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Performance Evaluation: Four Chemiluminescent SARS-Cov-2 Immunoassays and Rapid-Card Test in Mild Disease and Seroprevalence of SARS CoV-2 in Frontline Healthcare Workers

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Authors' contributions

This work was carried out in collaboration among all authors. Author RS designed the study, wrote the protocol, and wrote the first draft of the manuscript. Authors MD and GPT performed the statistical analysis. Authors AH, RY, PB and RKK managed the analyses of the study. Author AER managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: The COVID-19 pandemic raised a host of challenges to modern medicine. Key amongst these were in diagnostics, as most SARS-CoV-2 assays had been rapidly developed and released under emergency-use authorization with limited validation on clinical samples and secondly, an increased risk of COVID-19 infection to healthcare workers (HCW). There are limited inter-assay comparisons to detect SARS-CoV-2 antibodies in cases with milder symptoms of COVID-19, necessary to evaluate whether assays can detect SARS-CoV-2 antibodies in patients with mild infection.

Aim: Therefore this study aimed to evaluate the performance of four chemiluminescence immunoassays and a rapid immunochromatographic assay in 100 rRT-PCR diagnosed-recovered frontline HCW with milder COVID-19 disease and secondly to evaluate the seroprevalence of SARS-CoV-2 infection in the asymptomatic frontline HCW at a multispeciality hospital in Delhi, India.

Study Design: Serum and plasma samples were obtained from 100 rRT-PCR diagnosed-recovered frontline HCWs with mild disease working across the hospital, and performance of four common chemiluminescence immunoassays evaluated. Also samples of 505 asymptomatic, frontline HCWs working in hospital, who had not developed or shown any symptoms of COVID-19 infection to date was collected and the seroprevalence of infection was evaluated.

Place and Duration of the Study: A study was conducted at BLK Superspeciality Hospital, New Delhi from September to October 2020.

Methods: Four chemiluminescence immunoassays [Abbott SARS-CoV-2 IgG (Nucleocapsid), Roche Elecsys® Anti-SARS-CoV-2 Total (Nucleocapsid), Ortho-Clinical Diagnostics: VITROS Anti-SARS-CoV-2 IgG (Spike) and Anti-SARS-CoV-2 Total (Spike)] and a rapid assay [Medsource Ozone Biomedicals] were evaluated in 100 rRT-PCR diagnosed-recovered frontline HCW with mild disease. Also, seroprevalence was studied in 505 asymptomatic, frontline HCW.

Results: At manufacturers' thresholds, overall sensitivity for Abbott was 71%, Roche 96%, Ortho (both total and IgG(S) 99% and rapid card 56%. Seroprevalence in asymptomatic frontline HCW was found to be 17.6%, with positivity being higher in the HCW group not facing patients directly compared to direct patient caregivers (P = 0.0034).

Conclusion: Assay performance depends on assay design (total IgM & IgG antibodies versus IgG alone), choice of antigen, and time of sample testing from the onset of disease. In our study, Ortho Vitros total-Ab; IgG (Spike), and Roche Elecsys total-Ab (Nucleocapsid) assays were found to have optimal sensitivity. A seroprevalence study in the frontline HCWs at our institute showed that seroprevalence was higher (17.6%) in HCWs in comparison to the community.

Keywords: Chemiluminescent assay; COVID-19; frontline healthcare workers; SARS-CoV-2 antibody; seroprevalence.

ABBREVIATIONS

CMIA : Chemiluminescent Microparticle Immuno Assay (CMIA)
CLIA : Chemiluminescence Immunoassay
ECLIA :Enzyme Chemiluminescence Immuno-assay
ICA : Immunochromatographic Assays

1. INTRODUCTION

In early December 2019, the novel coronavirus SARS-CoV-2 causing coronavirus disease 2019 (COVID-19) emerged in China (Wuhan) leading to the ongoing pandemic [1], of paramount global health concern. Most countries are facing the second wave of the pandemic and as per "Worldometer" (accessed 31st March, 2021) 219 countries and territories have reported a total of 2,818,445 deaths [2]. Serological tests are important tools for the estimation of seroprevalence, and are gaining relevance in settings for i) Diagnostic purpose in cases who seek medical attention more than seven days after the onset of symptoms ii) to differentiate acute infection versus recent infection iii) for estimating potential immunity and risk of infection iv) identification of convalescent plasma donors and v) seroepidemiological studies to understand the extent of COVID-19 spread and monitoring immunization following vaccination.

