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FULL PAPER

Comparison between rapid and laboratory serological tests in the context of the first responders during the SARS-CoV-2 outbreak: are the two tests interchangeable?

Simone Murganti[1,†], Edoardo Cavalieri d'Oro[2,3,†], Matteo Villa[4], Antonio Papagni[5], Andrea Malizia[6]

[1]*University of Milano-Bicocca– Department of Earth and Environmental Science, Milan, Italy;*
[2]*CBRN Unit and Laboratories of the Lombardy region, Italian National Fire and Rescue Service, Milan, Italy;*
[3]*Department of Industrial Engineering, University of Rome Tor Vergata, Rome, Italy;*
[4]*University of Milano-Bicocca – School of Medicine and Surgery, Milan, Italy;*
[5]*Department of Materials Science at the University of Milano-Bicocca, Milan, Italy;*
[6]*Department of Biomedicine and Prevention, University of Rome Tor Vergata, Rome, Italy;*
[†]*These authors contributed equally as first authors.*

SUMMARY

The SARS-CoV-2 virus appeared and was discovered in the year 2019, marking its significance. The spread of the virus also had serious consequences for national safety; members of the Police and Fire Brigade contracted the infection and therefore the efficiency of their operational activity decreased. Since the beginning of 2020, the biological laboratory of the Chemical Biological Radiological Nuclear (CBRN) unit of Milan's Fire Brigade headquarters performed thousands of serological tests to monitor the health of the Fire Brigade and various branches of the Police Forces.
The aim of this study is to evaluate the degree of concordance and interchangeability between a lateral flow immunochromatographic assay (LFIA) and an automated laboratory immunoassay with different viral targets by comparing the data gathered from a sample group of firemen and policemen participating in a serological screening campaign. The serological tests used in this study are the LYHER® Novel Coronavirus (2019-nCoV) IgM/IgG Antibody Combo Test Kit and the Elecsys® Anti-SARS-CoV-2. The degree of concordance was computed using Cohen's kappa, with a result of 0.78 (CI 95%, 0.661-0.898), which is equivalent to a substantial agreement measured between the two tests. Additionally, the sensitivity of both serological tests was found to be 97%.

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INTRODUCTION

In December 2019, the novel coronavirus SARS-CoV-2 emerged in the Chinese city of Wuhan and rapidly spread globally (Wu *et al*., 2020). The first case in Italy appeared in January 2020, leading to significant health and economic impacts: starting in February of that year, the situation worsened considerably: the government shifted to remote schooling and administrative work, closing all activities considered non-essential, such as restaurants, gyms, and theaters, while also imposing restrictions on tourism. In fact, it is reported that GDP in 2020 was lowered by up to 3%. In addition, the national health system, due to the high number of contagions, suffered an immediate saturation of intensive care units and a difficulty in obtaining PPE (Personal Protective Equipment) and swabs even for medical personnel (Ortenzi *et al*., 2020). The country experienced two major pandemic waves in 2020: a spring peak in March with around 33,000 daily cases and a later surge peaking at nearly 60,000 daily cases in November. Notably, no Variants of Concern (VOC) were detected in Italy in 2020; the Alpha variant (B.1.1.7) became dominant only in 2021 (Ferrante *et al*., 2022). Additionally, during an epidemic, the transmissibility index R(t) is crucial: during the initial phases of the first wave, it was very high, but later dropped below 1. From mid-June until the end of 2020, the R(t) index fluctuated but showed a growing trend, consistently remaining above 1 (Naimoli *et al*., 2022).
Despite this, essential services, including Police and Fire Brigades, continued operating. Starting in February 2020 the Fire Brigade's CBRN (Chemical Biological Radiological Nuclear) unit in Milan, collaborating with the laboratory of Luigi Sacco Hospital, started monitoring essential workers' health using

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Corresponding author:
Simone Murganti
E-mail: murgantisimone@gmail.com

diagnostic tools like molecular swabs, rapid antigen tests, and serological tests; in fact, the spread of SARS-CoV-2 was affected their health and, with it, their ability to guarantee safety and security in the territory (McAlearney *et al.*, 2022).

Serological testing serves multiple purposes: the most important is epidemiological (Winter *et al.*, 2020), which aims to identify individuals with antibodies to understand how the infection spreads within the population. The second one is providing the possibility to calculate seroprevalence and also to understand when herd immunity is reached. Immunoassays also provide the opportunity to understand the effectiveness of preventive measures (e.g., social distancing and PPEs use), a very important aspect especially for strategic emergency services like healthcare workers (HCW) and Law Enforcement members (Fire Fighters and Police corps mainly). They are in direct contact with the community to carry out emergency and health services. Law Enforcement members come into contact with individuals who may be infected during their daily emergency calls, while HCW are in a very high-risk category because they regularly work in environments with SARS-CoV-2 infected individuals as part of their duties. When a member of the HCW comes into contact with a patient infected with SARS-CoV-2, he or she may become infected and infects another colleague or another patient who is passing through the hospital for other needs. This cycle of contagion is deleterious since, due to illness, more and more emergency service personnel are no longer available; this reasoning also applies to Law Enforcement members. In addition, by returning home after duty, HCW and Law Enforcement members transmit the infection to their family members, thus extending the chain of contagion. Hence, it is imperative to monitor the health of HCW, such as members of the Law Enforcement branch, to prevent the efficiency of the emergency services from being compromised.

