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Low sensitivity of rapid tests detecting anti-CoV-2 IgG and IgM in health care workers' serum for COVID-19 screening

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Abstract

Background: *the sensitivity and specificity of a rapid antibody test were investigated for the screening of healthcare workers.* ***Methods:*** *the serum of 389 health care workers exposed to COVID-19 patients or with symptoms, were analysed. All workers underwent monthly the screening for SARS-CoV-2 with detection of viral RNA in nasopharyngeal swabs by RT-PCR. IgG antibody detection in serum was performed by Chemiluminescence Immunoassay (CLIA) and by the Rapid test (KHB diagnostic kit for SARS CoV-2 IgM/IgG antibody after a median of 7.6 weeks (25°-75° percentiles 6.6-11.5).* ***Results:*** *the rapid test resulted positive in 31/132 (23.5%), 16/135 (11.8%) and 0/122 cases in COVID-19 positive individuals, in those with only SARS-CoV-2 IgG antibodies and in those negative for both tests, respectively. Sensitivity was 17.6% (CI95% 13.2-22.7) and 23.5% (CI95% 16.5-31.6), and specificity was 100% (CI95% 97-100) and 100% (CI95% 97-100) considering Rapid test vs CLIA IgG or Rapid test vs SARS-CoV-2 positive RNA detection, respectively.* ***Conclusion:*** *the KHB Rapid test is not suitable for the screening of workers with previous COVID-19 infection.*

Introduction

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is the etiological agent of the Coronavirus Disease 19 (COVID-19) causing millions of cases and deaths all over the world. The gold standard test to diagnose COVID-19 is the detection of SARS-CoV-2 RNA using RT-PCR (Reverse Transcription Polymerase Chain Reaction) from a nasopharyngeal swab. Serological tests detecting IgM, IgG and IgA against the virus are available allowing surveys in different populations, in particular healthcare workers. Serological tests permit to verify antibodies presence in exposed

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populations to find out undiagnosed people after the active infection, since antibodies are detectable in almost all people who have been in contact with SARS-CoV-2 after two or more weeks depending on the severity of the disease (1,2). The WHO in March 2020 recommended serological testing in addition to molecular diagnosis, for investigating ongoing outbreaks as well as for the diagnosis of individuals strongly suspected of SARS-CoV-2 infection with a negative molecular test for SARS-CoV-2 RNA (3). Antibody tests for SARS-CoV-2 have been considered one of the keys to fight the SARS-CoV-2 epidemic (4) and positivity to antibodies against SARS-CoV-2 would permit the identification of previously infected individuals. Moreover, the role of antibodies in the protection against COVID-19 disease is debated (5), but neutralizing antibodies have a crucial role in the protection against SARS-Cov-2 infection together with a robust T-cell response (6). IgG, IgM and IgA against the virus can be detected using chemiluminescent microparticles in automated immunoassays (7,8), and increasingly data are available for rapid lateral flow test (8-13), which are suggested for the screening of large population of workers. However, manufacturers tested specificity and sensibility of the tests mostly using serums coming from hospitalized patients and from healthy controls, but few data are available from asymptomatic or paucisymptomatic workers without a severe COVID-19 disease, at best of our knowledge (14). Ong et al. 2020 analysed various rapid tests finding large variability in diagnostic test performance between them with an overall limited sensitivity and high specificity in acutely admitted patients (15). The Cochrane review done in serological tests recognize limitations in sensitivity, mainly if used in the first weeks after the Covid-19 infection (16). Moreover, if the presence of antibodies in hospitalized patients with COVID-19 seems to be a consolidated observation, the majority of subjects who developed an asymptomatic SARS-CoV-2 infection do not present antibodies after 8 weeks of follow-up (17).