Amongst the 4 coronavirus structural proteins, spike (S) and nucleocapsid (N) proteins are the main immunogens. Studies have reported a

strong positive correlation between clinical severity and antibody titer after the illness onset [3]. The sensitivity, as well as specificity of any serological assay can also be affected by the target antigen. Studies have shown that S (Spike) protein (produced in a more advanced stage of SARS-CoV-2 infection) showed lower levels of sensitivity and more specificity (especially the S1 subunit) as compared to the N (Nucleocapsid) protein [4]. Therefore, the selection of an assay for a specific purpose, decision-making should include available knowledge of antibody specificities, kinetics, and functions [5,6]. However, due to urgent demands, majority of the serological tests have been rapidly developed and made available on the market under emergency use authorization with only limited validation on clinical samples. Most of the comparative analyses on various serological assays have been done on majorly the hospitalized patient groups with moderate to severe disease. There are limited inter-assay comparisons to detect SARS-CoV-2 antibodies in cases with milder symptoms of COVID-19, necessary to evaluate whether assays can detect SARS-CoV-2 antibodies among the most common type of patient with SARS-CoV-2 infection. The study provides an insight for seroepidemiological investigations and seroprevalence studies to assess the risk of infection in asymptomatic HCW.

The study aimed to evaluate the performance of four high-throughput commercial chemiluminescence immunoassays frequently used for healthcare settings, using samples collected from rRT-PCR confirmed COVID-19 infected and recovered frontline healthcare workers (HCW) with milder COVID-19 disease. Head-to-head comparisons were done in terms of various statistical parameters like sensitivity, specificity, and Cohen's kappa agreement. We also evaluated a rapid immunochromatographic card test to check for sensitivity for rapid test to be utilized for mass population screening. A seroprevalence study was simultaneously undertaken to estimate SARS-CoV-2 infection seroprevalence in asymptomatic frontline HCW working in the hospital in the pandemic peak.

2. METHODS

2.1 Participants Recruitment for Serological Assay Comparison and Seroprevalence

Frontline HCWs working at our hospital, a large tertiary care COVID hospital in North India, were recruited in this prospective cross-sectional monocentric study. The study was approved by the Institutional Ethics Committee and informed written consent was obtained from each subject before sample collection. A standardized questionnaire was answered by each subject. Participant selection was randomly done to cover staff from all sections of the hospital having a direct interface with patients and/ or their attendants and they were grouped depending on the frequency of contact to patients/ attendants visiting the hospital into the following groups:

i. High-risk Group with daily contact to COVID-19 patients in designated wards and in intensive care units;
ii. Intermediated-risk Group with daily non−COVID-19 patient contact;
iii. Low-risk Group without daily patient contact or working in areas like reception/ OPD pharmacy/ security.

605 frontline HCWs were recruited for the study to cover two limbs of the study.

The study population comprised of two groups:

(i) Known Positive (infected and recovered) HCW: 100 frontline HCW rRT-PCR (from naso and oropharyngeal swab) confirmed COVID-19 disease after the end of quarantine or hospitalization at ≥10 days from the positive test result and not more than 2 and half months beyond rRT-PCR positivity. Timelines of ≥10 days to 2.5 months were selected as studies have shown that median time for seroconversion is about 10-14 days and start declining after 3 months of infection[4]. Time of illness (TOI) of participants was calculated from the date of testing. Samples from this group were used to a comparative analysis of performance of four high throughput commercial chemiluminescent immunoassays and one rapid immunochromatographic assay.

COVID-19 negative panel was built from plasma collected in the pre-pandemic period before December 2019 from 100 healthy blood donors, who were considered true negative.

(ii) Asymptomatic frontline HCW: 505 asymptomatic frontline health care workers, working in hospital and had not developed or shown any symptoms of COVID-19 infection to date were recruited separately for the study to estimate the seroprevalence of SAR-Cov-2 infection in healthcare workers.

2.2 Sample Collection

Twelve milliliters (ml) samples were obtained from each participant in EDTA and serum separating vacutainers in the same draw using strict aseptic techniques. Serum and plasma were aliquoted and frozen at -80°C.

2.3 Index Test Methods

Performance of diagnostic accuracy of four high throughput commercial chemiluminescent immunoassays (FDA-EUA Authorized) and one rapid immunochromatographic assay (ICA) was performed (Table 1). These assays use either S or N protein antigens and all the assays generate a qualitative positive/negative result based on assay-dependent signal thresholds. Tests were performed by experienced laboratory technicians following manufacturers' protocols with cut-off values. One positive and negative control was run once, before each batch of antibody testing.

Assays evaluated (All assays were Indian Council of Medical Research (ICMR) Certified) [7].

1. Abbott Architect SARS-CoV-2 IgG assay: detects anti-N IgG using a two-step chemiluminescent microparticle immunoassay (CMIA) method with an acridinium-labeled anti-human IgG.
2. Roche Elecsys® Anti-SARS-CoV-2 total assay is a two-step bridging electro-chemiluminescent immunoassay (ECLIA) using ruthenium-labeled and biotin-conjugated N protein.
3. Ortho-Clinical Diagnostics VITROS Anti-SARS-CoV-2 IgG test is a two-step bridging CLIA method that detects antibodies against the RBD of the spike protein.
4. Ortho-Clinical Diagnostics VITROS Anti-SARS-CoV-2 Total qualitatively measures total antibody {including IgA, IgM, and IgG (S1)} to SARS-CoV-2.
5. ICA from Medsource Ozone Biomedicals Pvt Ltd: A rapid card to test SARS Cov-2 total antibodies (Total IgG and IgM).