Moreover, serological tests are also useful in the context of vaccination against SARS-CoV-2, for vaccine development, and for assessment of humoral response to vaccination (Devi *et al.*, 2022). In the context of clinical diagnosis, some studies highlight a complementary role of serological test for cases of false negatives in RT-PCR (Alamri *et al.*, 2023; Wang *et al.*, 2020).

The CBRN unit conducted serological screening using two test types: rapid tests detecting IgG/IgM antibodies against the S1 sub-unit of the Spike glycoprotein (S protein) and automated immunoassays for pan-Ig (IgG, IgA and IgM) antibodies against the Nucleocapsid protein (N protein). The kinetics of antibodies, which is the trend of the titre of immunoglobulins over time, is different, especially that of IgG: in fact, several weeks after infection, the amount of IgG antibodies directed against the N protein tends to decrease over time, while those that bind to the S protein tend to increase over time (Fenwick *et al.*, 2021; Choudhary *et al.*, 2021; Yassine *et al.*, 2021). Due to this distinction, if an individual undergoes a serological test after a potential prior infection, they might test positive for antibodies directed against one antigen but not the other. For example, if an individual is tested for antibodies against the Nucleocapsid protein at the beginning of convalescence after the acute phase, they may test positive; however, if the test is performed several months later, it may be negative. Antibodies direct against the Spike glycoprotein, on the other hand, have opposite kinetics, but in each case there is a question of when to test and consequently which viral target to use for antibody detection. In fact, the probability of detection changes over time depending on the viral target used in the test and this aspect is problematic during a massive screening after an epidemic wave, precisely because antibody kinetics is antigen dependent.

This aspect is a crucial factor for identifying individuals with antibodies resulting from a SARS-CoV-2 infection in a serosurveillance system, considering the various purposes of the serological tests described above. In fact, the probability of detection changes over time depending on the viral target used in the test and this aspect is problematic during a massive screening after an epidemic wave, especially because many people have contracted the virus asymptomatically and do not know when they had the onset of symptoms.

This study aims to assess the agreement of a rapid serological test and an automated serological immunoassay, considering differences in terms of viral targets and thus of antibody kinetics. To evaluate the concordance of the two tests, Cohen's kappa is used to measure test correspondence. Furthermore, confirmed positive cases from RT-PCR were considered for sensitivity comparison.

MATERIALS AND METHODS

Pool of volunteers

The people recruited for this study consisted of a group of individuals serving in essential services working in the city of Milan who voluntarily accepted to participate in this study *(see supplementary materials S1 for recruitment process)*. The majority worked in the Fire Brigade and a smaller portion in the State Police. The total number of individuals in the pool was 133, ranging from 23 to 65 years of age (average age was about 50), with a distribution of 117 males and 16 females. The data was collected from January to March 2021, the period during which the vaccine was made available in Italy. The volunteers were selected from individuals privileging those who tested positive on past serological test

or on RT-PCR; in this second case, blood samples could be collected only at least 14 days after the date of RT-PCR positivity since there are significantly higher seroconversion rates after 14 days from the onset of infection and thus the sensitivity of serological tests is greater (Cheng *et al*., 2020); due to the possibility of having an in-house laboratory at the CBRN unit of the Milan Fire Brigade, the difference between symptom onset and RT-PCR positivity dates is a maximum of 3-4 days.
Furthermore, the individuals who had never shown SARS-CoV-2 symptoms and were willing to check if they had no contact with the virus, also underwent the tests. Having started the vaccination cycle against SARS-CoV-2 was also an exclusion criterion.

Ethical aspects

All samples were analysed anonymously. All participants provided informed written consent. All the biological assessments were carried out in accordance with the Declaration of Helsinki, and the study was approved by the local Ethics Committee of the Università degli Studi di Milano – Bicocca protocol n. 716, issued 14th July 2022.

Serological tests

The two serological tests used two different targets for the identification of antibodies: the Spike glycoprotein and the Nucleocapsid protein. As stated in the introduction, antibodies that bind to the two antigens have different kinetics.
The Roche Elecsys® Anti-SARS-CoV-2 test detects total IgG, IgA, and IgM against the Nucleocapsid protein, with results expressed as S/CO, and values ≥1 S/CO are considered positive. However, this kind of instrument is expensive, requires trained personnel and maintenance, and consequently can be used only in hospitals, research institutions, or private testing laboratories. This immunoassay is provided by Luigi Sacco Hospital and represents the only automated test available for the Milan Firefighters.
The chosen rapid serological test, LYHER® Novel Coronavirus (2019-nCoV) IgM/IgG Antibody Combo Test Kit by Hangzhou Laihe Bio-tech Co., detects IgG and/or IgM binding to the S1 subunit of the Spike glycoprotein. Rapid tests provide qualitative results indicating IgG and/or IgM presence. This LFIA is directly provided to the CBRN unit of the Milan Firefighters and serves as the frontline for serological screening.
The serological tests were performed by having each volunteer undergo both the rapid serological test and venous blood sampling in the same moment *(see supplementary materials S1 for sample collection, analysis and data process)*. Blood samples were collected using BD Vacutainer® SST™ II Advance Tubes and then centrifuged at 4.4 rpm (2850 x *g*) for 10 minutes using the Centrifuge 5702 (Eppendorf). The collected venous blood samples were subsequently analysed with the Elecsys® Anti-SARS-CoV-2 at Luigi Sacco Hospital, following the manufacturer's protocols. In parallel, personnel at the CBRN laboratories assisted volunteers with the rapid serological tests using the LYHER® Novel Coronavirus (2019-nCoV) IgM/IgG Antibody Combo Test Kit, in accordance with the manufacturer's instructions, while venous blood sampling was taking place. The medical officer conveys the result of the LYHER® test to the CBRN Unit's Head (Legal Data Controller) while the results of the Elecsys® test were always transmitted from the Luigi Sacco Hospital directly to the CBRN Unit's Head.