The aim of this study was to assess the analytical performances (sensitivity and specificity) and agreement of a rapid test for detecting antibodies against SARS-CoV-2 compared to an automated immunoassay in health care workers with and without COVID-19 infection in Trieste main hospital. On 4216 workers, 115 developed SARS-CoV-2 infection (2.7%) from March 1, 2020 to April 6, 2020 (18). The incidence of the infection in general population in Trieste was 46.5 cases/100.000 inhabitants from March 1, 2020 to April 24, 2020 (19).

Methods

Population studied

Preliminary evaluation

The KHB Rapid lateral flow test was initially tested against the Biomaxima product with 30 sera archived before 2019 and 37 sera from severe COVID-19 patients admitted to the Department of Pulmonology of the University Hospital of Cattinara for COVID-19 complications and with a positive SARS-CoV-2 molecular test.

Health care workers studied

The evaluation was performed on 389 serum samples collected among health care workers followed by the Unit of Occupational Medicine at University Hospital of Trieste (NE-Italy) because of symptoms suggesting COVID-19 infection or exposed to COVID-19 patients without suitable personal protective equipment for more than 15 minutes or for any time during aerosol generated procedures (20). Moreover, since April 15, 2020, all underwent periodically (monthly or weekly according to biological hazard exposure) detection of SARS-CoV-2 RNA into nasopharyngeal swabs. Workers serums were collected each month from May to September 2020 to evaluate the presence of IgG and IgM for SARS-CoV-2. Two hundred sixty-seven sera samples were collected among workers who presented an IgG value higher than 15 AU/mL obtained with chemiluminescence immuno-assay (CLIA) in July 2020. One hundred thirty-two of them had at least one positive nasopharyngeal swab (analysed by RT-PCR). The others (n. 122) were negative to SARS-CoV-2 nasopharyngeal swabs and IgG serum detection. Figure 1 reports the study design.

Blood collection and Ig analyses

In a preliminary evaluation we compared the lateral flow test KHB diagnostic kit for SARS CoV-2 IgM/IgG antibody (Colloidal Gold – Sample diluent) produced by Shanghai Kehua Bio-Engeneering Co.,Ltd. (Shanghai, P.R. China) and the 2019-nCoV IgG/IgM Rapid Test Cassette from Biomaxima S.A. (Lublin, Poland) according to the manufacturer's instruction. Blood samples were stocked in fridges at 4°C; using a Pasteur pipe, we separated the serum from the cellulated component, maximum 10 days after blood sampling. Once the sera were allocated into dedicated test tubes, we took 20 microliters and put them into the reservoir of the diagnostic kit; results were read and interpretated 15 minutes after depositing sera.

Rapid test sensitivity and specificity was compared to Chemiluminescence Immunoassay CLIA - Kit DiaSorin IgG (Liaison SARS-CoV2 S1/S2 IgG), confirmed by Maglumi (Snibe) 2019-n CoV IgM CLIA and 2019-n CoV IgG CLIA. Antibody concentration higher than 15 A.U. was considered as a positive result. The performances of the LIAISON®SARS-CoV-2 resulted the same of ELISA method in terms of sensitivities and specificities regarding the determination of the IgG (21).

Swab collection and SARS-CoV-2 analysis by Reverse Trascriptase-PCR

As gold standard, we considered SARS-CoV-2 RNA in nasopharyngeal and oropharyngeal specimens, collected with the swab technique. RNA was extracted and determined by rRT-PCR targeting the E, N and RdRp gene of SARS-CoV-2, according to the CDC and Charité laboratory protocols (22). The cycle threshold values of RT-PCR were used as qualitative indicators of viral load of SARS-CoV-2 RNA in specimens, with lower cycle threshold values corresponding to higher viral copy numbers. A cycle threshold value less than 30 was interpreted as positive for SARS-CoV-2 RNA.