Rapid test was rated optically by the strength of their reaction and was carried out with plasma sample according to the manufacturer's instructions. The lines were read after 15 min and classified according to their strength, from 0 to 4+, graded at an intensity equivalent to the control line. A picture card was used to standardize the interpretation of the result (Fig. 1).

2.4 Statistical Analysis

In absence of any gold standard for SARS-CoV-2 antibody immunoassay, an alternate reference standard was used for this study, which is SARS-CoV-2 rRT-PCR positivity with ≥10 days and not more than 2 and half month beyond rRT-PCR positivity. The results of antibody measurements were evaluated according to manufacturers' cut-off indices as positive or negative for all 4 immunoassays assays and simply positive or negative for the rapid test. Diagnostic sensitivity and specificity were calculated under the following assumption: all samples obtained before to the onset of the pandemic were considered as true negative. In analogy to a previous study by Alexander Krüttgen, the SARS-CoV-2 antibody status of a serum was defined as follows: Serum was regarded as SARS-CoV-2 antibody-negative if at least three of the four chemiluminescent assays compared here had a negative test result, applying manufacturer's interpretation criteria and a sample was regarded as SARS-CoV-2 antibody positive if at least two of the four chemiluminescent assays had a positive test result [8]. Concordance analyses (Cohen's Kappa) and percent agreement (overall, positive/negative) were performed to compare the results of each antibody assay. To interpret results, following kappa values were considered: <0: less than chance agreement; 0.01-0.20: slight agreement; 0.21-0.40: fair agreement; 0.41-0.60: moderate agreement; 0.61-0.80: substantial agreement: 0.81-0.99, almost perfect agreement. Analyses were performed using Statistical Package for Social Sciences (SPSS) version 26.0.

Seroprevalence was stratified by high- versus low-risk work environment and healthcare role (i.e. doctors, nurses, lab technician/other technicians, housekeeping, security staff, others). Comparative rates are reported as relative risk (RR) with 95% confidence intervals (CIs), calculated using a Taylor series.

Table 1. Details of index test methods

Assay Name	Abbott Architect SARS-CoV-2 IgG assay	Ortho-Clinical Diagnostics VITROS Anti-SARS-CoV-2 IgG	Roche- Elecsys® Anti-SARS-CoV-2 Total antibody assay	Ortho-Clinical Diagnostics VITROS Anti-SARS-CoV-2 Total	Medsource Ozone Biomedicals Pvt Ltd
Assay Principle	CMIA	CLIA	ECLIA	CLIA	ICA
Target Antigen	N	Spike (S1)	N	Spike (S1)	Not specified
Sample type	Serum, plasma	Serum	Serum, plasma	Serum	Serum, plasma, whole blood (WB)
Volume of sample	25 µL	20 µL	20 µL	20 µL	10µL (Serum/ plasma) 20 µL(WB)
Type of antibody detected	IgG	IgG	IgA, IgM, and IgG	IgA, IgM, and IgG	IgM, and IgG
Result calculation index	S/CO	S/CO	COI	S/CO	Positive
Positive cut off threshold	≥ 1.4	≥ 1.0	≥ 1.0	≥ 1.0	Positive test
ICMR approved	Yes	Yes	Yes	Yes	Yes
Operation type	Continuous random access	Continuous random access	Continuous random access	Continuous random access	Point of care test

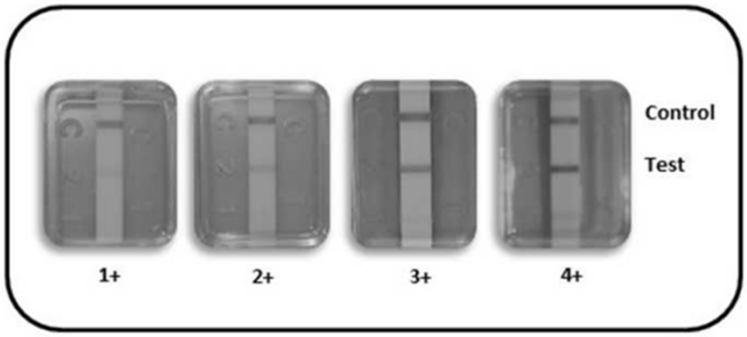

Fig. 1. A picture card to standardize interpretation of the result of rapid card test

3. RESULTS

3.1 Baseline Characteristics of Study Participants

A detailed history was taken from the 100 known positive (infected and recovered) HCW to determine the date of symptom onset and the date of first SARS-CoV-2 rRT-PCR positive result, nature of symptoms, the disease course, and area of working (Table 2).