Statistical analysis

The volunteers enrolled in the study were grouped in the different analyses by age, antibody titre, and positivity to the virus.
Statistical differences were evaluated and analysed by t-test, using GraphPad Prism software (GraphPad Software, Inc.) and the related figures were generated using the same software. Results are reported in the next chapter considering p-value ≤0.05 (* p-value ≤0.05; ** p-value ≤0.01; *** p-value ≤0.001; ns, p-value >0.05).

RESULTS

Data collection and Cohen's kappa test

In total, we performed both Elecsys® and LYHER® serological tests on 133 volunteers. Out of 133 people, 67 (50.4%) tested positive for SARS-CoV-2 in the past (as ascertained by RT-PCR) with an average number of 92 days elapsed between positive RT-PCR and serological tests (the range starts from 26 days up to a case of 321 days). People in this subgroup were infected mainly during the so-called "second wave of COVID-19" from October to December 2020.
In addition, 11 individuals out of the 133 cases analysed (8.3%) had tested positive for serological tests in the past, while the remaining 55 out of 133 (41.3%) had never shown symptoms of COVID-19. The results collected were expressed in form of the confusion matrix reported in *Table 1*.
The interchangeability between the two tests was evaluated by considering the observed agreement, or the reproducibility of a result, between the two tests. Rather than simply measuring it via a simple percent agreement calculation, it was preferred to compute the Cohen's kappa coefficient, which provides a more

Table 1 - *Serological test data collected in the confusion matrix.*

		Elecsys®		
		POSITIVE	*NEGATIVE*	*TOT*
LYHER®	POSITIVE	89	8	97
	NEGATIVE	4	32	36
		93	40	133

robust evaluation by taking into account the possibility of the agreement occurring by chance.
Cohen's kappa fundamentally views two raters as different versions of a test, with their evaluations resembling the scores produced by that test. Recognized as a chance-corrected measure of inter-rater reliability, Cohen's kappa evaluates whether the level of agreement between the two raters exceeds what would be expected by random chance. It is based on several key assumptions: the subjects being rated are independent of one another, the rating categories are independent, mutually exclusive, and collectively exhaustive and the two raters function independently. Additionally, Cohen's kappa assumes that the correctness of the ratings cannot be determined in typical situations, and it considers the raters to be equally competent in making judgments based on prior knowledge (Sun S., 2011).
The calculated agreement between the two tests was 91%, while Cohen's kappa was 0.78 (CI 95%, 0.661-0.898). According to the score scale provided by Landis and Koch (Landis *et al.*, 1977), the calculated Cohen's kappa is placed in the "substantial" score range. This result shows a good degree of interchangeability between the two serological tests. This aspect is also confirmed using the PPA (Positive Percentage Agreement) and the NPA (Negative Percentage Agreement), which are 0.96 and 0.80, respectively. These two last parameters correlate the results of the Elecsys® and LYHER® tests but consider the first Immunoassay as the standard of the analysis; a high value of PPA is related to a high matching degree of positive detection between the two Immunoassays. Considering the NPA, a high degree of matching is related to a good level of concordance in terms of negative detection of the two Immunoassays.
Out of 97 individuals testing positive to SARS-CoV-2 by the LFIA test, 58 volunteers were detected positive for IgG (59.8%), 35 (36.1%) positive for IgG/IgM, and 4 (4.1%) for IgM only. IgM is characterized by a low affinity towards antigens and therefore its detection could be a sign of an infection still in progress; for safety reasons, each person who tested positive for IgM or IgG/IgM was also subjected to an RT-PCR analysis, and none of the 39 people tested positive for this type of analysis. This aligns with existing literature (Glück *et al.*, 2021; Ghasemi *et al.*, 2022) and indicates that the detection of IgM antibodies alone is not a reliable indicator of active infection (Kučinskaitė-Kodzė *et al.*, 2021). Additionally, IgA could be assessed as a serological marker for diagnosing acute infections, along with other biomarkers detectable through blood tests (Esmat *et al.*, 2024; Zervou *et al.*, 2021; Battaglini *et al*, 2022)

Antibody titre

The 67 individuals who tested positive to the virus in the past (i.e., they had a PCR-RT confirming it) were divided into two groups based on their median age (50 years) and the antibody titre was analysed in this subgroup using Elecsys®. The temporal difference in days between the confirmation of RT-PCR positivity and serological testing was analysed using t-test. Given that the p-value > 0.05, no statistically significant difference was observed between the two groups (*see supplementary material S2*).
Always through t-test, it was evaluated whether there was a significant difference between the means of anti-Nucleocapside antibody levels among the two groups: a higher antibody titre was observed in subjects who were older than 50 years (**p-value ≤0.01), as illustrated by ***Figure 1*** (the figure was generated using the GraphPad software), and this interesting result is in line with the literature (Chansaenroj *et al.*, 2021; Tutukina *et al.*, 2021; Weisberg *et al.*, 2021).
Furthermore, of the 67 previously positive subjects only 2 (3%) had an Elecsys® test result lower than 1 S/CO.