Statistical analysis

Data analysis was performed with the software STATA™ v. 14.0 (Stata Corp., LP, College Station, TX, USA). Comparison between groups was performed using Mann-Whitney test for continuous variables and chi-square test for proportions. Youden's J index was used as a summary measure of performance of a diagnostic test (23). This index, calculated as the sum of sensitivity plus specificity minus 1, gives the proportion of diseased and healthy individuals who are correctly classified by a

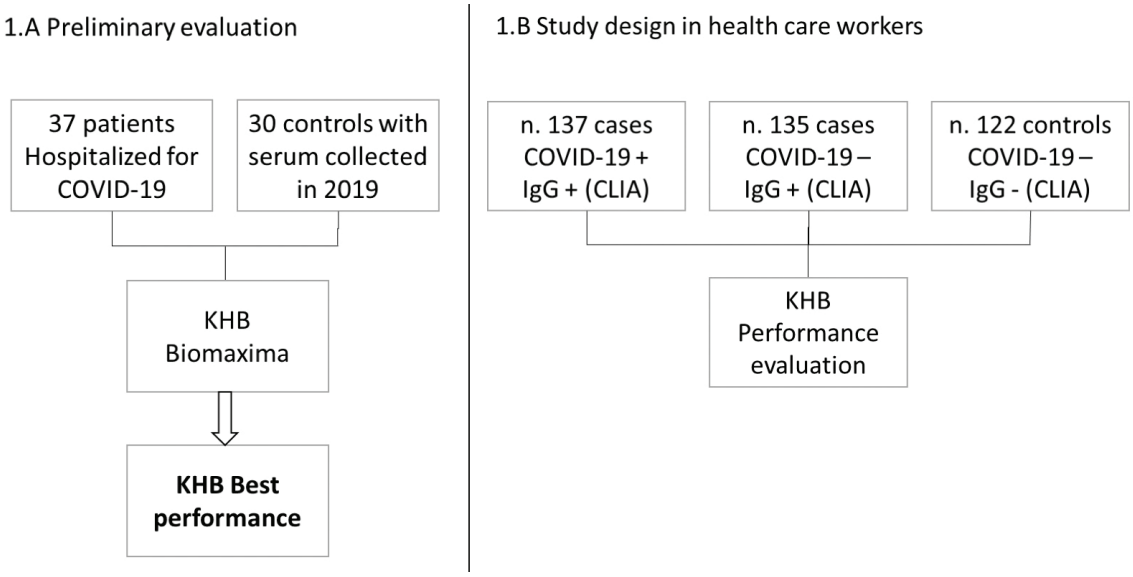

Figure 1. Design of the study

diagnostic test. The maximum value of the Youden index is 1 (perfect test) and the minimum is 0 when the test has no diagnostic value. A p-value of <0.05 was established as the limit of statistical significance.

Ethics

The study was approved by the Local Ethical Committee (CEUR- 2020-Os-072) on 16.04.2020.

Results

Preliminary evaluation of the KHB Rapid test

On 37 sera from severe COVID-19 patients with a positive SARS-CoV-2 molecular test, 33 resulted positive with the KHB lateral flow test, with a sensitivity of 89%. Considering that a certain time is required for seroconversion from the initial infection, we considered those tested at T0 (day of admission = 8/33 positives, sensitivity 67%) or T>0 (collection at T7, T14 and at discharge = 25/25 positives, sensitivity 100%). These data are consistent to what has been reported by the Company from a study on Italian patients (Table 1). Deconvolving data for IgG and IgM, the results shown above are maintained for IgG, while for IgM only 16/37 showed a positive signal (sensitivity 43% for IgM). Also considering the sera collected at T0, only 7/12 turned positive for IgM. Specificity for IgG was 100%, while for IgM 1 sample showed a positive signal (specificity of 96,6%). Since results for the Biomaxima lateral flow were similar, but with a lower IgM specificity (Table 1), we proceeded with the KHB test only.

