had a mean Ct of 34.82 (CI 95%: 33.68-35.99). Four specimens negative to molecular testing, were confirmed by both antigenic tests. Since the sensitivity of AFIAS Ag tests had Ct value around 30 (Boditech internal data), the positive data were collected in two tables, one considering only Ct <30 (Table 4a, Table 4b) and the other with Ct >30 (Table 5a, Table 5b).

Positive specimens with Ct<30

The AFIAS Combo Ag assay confirmed 22 of 23 positive results (95.6%; CI 95%: 77.3->99.9%), (one specimen was discarded because of a technical problem) while 21 of 23 (91.3%; CI 95%; 72.0->98.7%) positive results for the AFIAS Ag test. One molecular positive specimen not confirmed by AFIAS Combo Ag test had a COI value close to 1 (0.92), whereas the two from the AFIAS Ag test had significantly lower COI values (0.44 and 0.28).

Positive specimens with Ct>30

The AFIAS Combo Ag test seems to better than the AFIAS Ag test: two confirmed positive out of 32 (6.2%; CI 95%: 0.7->21.2%), against no confirmed positive by AFIAS Ag test. Those two had a Ct of 35.20 and 37. Both antigenic methods confirmed 4 negative specimens.

Analysis on the negative data (with RLU<1000) provided overlapping results between the two AFIAS Ag methods. (Table 6a, Table 6b).

Forty-three negative specimens for molecular testing out of 44 (97.7%; CI 95%: 87.1->99.9%) were confirmed by AFIAS Combo Ag test, whereas the AFIAS Ag test confirmed 45 out of 46 (97.8%; CI 95%: 86.6->99.9%). The discrepant value noted with an asterisk relates the specimen negative for molecular testing but positive for both antigenic tests. Table 7 shows overall positive and negative results (Ct <30 and RLU>1000 and negative data).

In Table 8 we illustrate the quality parameters (sensibility, specificity, VPN= negative predicted value, VPP= positive predicted value, agreement (Cohen K)) calculated on the data obtained.

Discussion

Our data suggests a general improvement in the performance of the AFIAS Combo Ag test. The cohort recruited was statistically representative and homogeneous. Figure 2 shows that no trend exists in the RLU values calculated on the negative specimens dur-

Table 4. a, b) Qualitative correlation between positive specimens with Ct<30 and the AFIAS Combo Ag and AFIAS Ag tests.

		Ct (N)	
		POS (Ct>30)	NEG
AFIAS Combo Ag (COI)	POS	22	0
	NEG	1	0
		23	0
AFIAS Ag (COI)	POS	21	0
	NEG	2	0
		23	0

Table 5. a, b) Qualitative correlation between weakly positive specimens with Ct>30 and AFIAS Combo Ag and AFIAS Ag tests.

		Ct (N)	
		POS (Ct>30)	NEG
AFIAS Combo Ag (COI)	POS	2	0
	NEG	30	4
		32	4
AFIAS Ag (COI)	POS	0	0
	NEG	32	4
		32	4

Table 6. a, b) Qualitative correlation between negative specimens to molecular testing and AFIAS Combo Ag and AFIAS Ag tests.

		RLU	
		POS	NEG
AFIASAg (COI)	POS	1	1*
	NEG	1	45
		2	46
AFIAS Combo Ag (COI)	POS	1	1*
	NEG	1	43
		2	44

*Correlation close to perfect.

Table 7. a, b) Overall qualitative correlation of positive and negative molecular test results with those of AFIAS Combo Ag and AFIAS Ag tests.

		Ct/RLU	
		POS	NEG
AFIAS Combo Ag (COI)	POS	23	1
	NEG	2	47
		25	48
AFIAS Ag (COI)	POS	22	1
	NEG	3	49
		25	50

Table 8. Summary of quality parameters for the AFIAS Combo Ag and AFIAS Ag tests.

	Sensibility	Specificity	VPN	VPP	Agreement	Cohen's K[3]
AFIAS Combo Ag	92,0% (73,9%-98,9%) (23/25)	97,9% (88,1%->99,9%) (47/48)	95,9% (85,5%-99,6%) (47/49)	95,8% (78,1%->99,9%) (23/24)	95,9% (88,1%-99,1%) (70/73)	0,908*
AFIAS Ag	88,0% (69,2%-96,7%) (22/25)	98,0% (88,5%->99,9%) (49/50)	94,2% (83,7%-98,6%) (49/52)	95,6% (77,3%->99,9%) (22/23)	94,7% (86,7%-98,3%) (71/75)	0,878*

*Correlation close to perfect.

ing the test days. This demonstrates the stability of the specimens. The first correlation between molecular test and AFIAS Combo Ag test on positive specimens (Figure 3) shows that it is able to provide positive results up to Ct values of 37 the maximum Ct value detected as positive by the AFIAS Ag test (Figure 4), instead, was 27.50. This suggest how the AFIAS Combo Ag test is more sensitive than the AFIAS Ag test.

As regards to the negatives, no difference was noted in the performance of the two antigen tests (Figure 5, Figure 6). All specimens negative by molecular method were confirmed with the exception of one sample. The best performance of the AFIAS Combo Ag test also results from the analysis of the correlation highlighted in Figure 7. Three RT-PCR positive specimens plotted in the 4th quadrant of the distribution were confirmed by the AFIAS Combo Ag test but negative with the AFIAS Ag test. There was statistically significant difference in the mean COI values of the two datasets (p=0.0074) and the correlation line intercept value (-0.3354, not shown), indicating greater sensitivity of the AFIAS Combo Ag test against the AFIAS Ag test.

The qualitative analysis on positive specimens provided a correlation of 43.6% with the AFIAS Combo Ag test, and 39.3% with the AFIAS Ag test (Table 3a, Table 3b). It should be noted that in this case all the positive specimens were considered, regardless of their Ct value (therefore both greater and less than 30). Subsequent tables show two groups of positive specimens based on their Ct value (Ct<30 Table 4a, Table 4b; Ct>30 Table 5a, Table 5b). When Ct<30 (Table 4a, Table 4b), the agreement with AFIAS Combo Ag test was 95.6%, against 91.3% of the AFIAS Ag test. All specimens with Ct>30 (Table 5a, Table 5b), were negative with the AFIAS Ag test, while the AFIAS Combo Ag test identified 2 specimens as positive with Ct values 35.20 and 37. Despite the greater sensitivity of the AFIAS Combo Ag test compared to the AFIAS Ag test, the specificity remains the same in the two methods (Table 6a, Table 6b 97.7-97.8%). Table 8 shows an overall qualitative performance data of the two tests. The AFIAS Combo Ag test is more sensitive than the AFIAS Ag test (92.0% *vs* 88.0%) with the same specificity (97.9-98.0%). While the VPP value is the same for the two methods (95.8-95.6%), the VPN value is greater in the AFIAS Combo Ag test (95.9% *vs* 94.2%). Its sensitivity and VPN values enable to remove negative specimens from further investigation relieving the pressure on healthcare system. A further point that should be considered is the agreement resulting from the Cohen's K value (0.908).

Conclusions

The aim of this work was to evaluate the performance of the new AFIAS Combo Ag test compared to AFIAS Ag test. AFIAS Combo Ag test has a clinical sensitivity and a VPN higher than those of the AFIAS Ag test, keeping the specificity value unchanged. Considering its qualitative improvement, the possibility of rapid, inexpensive and early detection of the most infectious COVID-19 cases in appropriate settings, we suggest AFIAS Combo Ag test as a valid alternative to the reference lateral flow test (AFIAS Ag) and an adequate screening test (17,1,23,6).

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