Sensitivity estimation

As mentioned above, 67 (50.4%) of the 133 volunteers enrolled had a positive RT-PCR for the virus prior to

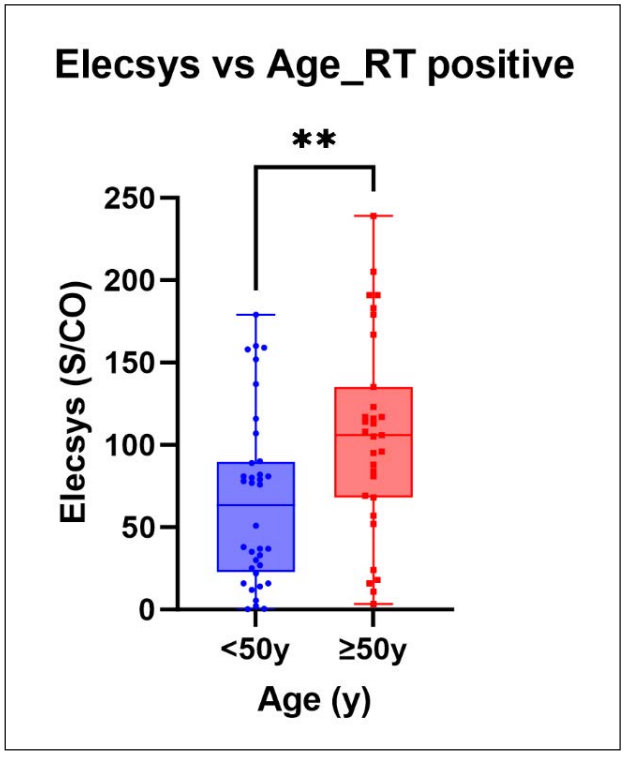

Figure 1 - *The 67 individuals who had previously tested positive for RT-PCR were divided into two groups according to their median age; the y-axis shows the antibody titre measured by Elecsys®. The t-test showed a significant difference (**p-value ≤0.01) between the mean antibody titres measured by Elecsys®. Thus, persons over 50 years of age have on average a significantly higher titre than the group with persons under 50 years of age.*

Table 2 - *Evaluation of the clinical sensitivity of serological tests.*

		POSITIVE RT-PCR			
Elecsys®	POSITIVE	65	65	POSITIVE	LYHER®
	NEGATIVE	2	2	NEGATIVE	
		67	67		

the study. From the test results, it was possible to calculate the clinical sensitivity for both serological tests used in this study.
In *Table 2*, both tests show 97% sensitivity (i.e., 65/67) in detecting immunoglobulins resulting from a previous SARS-CoV-2 virus infection.

Cohen's kappa disagreements

As shown by the sum of the number of individuals on the anti-diagonal of the confusion matrix presented in *Table 1*, there are 12 volunteers with discordant serological results. *Table 3* summarizes all the data concerning these individuals. Potential causes of discrepancies among these 12 volunteers will be discussed following *Table 3*.
Individuals No. 1 and No. 3 tested positive with the LYHER® test but not with the Elecsys® test after nearly one year from positive RT-PCR; this result is in line with what was previously stated about antibodies kinetics.
Individuals No. 2, 5, 6, 7 claim no past symptoms of Covid-19 but tested positive only on the rapid test. Additionally, Individual No. 9 mentioned close contact with a positive person. Considering the specificity of IgG presence, the rapid test correctly detected these individuals who had contracted SARS-CoV-2 infection further in the past.
The explanation for the failed detection of antibodies by the LYHER® test regarding subjects No. 4, No. 8 and No.10 will be provided in the discussion section.
Individual No. 11 tested positive for IgM antibodies from the rapid serological test: these kinds of antibody have low affinity and so this false positive result can be related to cross reaction dynamics with other pathogens; IgM are produced in the very early stages of infection and they have the ability to bind to different epitopes but with very low affinity constants, hence the possible cause of false positives due to cross-reactions with other pathogens. (Kadkhoda, 2022; Latiano *et al*., 2021). Additionally, as said in 3.1 chapter, they are not a reliable indicator of an ongoing SARS-CoV-2 infection if considered alone (Li Q *et al*., 2023; Trenti *et al*., 2021).
Endogenous factors may also cause false-positive results. In particular, the presence of molecules such as rheumatoid factor (RF), heterophil antibodies (HA), human anti-animal antibodies (HAAA), lysozyme and complement can cause false-positive results as reported by Ye et al. Rheumatoid factor (RF) is a class of autoimmune antibodies and the most common is the autoimmune IgM type. Rheumatoid factor is a marker of inflammation but it can also be detected in healthy individuals and may cause IgM false-positive results. In fact, it can bind through non-specific binding, and the immune complexes it forms can be captured by anti-IgM antibodies, resulting in a positive signal. For HAAA and HA there is a similar behaviour producing false positive results (Ye *et al*., 2021; Kharlamova *et al*., 2021).
Moreover, the LFIA test, as mentioned, targets the viral subunit S1 of the Spike glycoprotein: it is therefore possible to qualitatively estimate the presence of