Table 1. Preliminary evaluation of KHB Rapid lateral flow test and BIOMAXIMA test vs CLIA IgG

	KHB lateral flow					BIOMAXIMA				
	manufacturer's data	T>7	T0	IgG all	IgM all	manufacturer's data	T>7	T0	IgG all	IgM all
True Positive	25	25	8	33	16	37	29	10	38	19
False Positive	2	1	1	0	1	3	2	2	0	2
True Negative	52	29	29	30	29	49	28	28	30	28
False Negative	2	0	4	4	21	3	0	4	5	24
Sensitivity %	93	100	67	89	43	93	100	71	88	44
Specificity %	96	97	97	100	97	94	93	93	100	93%
Positive predictive value %	93	96	89	100	94	93	94	83	100	90%
Negative predictive value %	96	100	88	88	58	94	100	88	86	54%

Table 2. Characteristics of the population studied

	Positive swabs	**Positive CLIA**	**Controls**	**Total**
N. (%)	132 (33.9)	135 (34.7)	122 (31.4)	389 (100)
Women n. (%)	88 (66.7)	93 (68.9)	66 (54.1)	247 (63.5)
Age years Median (25°-75° percentiles)	41 (30-51)	44 (34-53)	47.5 (35-57)	44 (33-54)
Symptoms n. (%)	112 (84.8)	13 (9.6)	0	125 (32.1)
SARS-Cov-2 RT-PCR positive n. (%)	132 (100)	0	0	132 (33.9)
IgG CLIA positive n. (%)	132 (100)	135 (100)	0	267 (68.6)
CLIA Values A.U./mL median (25°-75° percentiles)	51.2 (30-74.8)*	29.1 (20-55.1)	0	23.6 (0-54.9)
Weeks after SARS-Cov-2 RT-PCR median (25-75 percentiles)	7.7 (6.6-11.5)	7.5 (3.6-7.6)	7.5 (5-8)	7.6 (6.6-11.5)
IgG KHB Rapid test positive n. (%)	31 (23.5)	16 (11.8)	0	47 (12.2)
IgM KHB Rapid test positive n. (%)	6 (4.6)	3 (2.2)	1 (0.8)	10 (2.6)

Population studied

Table 2 reported the characteristics of the population studied. One hundred thirty-two cases have had a nasopharyngeal swab positive for SARS-CoV-2 RNA and 84.8% of subjects reported symptoms, mainly mild, 8.3% (n.11) had had pneumonia, one required hospitalization, none died. All resulted positive to CLIA immunoassay, with values of the test significantly higher compared to those found in subjects with negative nasopharyngeal swab (median; 25°-75° percentiles 51.2; 30-74.8 A.U./mL vs 29.1; 20-55.1 A.U./mL, respectively p<0.001). IgG rapid test resulted positive in the 23.5% of the group and only 4.6% had detectable IgM. The tests were performed after in median 7.7 weeks from the nasopharyngeal swab positive for SARS-Cov-2 RNA. The second group was composed by 135 health care workers that resulted positive only to IgG in serum by CLIA immunoassays and the detection of SARS-Cov-2 RNA in nasopharyngeal swabs was every time negative. The majority of them were asymptomatic (90.4%). Their IgG level in serum was lower compared to those with a positive swab, and KHB Rapid IgG test resulted positive in 11.8% of cases. In three cases, IgM antibodies were present. Controls (n=122) were negative for IgG while one subject was positive to IgM.

Figure 2 reports box-plots with median values (25°-75° percentiles), minimum and maximum and outliers of CLIA IgG values in subjects negative and positive to KHB Rapid test. The CLIA values are significantly higher in subjects with KHB Rapid test positivity (median 63.2; 25-75° percentiles 14.8-400 A.U./mL) compared to those negative (median 20.1; 25-75° percentiles 0-43.6 A.U./mL) (p<0.001).