3.2 Specificity, Sensitivity and Agreement of Four Commercial Chemiluminescent Immunoassays Assays

Samples of all 100 known positive (infected and recovered) HCW were run on all four chemiluminescent immunoassay platforms and rapid immunochromatographic assay (Fig. 2).

Intra-assay sensitivity for each assay as detailed in, to account for the differences in time post-rRT-PCR positivity. Sensitivity across 20 days rolling time window was studied. Assay from Abbott had sensitivities of 73.08% at 10-30 days, 76.08% at 31-50 days, and 60.71% at >50 days post-rRT-PCR test, while Roche had sensitivity 100% at 10-30 days, 93.48% at 31-50 days and 96.43% at >50 days post-rRT-PCR-positive test. Ortho showed 100% sensitivity at 10-30 days and 31-50 days and 99% at >50 days post-rRT-PCR-positive test. One case tested negative by all 4 assays, was a nursing staff who was day 56 post-rRT-PCR positive result. The staff had been asymptomatic and diagnosed during contact tracing. Sero-negativity could be due by several factors, including mild disease, only transient antibody response, no antibodies produced or produced at non-detectable levels, or possibly false-positive rRT-PCR result. Relative sensitivities of all assays changed with time. Specifically, the sensitivity of the Abbott assay declined to 60.71% in the >50-day window, possibly as Abbott assay is an anti-nucleocapsid IgG assay and anti-N antibodies appear early in the disease and decline with time[4]. The sensitivity of the rapid immunochromatographic assay was only 56%. To evaluate the diagnostic specificity of SARS-CoV-2 antibody assays we used control samples of blood donors collected before December 2019 (Table 3).

Comparing qualitative results of SARS-CoV-2 antibody assays, the Abbott Architect IgG (N) assay showed a substantial agreement of 82% (Cohen's Kappa 0.64, 95% CI) with Roche Elecsys® Total (N) assay, the Roche Elecsys® Total (N) assay showed an almost perfect agreement of 98 % (Cohen's Kappa 0.96, 95% CI) with the Ortho Vitros Total (S) assay. Abbott Architect IgG (N) assay showed a moderate agreement of 80% (Cohen's Kappa 0.60, 95% CI) with the Ortho Vitros IgG(S) assay (Table 4).

3.3 Seroprevalence of SARS Cov-2 Infection in Asymptomatic Frontline HCW

Samples of 505 healthy frontline HCW were collected after taking detailed history for any flu-like symptoms in the last 4 months and run on two chemiluminescent immunoassays targeting different target proteins [Roche Elecsys® Anti-SARS-CoV-2 total assay (Anti-N) and Ortho Vitros IgG(S)]. None of the 505 HCW in this group had reported any significant flu-like symptoms. Only those HCW who tested positive on both chemiluminescent immunoassays were considered to be truly positive. The average age of participants was 35 years and 307 (60.8%) were male and 198 (39.2%) were females. 89 HCW (17.6%) tested positive for SARS-CoV-2 on both 2 chemiluminescent assays.

Table 2. Baseline characteristics of study participants

Characteristic	Know positive (infected) HCWs N (%)	Asymptomatic HCWs N (%)
Total Numbers	**100**	**505**
	Sex	
Male	76 (76)	307(60.8)
Female	24 (24)	198 (39.2)
Age, median (range) years	34 (21-53)	35 (20-64)
	Staff category	
Doctors	14 (14)	101 (20)
Nurses	23 (23)	136 (26.9)
Lab technician/other technician	9 (9)	81(16.0)
Housekeeping staff	9 (9)	51 (10)
Security staff	7 (7)	20 (3.9)
Others	38 (38)	116 (22.9)
	Risk Stratification	
High-risk group	33 (33)	70 (13.8)
Intermediated-risk group	28 (28)	318 (62.9)
Low-risk group	39 (39)	117 (23.1)
	Symptoms	
Asymptomatic	10 (10)	-
Fever	70 (70)	-
Cough and sore throat	33 (33)	-
Shortness of breath	7(7)	-
Weakness and malaise	19 (19)	-
Headache	21 (21)	-
Anosmia and ageusia	11(11)	-
Gastrointestinal Symptoms	5 (5)	-
	Severity of symptoms	
Mild (Requiring home isolation)	83(83)	-
Moderate (Requiring hospitalization-ward)	17 (17)	-
Severe (Requiring ICU admission)	0(0)	-