Table 3 - *Discordant results observed between the LYHER and the Elecsys® tests. Time range is the number of days between the confirmation of positivity by RT-PCR, if available, of a given individual and the performance of serological tests on the same individual. The "Information available" column includes information on previous molecular or serological tests that may supplement discordant serological results. If there is no previous information and the subject does not complain of any symptoms related to COVID-19 at the time of testing, volunteers are considered an asymptomatic subject.*

ID.	*LYHER® results*	*Elecsys® results (S/CO)*	*Time Range (Days)*	*Information available*
1	IgG	0,3	300	confirmed positive by RT-PCR
2	IgG	0,1	/	asymptomatic subject
3	IgG/IgM	0,6	304	confirmed positive by RT-PCR
4	Negative	95	78	confirmed positive by RT-PCR
5	IgG	0,1	/	asymptomatic subject
6	IgG	0,9	/	asymptomatic subject
7	IgG	0,1	/	asymptomatic subject
8	Negative	25	77	confirmed positive by RT-PCR
9	IgG	0,1	/	refer previous contact with positive patient
10	Negative	7,4	201	previous positive serological test
11	IgM	0,1	/	false positive
12	Negative	21	/	asymptomatic subject

neutralizing antibodies as well (Danh *et al.*, 2022; Cerutti *et al.*, 2021; Cerutti *et al.*, 2022).
Individual No. 12, despite showing no symptoms of SARS-CoV-2 and no previous RT-PCR positivity, tested positive only for antibodies binding to the N protein, suggesting a recent infection. However, Elecsys® positivity may be attributed to another reason. The N protein's high conservation among coronaviruses raises the risk of detecting antibodies from previous infections unrelated to SARS-CoV-2, potentially resulting in a false-positive result (Michel *et al.*, 2020). The negative result in the rapid serological test may be interpreted either as correct or false negative; the causes of potential false negative results are similar to those discussed for individuals No. 4 and No. 8 in the discussion section.

DISCUSSION

As stated in the introduction, the detection of antibodies can be achieved using serological tests with a single viral target (the Spike glycoprotein or the Nucleocapsid protein); depending on when the serological test is performed and the viral target selected, the probability of detection changes. The following discussion pertains to the use of Cohen's kappa to compare different technologies and different viral targets.

Cohen's kappa applicability

Regarding the Cohen's kappa result, it has a value of 0.78, consistent with, or surpassing, literature benchmarks in comparing automated immunoassay and rapid serological tests. Specifically, Gambino *et al.*'s study, using viral Spike protein-targeted rapid tests and a combination of Nucleocapsid and Spike proteins for automated tests, reported a Cohen's kappa of 0.71 for IgM and IgG detection (Gambino *et al.*, 2020). The higher Cohen's kappa value in our study, likely due to the Elecsys® test's measurement of total immunoglobulins without distinguishing between IgG, IgM, and IgA. Such feature is highly useful for mass screening by identifying any antibody type, confirming past infection.
Nyabi *et al.* carried out a study on serological tests also conducted on HCW by means of a mobile laboratory in Piedmont: using an LFIA with Spike protein RBD as viral target, they analysed capillary whole blood and serum from same individuals; they found a Cohen's kappa of 0.77 for IgG and negative for IgM, potentially due to a matrix effect. This result is in line with ours for IgG. They also compared the results of the same LFIA with a serological test also detecting anti-Nucleocapsid IgM and IgG with serum samples from the same subjects. They found a Cohen's kappa for IgG of 0.61 and negative again for IgM (Nyabi *et al.*, 2021). Serre-Miranda et al. found Cohen's kappa values comparable to this study, despite utilizing different test combinations targeting Spike and Nucleocapsid protein (Serre-Miranda *et al.*, 2021). Plebani et al.'s comparison of a rapid test similar to ours with various automated tests yielded modest Cohen's kappa values, peaking at 0.62. A specific comparison with the Abbott test showed a Cohen's kappa of 0.57, which was again higher in our case, potentially due to the Elecsys® test's broader antibody detection. (Plebani *et al.*, 2021). Porru et al. conducted a serological survey of more than 5000 HCW and evaluated Cohen's kappa between automated and rapid tests, obtaining similar results to ours (Porru *et al.*, 2021). Coyle et al. conducted a serosurvey of about 700 patients and obtained a discordance rate of 8.5 %. This result is in line with ours (about 9 %) but they obtained a Cohen's kappa between Elecsys® and a LFIA test similar to the one we used of 0.81 (Coyle *et al.*, 2022). Buonocore et al. conducted a study using an LFIA with Nucleocapsid protein as viral target and a laboratory immunoassay that detects antibodies against the S1 and S2 subunits of the Spike glycoprotein. This study, like the one conducted by Porru *et al.*, was performed on HCW and their result is a Cohen's kappa of 0.86 and they used Elecsys® as a third rater for discordance. In their case, the percentage of discrepancies in the total is 5.8%, which is lower than ours. They too understand that serological analyses are antigen dependent and that therefore antibodies against Spike glycoprotein and Nucleocapsid protein vary their concentration over time according to different kinetics. This aspect is fundamental for understanding the outcome of serological analyses (Buonocore *et al.*, 2021). Considering the studies mentioned above, it must be emphasised that in each case there are discrepancies between serological tests with different viral targets, and this is an important factor to consider during a serological campaign. An interesting study was performed by Mafi et al. where they reported Cohen's kappa values of 0.85 between a LFIA and Alinity-i SARS-CoV-2 IgG (Abbott). In their study, they point out that the rapid test is not affected in terms of performance by the presence of variants such as Alpha, Beta and Delta (Mafi *et al.*, 2023); this is a very interesting aspect because the same tests could also be used with variants different from the wild-type, further lowering operating costs. Considering the cited studies and the results we obtained in terms of overall agreement and Cohen's kappa, which indicate a significant degree of interchangeability, the rapid serological test can serve as an alternative to the automated immunoassay when used on a statistically significant number of individuals. Moreover, rapid tests are easy to use and do not require specialized training. However, the Cohen's kappa obtained here differs from studies comparing automated laboratory serological tests, where kappa values often fall within the excellence range (0.81-1.00) due to their high-