Sensitivity assessment

Sensitivity of the KHB Rapid diagnostic kit for SARS CoV-2 IgM/IgG antibody was assessed on the 267 samples with a positive IgG using CLIA, of which only 47 resulted positive for IgG, with a sensitivity of 17.6% (95% CI 13.2-22.7). Considering as gold standard the SARS-CoV-2 RNA detection in nasopharyngeal swab using RT-PCR, the Rapid test sensitivity was assessed at 23.5% (95% CI 16.5-31.6) for IgG and at 12.1% (CI95% 7-18.9) for IgM (table 3). Table 4 reports the prevalence of KHB Rapid IgG vs SARS-CoV-2 RNA detection in nasopharyngeal swab in symptomatic and asymptomatic workers. In

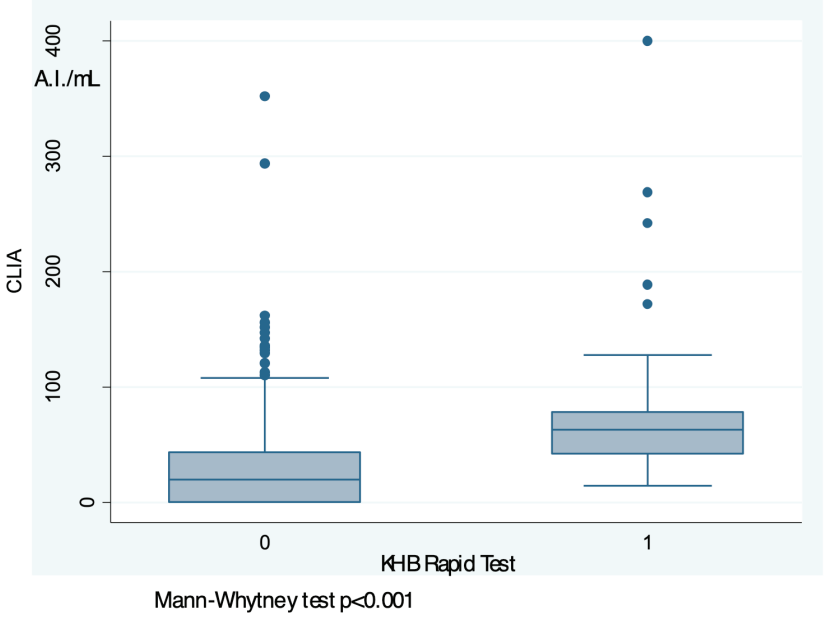

Figure 2. Box plots of CLIA values (median, 25-75° percentiles, minimum and maximum and outliers) in patients negative or positive to KHB Rapid test

Table 3. Rapid test performance against CLIA IgG or PCR-SARS-CoV-2 in the study population

	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	Youden J index
KHB Rapid IgG vs CLIA IgG	47/267 17.6% (13.2-22.7)	122/122 100% (97-100)	47/47 100% (92.5-100)	122/342 35.7% (30.6-41.0)	0.176
KHB Rapid IgG vs SARS-Cov-2 RNA swab	31/132 23.5% (16.5-31.6)	122/122 100% (97-100)	31/31 100% (88.8-100)	122/223 50.2% (43.5-57.0)	0.235

Table 4. KHB diagnostic kit for SARS-CoV-2 IgG Antibody (Colloidal Gold) prevalence by symptoms and SARS-Cov-2 swab findings

	Positive swabs n/total % (95% CI)	Negative swabs n/total % (95% CI)	Overall n/total % (95% CI)
KHB Rapid IgG with symptoms	29/112 25.9% (18.1-35.0)	1/13 7.7% (0.2-36.0)	30/125 24% (16.8-32.5)
KHB Rapid IgG without symptoms	2/20 10.0% (1.2-31.7)	15/122 12.3% (7.0-19.5)	17/142 12.0% (7.1-18.5)

symptomatic subjects swab positive and negative, the prevalence of KHB rapid IgG was 25.9% (95% CI 18.1-35.0) and 7.7% (95% CI 0.2-36.0), respectively, while in asymptomatic workers the prevalence was 10.0% (95% CI 1.2-31.7) and 12.3% (95% CI 7.0-19.5), respectively.