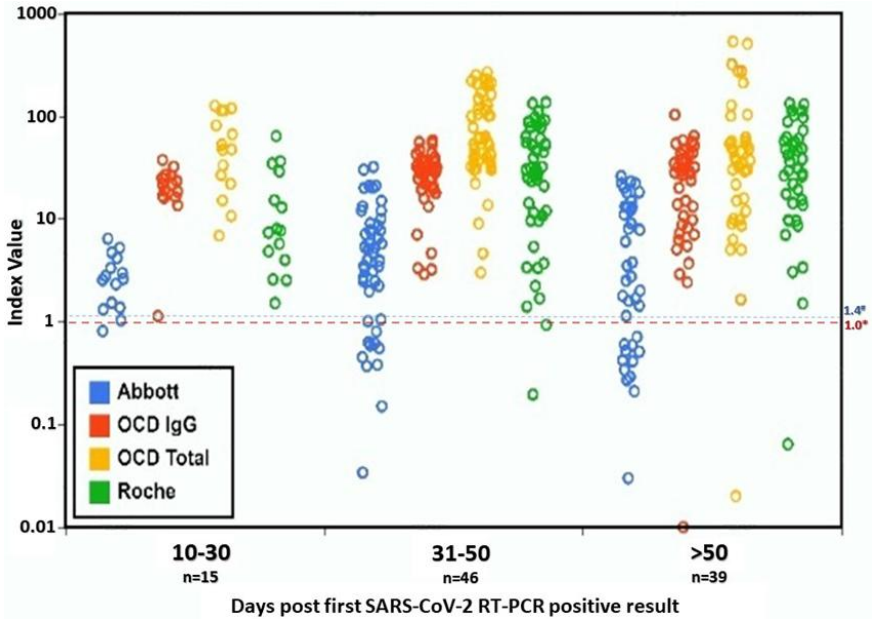

Fig. 2. SARS-CoV-2 antibody levels on 4 chemiluminescent immunoassays across 20 days rolling time window in 100 RT-PCR diagnosed-recovered frontline healthcare workers with mild disease

Table 3. Sensitivity (CI 95%) calculated for different immunoassays (Abbott, Roche- Elecsys, Ortho-Clinical Diagnostics VITROS IgG, Ortho-Clinical Diagnostics VITROS (Total), Med source biomedical across 20 days rolling time window: 10–30 days (n:15), 31-50 days (n=26) and >50 days (n=16) post positive RRT-PCR(known positive). The overall sensitivity (CI 95%), specificity (CI 95%), positive predictive value as well as negative predictive value (CI 95%) for assessing SARS-CoV-2seroconversion is reported for each immunoassay

	Abbott Architect	Roche- Elecsys®	Ortho-Clinical Diagnostics VITROS IgG	Ortho-Clinical Diagnostics VITROS (Total)	Med source biomedical
			Sensitivity %		
10-30 (n=26)	73.08 (19/26)	100(26/26)	100(26/26)	100(26/26)	34.6 (9/26)
31-50 (n=46)	76.08 (35/46)	93.48 (43/46)	100 (46/46)	100 (46/46)	71.73 (33/46)
>50 (n=28)	60.71 (17/28)	96.43(27/28)	96.43(27/28)	96.43(27/28)	50.00 (14/28)
Over all Sensitivity % (95% CI)	71.00% (61.07 to 79.64%)	96.00% (90.07 to 98.90%)	99.00% (94.55 to 99.97%)	99.00% (94.55 to 99.97%	56.00% (45.72 to 65.92%)
Negative Predictive valve (95% CI)	77.17% (71.29 to 82.14%	96.12% (90.45 to 98.48%)	99.01% (93.43 to 99.86%)	99.01% (93.43 to 99.86%)	69.44% (64.56 to 73.93%)
Accuracy % (95% CI)	84.50% (78.73 to 89.22%)	97.50% (94.26 to 99.18%)	99.50% (97.25 to 99.99%)	99.50% (97.25 to 99.99%)	78.00% (71.61 to 83.54%)
Positive Predictive valve% (95% CI)	97.26% (89.95 to 99.29%)	98.97% (93.17 to 99.85%)	100.00%	100.00%	100.00%
Specificity % (95% CI)	98.00% (92.96 to 99.76%)	99.00% (94.55 to 99.97%)	100.00% (96.38 to 100.00%)	100.00% (96.38 to 100.00%)	99.00% (94.55 to 99.97%)

Table 4. Concordances among the SARS-CoV-2 antibody assays

	Agreement	Kappa	Interpretation	CI
Abbott Architect IgG (N) Versus Roche Elecsys® Total (N)	82%	0.64	Substantial agreement	95%
Roche Elecsys® Total (N) to Ortho Vitros Total (S)	98%	0.96	Almost perfect agreement	95%
Abbott Architect IgG (N) to Ortho Vitros IgG(S)	80%	0.60	Moderate agreement	95%

Table 5. Overall distribution detectable SARS Cov-2 antibodies in asymptomatic healthy frontline HCW with relative risk of infection in different groups of caregivers