er detection efficacy and advanced automation (Nasrallah *et al.*, 2021; Einhauser *et al.*, 2021; Hörber *et al.*, 2020).

Explanation of most controversial serological discordances

Individuals No. 4 and No. 8 tested positive in the RT-PCR respectively 77 and 78 days before the blood collection data, and were confirmed only by Elecsys®. It is unusual that individuals who contracted the infection about two months earlier showed no detectable anti-Spike antibodies; these kinds of results in rapid serological testing can be caused by operational and biochemical factors. Operational factors are related to test execution, but adherence to rules in the IFU (Instructions for Use) minimizes the likelihood of errors. However, two main types of operational factors can generate a possible false negative in an LFIA test, and they are operator dependent. A first possible cause lies in a sort of sample dilution: once the distal digit of a finger is pricked with a sterile needle, the blood must flow out with a good flow rate, otherwise there could be a dilution of the sample with tissue fluid. Furthermore, it is important not to prick in hematoma sites and without too much pressure to try to force the blood flow rate, otherwise there would always be a dilution with tissue fluid. Not discarding the first drops of blood, which always contain an excess of tissue fluid, would lead to a dilution of the sample. All these possible causes lead to a dilution of the sample and therefore the concentration of antibodies could fall below the detection limits of the rapid serological test. The second operational factor is due to the sanitization of the sampling site, generally performed with isopropyl alcohol; if the skin is not dried to remove the alcohol after the puncture with a sterile needle, it can cause hemolysis of the blood flowing out. Hemolysis interferes with the reaction that occurs between the antigen and the antibody and signal generation in rapid serological tests; in fact, hemolysis causes a release of proteolytic enzymes that can degrade proteins and this can falsify the result (D. Wild, chapter 6.1, Elsevier, The Immunoassay Handbook, Oxford, 2013). Other operator-dependent factors that may cause a false negative may be insufficient sample quantity or an early reading of the result compared to the timing dictated by the manufacturer in the IFU. Environmental factors such as temperature and relative humidity could influence the test (Mouliou *et al.*; 2021 a). Furthermore, Wang J *et al*. (Wang *et al.*, 2012) noted in their work that temperature above 70°C can affect antibody activity. Humidity, on the other hand, is relevant at 80°C as it has a greater effect on the water solvation of the antibodies and they therefore undergo structure unfolding. Similar considerations on humidity were made by Huang *et al*. (Huang *et al.*, 2018). Laboratory tests such as Elecsys® are used in professional contexts and therefore both sample storage and the conditions under which the immunoassay is used must be optimal. Due to the use of rapid serological in various contexts, studies on the effects of environmental factors on these tests could be a valuable line of research, especially after their widespread use during the SARS-CoV-2 outbreak. In fact, it is interesting to note that, although using antigenic and not rapid serological tests, two studies conducted with slightly different environmental conditions led to diverging conclusions (Gick *et al.*, 2023; Haage *et al.*, 2021).

Biochemical factors like genetics, gender, and the presence of substances like cholesterol, can influence serological test outcomes (Mouliou *et al.*; 2021 b) Regarding to gender, it is reported that women have a more intense immune response, also in terms of antibody production. This difference seems to be attributed to genetic and hormonal factors that modulate the immune response (Plebani *et al*. 2022; Ciarambino *et al.*, 2021)

Anticoagulants, high haematocrit and the presence of molecules like triglycerides or cholesterol can change the viscosity of the blood and thus distort the result as the speed of blood migration on the nitrocellulose strip influences the appropriate binding between antigen and antibody (Ernst *et al.*, 2021).

Similar consideration to individuals No. 4 and No. 8 can be made also for individual No.10 who voluntarily underwent a serological assay (DiaSorin LIAISON SARS-CoV-2 S1/S2 IgG) 201 days before blood collection date and resulted positive.

Considering only individuals No. 2, 5, 6, 7, 11, and 12 of Table 3, none of them showed symptoms of any kind at the time of the tests: they were unaware of having contracted the virus in the past, highlighting the further utility of serological campaigns conducted during an epidemic, i.e., detecting individuals who have come into contact with the virus unconsciously. Without detecting these types of individuals, there wouldn't be a true understanding of how the epidemic spreads. In addition, these subjects have discordant serological results, and therefore might not be correctly identified if only one type of viral target had been used. This aspect highlights the usefulness of serological screening performed with different viral targets.

Sensitivity of serological tests

The calculated sensitivities have values comparable to those declared by the manufacturers, which is 97% for both tests. LYHER® test failed to detect subjects No. 4 and 8 of Table 3, while the Elecsys® subjects No. 1 and 3, also from *Table 3*.