Specificity assessment

Specificity of KHB Rapid diagnostic kit for SARS CoV-2 IgM/IgG antibody was assessed on 122 negative CLIA tests. All of them resulted negative. Specificity was 100% (95%CI 97- 100) when the KHB rapid IgG were tested vs either CLIA IgG or SARS-CoV-2 RNA swab (table 3).

Overall evaluation

Predictive positive value (PPV) were 100% (95% CI 92.5-100) and 100% (95% CI 88.8-100) for KHB Rapid test vs CLIA or vs SARS-Cov-2 in nasopharyngeal swab, respectively. Negative predictive values (NPV) were 35.7% (95% CI 30.6-41.0) and 50.2% (95% CI 43.5-57.0) for KHB Rapid test vs CLIA or vs SARS-Cov-2 in nasopharyngeal swab, respectively.

Applying the Youden statistics to evaluate the KHB Rapid test vs either CLIA or SARS-Cov-2 RNA , the J index resulted 0.176 and 0.235, respectively, indicating that the test has poor diagnostic value (near 0).

Discussion

Our study investigated sensitivity and specificity of the KHB lateral flow rapid antibody test for the detection of IgG and IgM antibodies against SARS-CoV-2. In a preliminary test performed only with sera from patients hospitalized for severe COVID-19 and serum from controls collected before the COVID-19 outbreak, the kit demonstrated a good sensitivity (89%) and a good specificity (100%), in line with data declared by the producer. After the preliminary assessment, we proceeded to investigate the performance of the KHB Rapid test in a population of healthcare workers that were routinely screened using SARS-CoV-2 RNA detection in nasopharyngeal swab and IgG against SARS-CoV-2 detection in serum. We identify three groups: the first with positive swabs, the second with positive IgG for SARS-CoV-2 in serum identified with CLIA, the third negative for both SARS-CoV-2 detection in nasopharyngeal swab and IgG. Our population was characterized by workers that had very mild COVID-19 symptoms, 16.2% were completely asymptomatic, only 11 (8.3%) had pneumonia and one was hospitalized. We choose to perform the comparison test after 7-8 weeks from the positive swabs to be sure to find IgG that usually are detectable in serum after 3-4 weeks from the infection. Our population is different from those normally used to test sensibility and specificity of diagnostic tests in which hospitalized patients

and healthy controls are used (10-13). Our study revealed a very low sensitivity of KHB rapid test that identified a previous COVID-19 infection only in 23.5% of cases with a positive swab and in 17.6% of cases with IgG antibodies against SARS-CoV-2 in serum. On the opposite, specificity resulted very high reaching 100% comparing KHB Rapid test with SARS-CoV-2 RNA detection in nasopharyngeal swab or IgG antibodies against SARS-Cov-2 in serum. Considering symptomatic cases with previous COVID-19 disease, the sensitivity resulted extremely low (25.9%) and considering asymptomatic cases the sensitivity decreases to 10%.

Charpentier et al. (9) analysed performances of 2 other rapid tests (Covid- Presto® test rapid Covid-19 IgG/IgM and NG-Test® IgM-IgG COVID-19) using an automated immunoassay (Abbott SARS-CoV-2 IgG) for detecting anti-SARS-CoV-2 antibodies finding a good correlation between them (Sensitivity of Covid-Presto® test for IgM and IgG was 78.4% and 92.0%, respectively). Sensitivity of NG-Test® for IgM and IgG was 96.6% and 94.9%, respectively. Sensitivity of Abbott IgG assay was 96.5% showing an excellent agreement with the two rapid tests. However, they used serum coming from 54 subjects, among them 29 were hospitalized in intensive care, 11 in infectious diseases. They concluded that performances of these two rapid tests are very good and comparable to those obtained with automated immunoassay, except for IgM specificity with the NG-Test®. However, Charpentier et al. (9) recognized as a limitation of their study that most of the patients of the positive panel presented severe infections, since 74 % of them were hospitalized in infectious disease unit or in intensive care.