Characteristic	Total numbers (SARS-Cov-2 antibodies detected)	Relative Risk	95% CI	P-value
Gender				
Male	65 (73)			
Female	24 (24)			
Category				
Doctors	101 (4)	-		
Nurses	136(16)			
Lab technician/other technicians	82 (4)			
Housekeeping staff	51 (31)			
Security staff	19 (8)			
Others	116 (26)			
Total	89 (17.6)			
Risk Stratification				
High-risk group	70 (35)			
Intermediated-risk group	318 (42)			
Low-risk group	117 (12)			
Group stratification for comparison				
Males versus females	307 (61)vs 198 (28)	2.208	1.42 to 3.43	0.0004
Direct patient care giving employees versus non patient facing employees	369 (54)vs 136 (35)	0.57	0.39 - 0.83	0.0034
Nurses versus other direct patient caregiving employees	136 (15)vs 233 (39)	0.66	0.37 - 1.15	0.15
Housekeeping versus other direct care giving employees	51 (31)vs 318 (23)	8.40	5.35 - 13.20	< 0.0001

Male HCW showed higher adjusted seropositivity and relative risk as compared to female HCW and the difference in rates was statistically significant (P = 0.0004). HCW amongst the high-risk group had a positivity rate of 6.9% (35 of 505) compared to 8.5% (42 of 505) in the intermediate-risk group and 2.3% in low-risk (12 of 505) (Table 5).

Pre-planned comparisons were done amongst high-risk plus intermediate-risk versus low-risk settings (direct caregivers versus non-patient-facing employees). Nurses (being at higher risk owing to a longer period of direct patient contact) versus all others; housekeeping versus other direct patient-facing employees. The difference in rates was statistically significant (P = 0.0034) in group not facing patients directly versus direct patient caregiving employees [relative` risk [RR], 0.57; 95% Confidence interval (CI); 0.39- 0.83]. Nurses also did not show a higher seroprevalence as compared to other direct caregiving employees (p=0.15) [relative risk [RR], 0.68; 95% CI; 0.37- 1.15]. However, housekeeping (stretcher-bearers and waste management staff) had a higher rate of positive cases than other direct care giving employees-doctors, nurses, and technical staff, and difference in rates was statistically very significantly (P < 0.0001) (RR, 8.4; 95% CI; 5.35 to 13.20). None of these 89 asymptomatic, seropositive HCW had been diagnosed with COVID-19 infection. On taking a detailed history once again, 18 of them reported very vague symptoms like fatigue or mild headache lasting for less than a day, however, none of them had a

history of fever, cough, sore throat, gastrointestinal symptoms or anosmia.

4. DISCUSSION

This study presents a head-to-head comparison of 4 high-throughput, commercially available anti-SARS-CoV-2 serologic immunoassays from Abbott Laboratories, Roche Elecsys®, Ortho-Clinical Diagnostics and, one rapid ICA assay from Medsource Ozone Biomedicals Pvt Ltd [9] available at our institute, using convalescent-phase sera from frontline HCW working in the hospital. It also studies the seroprevalence of SARS-Cov-2 infection in, asymptomatic healthy frontline HCW in a hospital setting. We have studied seroprevalence and relative risk of COVID-19 infection in 505 asymptomatic HCWs across all categories of frontline HCW, which is one of the very few studies from the Indian subcontinent where seroprevalence in frontline HCW have been studied for the presence of both SARS-COV-2 antinucleocapsid and anti-spike antibodies. Additionally, we have evaluated and compared sensitivity amongst the 4 most commonly available chemiluminescent high throughput immunoassay platforms and a rapid ICA assay and in contrast to most previous evaluations of serological SARS-CoV-2 assays, the case panel was obtained from frontline HCW who had milder symptoms of COVID-19, evaluating whether the assays could detect SARS-CoV-2 antibodies among the most common type of patient with SARS-CoV-2 infection. Specificity was evaluated with pre-COVID-19 blood donor samples, making this study very solid in terms of clinical accuracy and agreement between the assays investigated.

In two of the evaluated assays (Abbott and Roche), a recombinant nucleocapsid antigen (rN) is used in the immunoassay, while in two assays (both from Ortho-Clinical Diagnostics) a recombinant spike antigen (rS) of the RBD is used; the immunochromatographic assay from Medsource Ozone Biomedicals Pvt Ltd did not specify the protein(s) used as the capturing antigen in the assay.

In our study, Abbott assay had sensitivities of 73.08% at 10-30 days, 76.08% at 31-50 days, and 60.71% at >50 days, while Roche had sensitivity 100% at 10-30 days, 93.48% at 31-50 days and 96.43% at >50 days. Ortho showed 100% sensitivity at 10-30 days and 31-50 days and 99% at >50 days.