A crucial aspect is that the rapid serological test interpretation was done visually by the operators without any aid. There were situations in which two operators disagreed when the positive line was faintly coloured; incorporating organic fluorescent substances could

enhance the visibility of the positive signal. In the past, substances such as fluorescein isothiocyanate were used (Kim *et al*., 2023), and during the SARS-CoV-2 outbreak there was a great development of LFIAs with the possibility of quantifying antibodies (Bian *et al*., 2024; Shurrab *et al*., 2022). Wang X et al. also show how quantification can be carried out with spectrophotometers that are not very expensive and can even report data using a smartphone (Wang X *et al*., 2023). Moreover, employing dual viral target rapid tests may improve concordance between the two tests (Jassam *et al*., 2021; Cook *et al*., 2021; Fogaça *et al*., 2024).

Limitations of the study

The limitations of the present study primarily include the number of subjects involved, their age (age ranges from 23 to 65 years old), their gender (e.g., with fewer than 10% being female), and the different timeframes between the occurrence of infection and the dates of serological testing.

However, these intrinsic aspects of the observed population are evidently linked to resource availability, which was challenging to reconcile with operational issues stemming from personnel diverted from the emergency sector during the pandemic.

Furthermore, it was not possible to perform serial sampling according to a precise timeline to study the kinetics of antibodies in volunteers and observe the variation of Cohen's kappa over time; this aspect could be considered for a future project.

CONCLUSIONS

In summary, our study demonstrates that the LYHER® Novel Coronavirus (2019-nCoV) IgM/IgG Antibody Combo Test Kit and the Elecsys® Anti-SARS-CoV-2 tests exhibit a good level of concordance and interchangeability through the use of Cohen's kappa in a pool of individuals. In our case, the pool of individuals belongs to the broader group of first responders (i.e., firefighters and police officers) participating in a screening campaign that started in January 2021, shortly before the vaccine became available in Italy. We also verified that both tests are characterized by high sensitivity in detecting previous exposure to the virus.

In practical terms, our results indicate that a portable, inexpensive, and non-invasive immunoglobulin detection device, specifically the LFIA, can effectively assess the extent of SARS-CoV-2 exposure among a given pool of individuals. Rapid serological tests are particularly valuable during periods of high demand on national healthcare services: in fact, they allow identification of first responders with a previous infection and guide them towards more complex laboratory tests without routing them through the healthcare system for the general population, as they need to return to service as soon as possible. Rapid serological tests have, however, disadvantages. Interpreting the results can be challenging, as the positive band on the nitrocellulose strip may be faint. At the beginning of the epidemic, only qualitative tests were available. Additionally, rapid tests may have a lower detection limit compared to automated tests like Elecsys®, which utilize advanced technology to read analytical signals, while rapid tests rely on visual assessment. Environmental factors such as temperature and humidity can also affect results, and the use of capillary blood may introduce interfering substances that distort the outcome. The rapid serological test employed in this study can also serve to assess the presence of effectiveness of vaccination, as it can detect antibodies generated in response to the vaccination regimen (Tsuchiya *et al*., 2023) and to assess the presence of neutralizing antibodies.

Moreover, checking for the presence of antibodies resulting from SARS-CoV-2 infection could lead to a more efficient allocation of vaccine supplies, especially in countries with limited availability (Castrejón-Jiménez *et al*., 2022; Spicuzza *et al*., 2023; Ayoub *et al*., 2021; Wang *et al*., 2022). LFIAs that detect anti-Nucleocapsid antibodies, which are markers for a previous infection, could be used in this context. People who test positive in such tests may not need additional vaccine or booster doses, and therefore the vaccine doses can be rationalised and concentrated towards those who have never contracted the disease. Furthermore, the use of a rapid test with the same viral target as ours is important to verify the individual's actual immunization (Ward et al., 2022), especially for people who have never contracted the virus in the past, and to monitor antibody levels over time for all vaccinated personnel.

Credit authorship contribution statement

Simone Murganti: Conceptualization, Methodology, Validation, Formal analysis, Writing - original draft.
Edoardo Cavalieri d'Oro: Validation, Formal analysis, Writing - review & editing, Supervision, (P.I.).
Matteo Villa: Methodology, Validation, Formal analysis, Writing - original draft.
Antonio Papagni: Supervision.
Andrea Malizia: Funding acquisition.

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Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon request (murgantisimone@gmail.com).