Also Hoffman et al (11) studied a test developed for rapid (within 15 minutes) detection of SARS-CoV- 2-specific IgM and IgG (Orient Gene Biotech Co Ltd, Huzhou, Zhejiang, China) by 29 PCR-confirmed COVID-19 cases and 124 negative controls. The results revealed a sensitivity of 69% and 93.1% for IgM and IgG, respectively. In this study, they used COVID-19 patients but they did not specify the gravity of the diseases. The same rapid test was evaluated by Delliere et al (12) finding a very good sensitivity (95.8; CI95% 89.6 to 98.8) for samples collected >10 days after the onset of symptoms, but again the 61% of positive subjects were COVID-19 cases requiring hospitalization.

More recently, Pere et al. (13) studied the analytical performances of five SARS-CoV-2 whole-blood finger-stick IgG-IgM combined antibody rapid tests. Using serum of hospitalized patients, they found a very good sensitivity and specificity for the five tests analysed. They found a sensitivity 95.8%, 91.6%, 92.3%, 97.9% and 91.4%, and a specificity of 98.1%, 86.5%, 100%, 98.1% and 84.6%, for BIOSYNEX COVID-19 BSS (IgG/IgM), Humasis COVID-19 IgG/IgM Test, LYHER COVID-19 IgM/IgG Rapid Test, SIENNA™ COVID-19 (IgG/IgM) Rapid Test Cassette and NG-BIOTECH COVID-19 (IgG/IgM), respectively. Other authors suggested multiplex screening, but again using serum from hospitalized patients (24). In our study, sensitivity of the Rapid test was high when tested using sera from hospitalized patients, but it was low using workers' sera in which COVID-9 infection was mild.

Finally, we need to consider that the utility of rapid tests is impacted by pretest probability and therefore changes in the different stages of the pandemic (25).

Regarding IgM found with KHB Rapid test, sensitivity resulted extremely low, but the test was performed in median after 7 weeks after the SARS-CoV-2 RNA positive swab tests, too late to detect IgM antibodies, which are detectable 3-4 weeks (24) after the infection and are going to disappear after 6-7 weeks. Moreover, it is known (9) that the presence of isolated IgM should be cautiously interpreted due to the possible false-positive reactions because of the cross-reactivity with sera containing reactivity malarial antibodies. For that reason, some authors excluded serum coming from people with malarial antibodies in control group (26).

Obviously, regarding specificity, the KHB Rapid test showed high values with 100% for IgG and 99.2% for IgM.

Analyzing the performance of KHB Rapid test using the Youden test, the value below 0.2 define a test useless to screen general or working population for previous COVID-19 infection. Using the Rapid test, we failed to detect the ¾ of health care workers

with previous COVID-19 diseases, also with symptoms.

We confirm the poor utility of these tests for seroprevalence surveys for public health management purposes, as recognized in a recent Cochrane review by Decks et al (16).

Our paper has some strengths: 1. the screening was done on working population, the same that is suggested for the screening; 2.the number of subjects included in the study is higher compared to those used for the evaluation of a test; 3. the periodical screening performed on health care workers had permitted to define previously infected workers in a precise way.

Moreover, our study has some limitations: 1. we did not perform the test repetitively after the COVID-19 on-set focusing only on one screening after in median 7.6 weeks after the disease; 2. in mild COVID-19 disease the antibody levels are lower than those detected in hospitalized patients (6); 3. we did not find IgM antibodies mainly because the analysis was done when IgM declined.

Conclusion

In conclusion, the KHB lateral flow rapid test resulted with a too low sensitivity to screen general or working population for previous COVID-19 infection.

Conflict of interest: No potential conflict of interest relevant to this article was reported by the authors

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