Overall sensitivities calculated from case samples with a known (Time from Infection) TOI >10 days (N=100) up to 2 and a half months was 99% in two of the assays from Ortho-Clinical Diagnostics and was 96% in the total anti-SARS-COV-s antibody assay from Roche, however, assay from Abbott Laboratories showed the lowest sensitivity (71%) in the Chemiluminescence assays evaluated (Table 4). Our findings indicate that majority of the infected individuals develop an immune response to SARS-CoV-2, irrespective of disease severity or the viral antigen used in the immunoassay. Second, this response seems to be at a peak in samples taken at approximately 3-4 weeks after TOI. A variation in sensitivity performance between the anti-SARS-CoV-2 assays was observed in the samples across the time range of testing from the TOI; however, it was notable that the lowest sensitivity was found in the rapid immunochromatographic assay followed by chemiluminescence based assay detecting only IgG antibodies to nucleocapsid protein. It is known that antibodies to nucleocapsid protein are the earliest to appear and also earliest to disappear and we did not include cases that were <10 days from TOI in the study. A study by Public Health England, showed the comparison between Abbott, Diasorin, Roche, and Siemens for convalescent patients (≥20 days of symptoms). At the manufacturers' thresholds, for the Abbott assay sensitivity was 92·7% (95% CI 90·2–94·8) and specificity was 99·9% (99·4–100%); for the DiaSorin assay sensitivity was 96·2% (94·2–97·7) and specificity was 98·9% (98·0–99·4); for the Oxford immunoassay sensitivity was 99·1% (97·8–99·7) and specificity was 99·0% (98·1–99·5); for the Roche assay sensitivity was 97·2% (95·4–98·4) and specificity was 99·8% (99·3–100), and for the Siemens assay sensitivity was 98·1% (96·6–99·1) and specificity was 99·9% (99·4–100%). All assays achieved a sensitivity of at least 98% with thresholds optimized to achieve a specificity of at least 98% on samples taken 30 days or more post symptom onset [10]. Tan SS et al in their study reported that Roche exhibited marginally better performance in the 21 days or more group, with a sensitivity of 90.6% versus an Abbott sensitivity of 84.4%, as well as in the 14- to 20-day group with a sensitivity of 85.7% versus an Abbott sensitivity of 81.0%. They reported that less than 14 days of symptoms group exhibited poor sensitivity at less than 50% for both assays [11]. Similar to our findings, Chua KYL et. al, in their study reported clinical sensitivity of 98.84% (95% CI 93.69-99.97) for Roche assay and

97.67% (95% CI 91.85-99.72) for Vitros assay [12]. Similarly, other studies too have reported that the sensitivity of each immunoassay is variable depending on the time of onset [13,14,15,16]. Our sensitivity data show that Roche outperforms Abbott through all time ranges. This could be due to the Roche Elecsys assay measuring total antibodies and Abbott assay specifically detecting IgG. Ortho also showed better performance than Abbott and marginally better performance than Roche in the 31-50 days group. The decline at 31-50 days in Roche assay could be because this assay detects antinucleocapsid antibodies in the individuals, which may either be in the declining phase in this group. We do not suggest that the chosen antigen (N vs S RBD) affects the assay performance but instead, we propose that differences in performance seem to be related to overall assay design (Total antibody versus only IgG) along with choice of antigen and the most important, time of sample testing from the onset of disease.

Though rapid immunochromatographic assays provide an easy solution for mass screening of the population for establishing seroprevalence infection, a negative result should be followed up on an assay with higher sensitivity before labeling the person as seronegative. US, the FDA (US Food and Drug Administration) requires a minimum sensitivity of 90% and specificity of 95% for emergency use authorization of serologic anti-SARS CoV-2 assays [17]. However, we chose a higher sensitivity at 96% and diagnostic specificity (≥99%) as our main criterion, since India has a low anti-SARS-CoV-2 seroprevalence (approximately 6·6%). Therefore we can say that two total antibody (N and S RBD) assays from Roche and Ortho and one IgG (S RBD) assay from Ortho amongst the assays that we evaluated, reached predefined criteria for acceptable performance.

The poor sensitivity of the Abbott assay was seemingly due to the manufacturer's setting with a higher assigned cut-off value. For example, adjusting the Abbott assay cut-off from 1.4 S/CO to 1.0 S/CO increased the sensitivity from 71.43 % to 92.9% without changing 100% specificity. Adjustment in cut-offs could potentially also improve the performance of SARS- COV-2 antibody assay from Abbott Laboratories. However, for this study, we used cut-offs as specified by the manufacturers.