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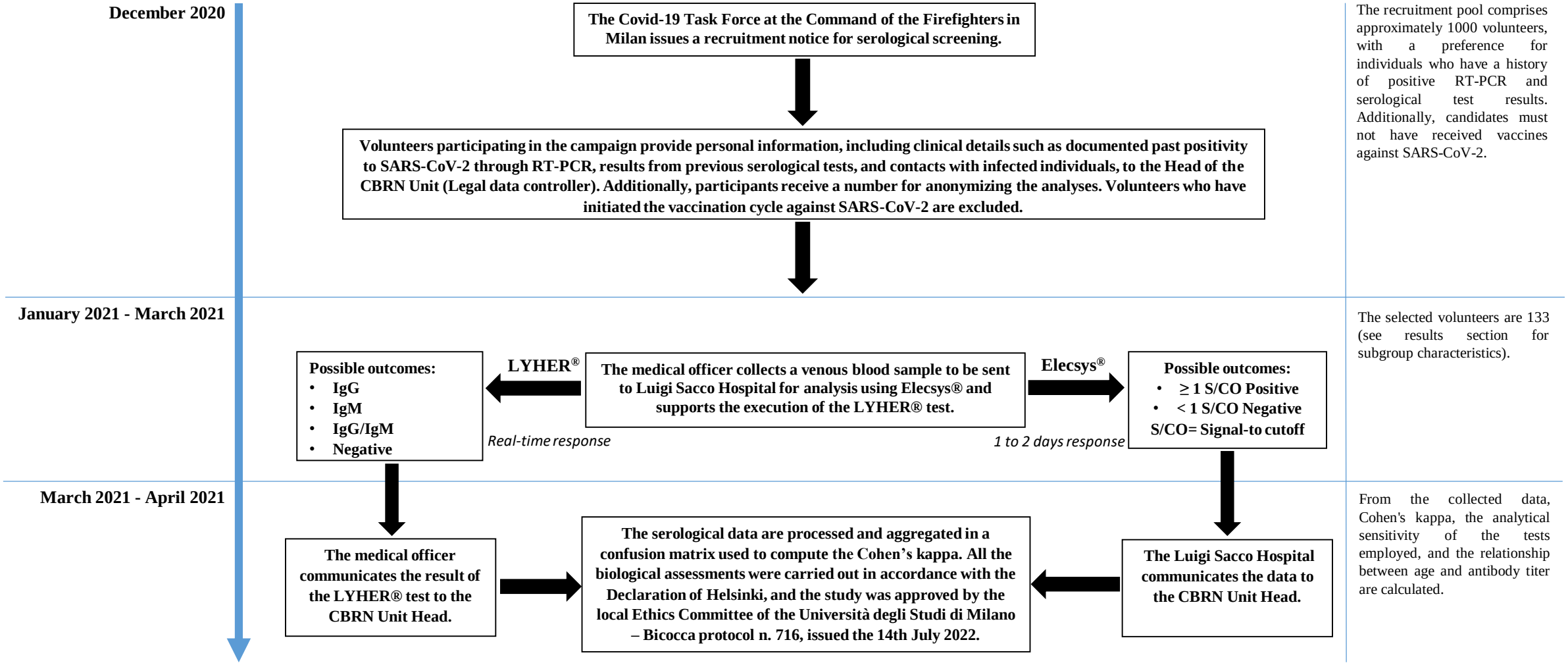
December 2020
The Covid-19 Task Force at the Command of the Firefighters in Milan issues a recruitment notice for serological screening.
Volunteers participating in the campaign provide personal information, including clinical details such as documented past positivity to SARS-CoV-2 through RT-PCR, results from previous serological tests, and contacts with infected individuals, to the Head of the CBRN Unit (Legal data controller). Additionally, participants receive a number for anonymizing the analyses. Volunteers who have initiated the vaccination cycle against SARS-CoV-2 are excluded.
The recruitment pool comprises approximately 1000 volunteers, with a preference for individuals who have a history of positive RT-PCR and serological test results. Additionally, candidates must not have received vaccines against SARS-CoV-2.
January 2021 - March 2021
The selected volunteers are 133 (see results section for subgroup characteristics).
Possible outcomes:
• IgG
• IgM
• IgG/IgM
• Negative
LYHER®
The medical officer collects a venous blood sample to be sent to Luigi Sacco Hospital for analysis using Elecsys® and supports the execution of the LYHER® test.
Elecsys®
Possible outcomes:
• ≥ 1 S/CO Positive
• < 1 S/CO Negative
S/CO= Signal-to cutoff
Real-time response
1 to 2 days response
March 2021 - April 2021
From the collected data, Cohen's kappa, the analytical sensitivity of the tests employed, and the relationship between age and antibody titer are calculated.
The medical officer communicates the result of the LYHER® test to the CBRN Unit Head.
The serological data are processed and aggregated in a confusion matrix used to compute the Cohen's kappa. All the biological assessments were carried out in accordance with the Declaration of Helsinki, and the study was approved by the local Ethics Committee of the Università degli Studi di Milano – Bicocca protocol n. 716, issued the 14th July 2022.
The Luigi Sacco Hospital communicates the data to the CBRN Unit Head.

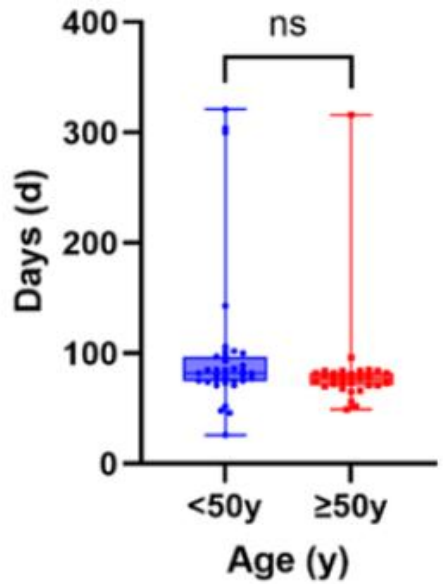

Figure 1. The 67 RT-PCR positive individuals were divided into two groups based on the median age. The y-axis represents the differences in days between the confirmation of RT-PCR positivity and the day of serological testing for this study. The t-test showed no statistically significant difference (ns, $p > 0.05$) in the time from RT-PCR positivity confirmation to serological testing between the groups.