In the seroprevalence study, we found significantly higher seropositivity in the asymptomatic health-care workers group (17.6%) as compared to the general Indian population seropositivity (6.6%) reported by Muhekar et.al [18]. This seroprevalence was based on the second nationwide household serosurvey conducted, by them, in the general population of India. This seroprevalence survey was conducted between 18th August and 20th September 2020, amongst the enrolled 29 082 individuals from 15 613 households and included individuals aged 10 years or older in the same 700 villages or wards within 70 districts from 21 states in India. The weighted and adjusted seroprevalence of SARS-CoV-2 IgG antibodies in individuals aged 10 years or older was 6·6% (95% CI 5·8–7·4). Among 15084 randomly selected adults (one per household), the weighted and adjusted seroprevalence was 7·1% (6·2–8·2). The findings of our study are similar to studies published from other countries that have reported seroprevalence of SARS-CoV-2infection to be higher for HCWs performing patient-related work in other countries as well [19,20,21] and front-line HCWs [22]. Rudberg et al. found that seropositivity of HCWs was much higher compared with the general population in London and Stockholm, respectively, indicating an occupational health risk among HCWs [23].

Lan F-Y et. al, in their study pertaining to work-related COVID-19 transmission in six Asian countries/areas, reported that amongst the 103 possible work-related COVID-19 transmission cases, 22% were HCW and they were found to be the most susceptible to acquire the infection from the workplace [24]. In the COVID-19 pandemic, the provision of adequate health care to patients is fundamental to keep mortality low; however provision of state-of-the-art health care is highly reliant on professional staff that feels safe and well protected during this period. The unexpected seroconversion found among asymptomatic frontline HCWs who are into patient care was 17.6%. There are very few studies from the Indian subcontinent that have offered systematic screening for antibodies against SARS-CoV-2 in frontline HCW in a population of this size and calculated relative risk across different groups of HCWs. The lower seroprevalence in the high-risk group might be an indicator that the infection control hygiene standard is effective. However, the higher seroprevalence in the intermediate group suggests that awareness of COVID-19 patient-to-staff transmission must be maintained, even in non−COVID-19 wards. High infectivity rate found in the housekeeping (stretcher-bearers - waste

management staff) compared to other direct caregiving employees-doctors, nurses, and technical staff. This may be attributed to not only non-adherence to infection control practices during patient handling but also during waste handling. Another explanation could be that those with higher rates were moving in and out of different hospital areas, whereas nurses and doctors were working in the well-defined designated location. In addition, the housekeeping staff belongs to the lesser educated group amongst all HCW, and infection control practices not only need to be monitored all the time but necessitate repeated training to re-inforce adherence to correct practices for their safety. High seroprevalence in HCW has been reported in other studies from India [25]. Goenka et al., too in their study have also reported higher seroprevalence in housekeeping staff, food and beverage staff, lab assistants, and technicians than doctors and nurses ($p < 0.0001$) [26]. The current study, however, did not evaluate adherence to infection control guidelines in groups of HCW studied. Healthcare workers with immunity against SARS-CoV-2 may be less vulnerable for SARS-CoV-2 infection. However, antibody detection is no assurance of protective immunity. The sex-related difference in seroprevalence might be due to unknown underlying patterns of transmission or to different behavior e.g., women might possibly follow recommendations more carefully. Vahidy FS et. al, in their study have found that males were more likely to test positive for COVID-19 infection and have higher mortality as compared to females [27]. Ahnstedt H et. al, have reported that men show more susceptibility to pathogens as compared to women. They have also reported that stronger antigenic response to infection, vaccines, and self-antigens as mounted by females compared to males, at a disadvantage of a higher prevalence of autoimmune disorders in the females [28]. Thus the difference could possibly be of a biological origin as differences in severity of disease or immunological response between sexes exist. However more research is needed to answer these questions.

5. LIMITATION

A head to head qualitative comparison of the 4 common chemiluminescent platforms has been performed in 100 rRT-PCR diagnosed-recovered frontline HCWs, with mild disease, however comparison with titters using neutralizing antibody assay could not be performed. It is recommended that further studies be performed on larger sample size along with correlation with neutralizing antibody titers.

6. CONCLUSION

Performance of immunoassays for SARS-CoV-2 antibody testing depends on the overall assay design (Total antibody versus only IgG), choice of antigen, and time of sample testing from the onset of disease. In our study, Ortho Vitros total-Ab; IgG (Spike) and Roche Elecsys total-Ab (Nucleocapsid) assays were found to have optimal sensitivity across the time range ≥10 days post rRT-PCR positive result and not more than 2 and half month beyond rRT-PCR positivity. The seroprevalence study in the frontline HCWs of the institute showed that seroprevalence was high (17.6%) in HCWs in comparison to the community. Housekeeping staff and waste handlers showed significantly higher positivity as compared to other groups of frontline HCW.

DISCLAIMER

All the authors declare that they have no competing financial interests or personal relationships that could have appeared to influence the work reported in this study. The authors did not receive any financial support for this work.

CONSENT

The study was informed written consent was obtained from each subject before sample collection.

ETHICAL APPROVAL

Institutional Ethics Committee (ECR/3/BLK/Inst/DL/2013/RR-19) approved this study. (Reference no: Ethics committee/AARCE/Letter/November/2020/11).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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