Original Research Article

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

Abstract

Aim: 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 second, increased risk of COVID-19 infection to healthcare workers (HCW). The study aimed to evaluate performance of four chemiluminescence immunoassay and a rapid immunochromatographic assay in 100 rRT-PCR diagnosed-recovered frontline HCW with milder COVID-19 disease and secondly to evaluate seroprevalence of SARS-CoV-2 infection in asymptomatic frontline HCW at multispecialty hospital in Delhi, India.

Study Design: Performance of four common chemiluminescence immunoassay was evaluated in 100 rRT-PCR diagnosed-recovered frontline HCW with mild disease. In addition seroprevalence studied in 505 asymptomatic frontline HCW.

Place and Duration of the Study: The study was conducted at BLK Superspeciality Hospital, New Delhi from September to October2020.

Method: Four chemiluminescence immunoassay [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] was evaluated in 100 rRT-PCR diagnosed-recovered frontline HCW with mild disease. Also seroprevalence studied in 505 asymptomatic frontline HCW.

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

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

1 Introduction

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], a major global health concern. As most countries are facing the second wave of the pandemic, as per "Worldometer" (accessed 31stMarch, 2021) 219 countries and territories have reported a total of 2,818,445 deaths [2]. Serological tests are important tools for 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) determining potential immunity and risk of infection ; iv) identification of convalescent plasma donors and v) sero-epidemiological 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 titre after illness onset [3]. Sensitivity as well as specificity of serological assays can also be affected by the target antigen. Studies have shown that S (Spike) protein (produced in 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, selection of an assay for a specific purpose, decision

making should include available knowledge on antibody specificities, kinetics, and functions [5, 6]. However, due to urgent demands, a lot of 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 hospitalized patient with moderate to severe disease. There limited interassay comparisons to detect SARS-CoV-2 antibodies in cases with milder symptoms of COVID-19 necessary to evaluate whether an 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 sero-epidemiological investigation and seroprevalence study to assess risk of infection in asymptomatic HCW.

The study aimed to evaluate performance of four high-through put 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 term 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 sero-prevalence 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 our hospital, a large tertiary care COVID hospital in North India were recruited in this prospective cross-sectional monocentric study. Study was approved by Institutional Ethics Committee and informed written consent was obtained from each subject prior to sample collection. Each subject answered a standardized questionnaire. Participant selection was randomly done to cover staff from all sections of the hospital having direct interface with patients and/ or their attendants and they were grouped depending on 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 the 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.

A total of 605 frontline HCWs were recruited for the study.

Study population was further divided into:

(i) Known positive (infected and recovered) HCW: 100 frontline HCW rRT-PCR (from naso and oro-pharyngeal swab) confirmed COVID-19 disease after end of quarantine or hospitalization at ≥10 days from positive test result and not more than 2 and half month beyond rRT-PCR positivity. Time of illness (TOI) of participants was calculated from date of testing

(ii) Known negatives (asymptomatic frontline HCW: 505 asymptomatic frontline health care workers who had not developed or shown any symptoms of COVID-19 infection till date.

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

2.2 Sample *collection*

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

2.3 Index test methods

Performance of diagnostic accuracy of four high through put commercial chemiluminescent immunoassays (FDA-EUA Authorized) [8] and one rapid immunochromatographic assay (ICA) was performed (Table I). These assays employ either S or N protein antigens and all the assays generate a qualitative positive/negative result based on assay-dependent signal thresholds. Tests were run 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) [9].

1. Abbott Architect SARS-CoV-2 IgG assay: detects anti-N IgG using a two-step chemiluminescent microparticle immunoassay (CMIA) method with an acridinium-labelled anti-human IgG.
2. Roche Elecsys® Anti-SARS-CoV-2 total assay is a two-step bridging electro-chemiluminescent immunoassay (ECLIA) using ruthenium-labelled 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).

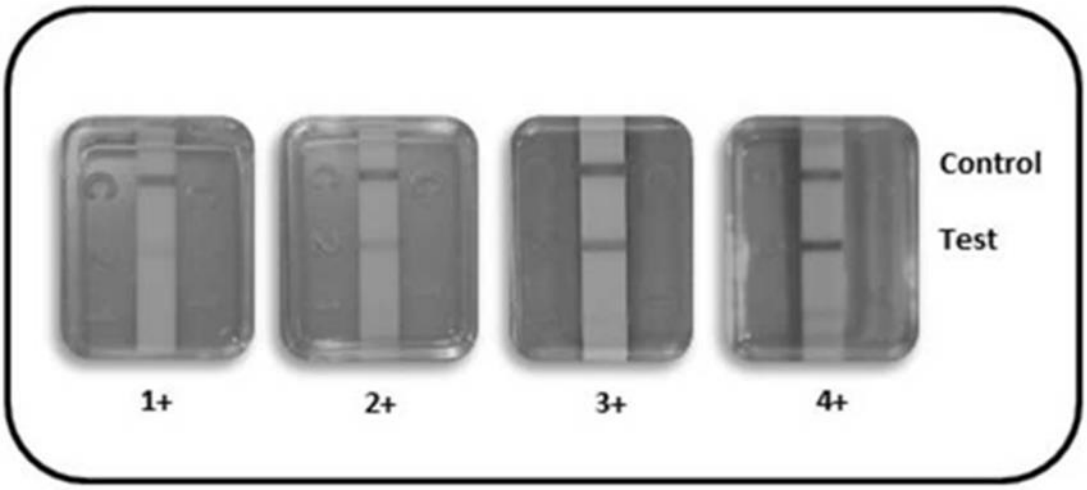
Table I: Details of Index test methods

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

Abbreviations: CMIA: Chemiluminescent Microparticle Immuno Assay (CMIA); CLIA: Chemiluminescence Immunoassay; ECLIA: Enzyme Chemiluminescence Immunoassay; ICA: immunochromatographic assays

Figure I: Picture card to standardize interpretation of the result of rapid card test



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

2.4 Statistical analysis

In absence of a 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. 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 following assumption: all samples obtained prior 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 sera 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 [7]. Concordance analyses (Cohen's Kappa) and percent agreement (overall, positive/negative) were performed to compare 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 staff, security staff, others). Comparative rates are reported as relative risk (RR) with 95% confidence intervals (CIs), calculated using a Taylor series.

2.5 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)

3 Results

3.1 Baseline characteristics of study participants

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

Table II: Baseline characteristics of study participants

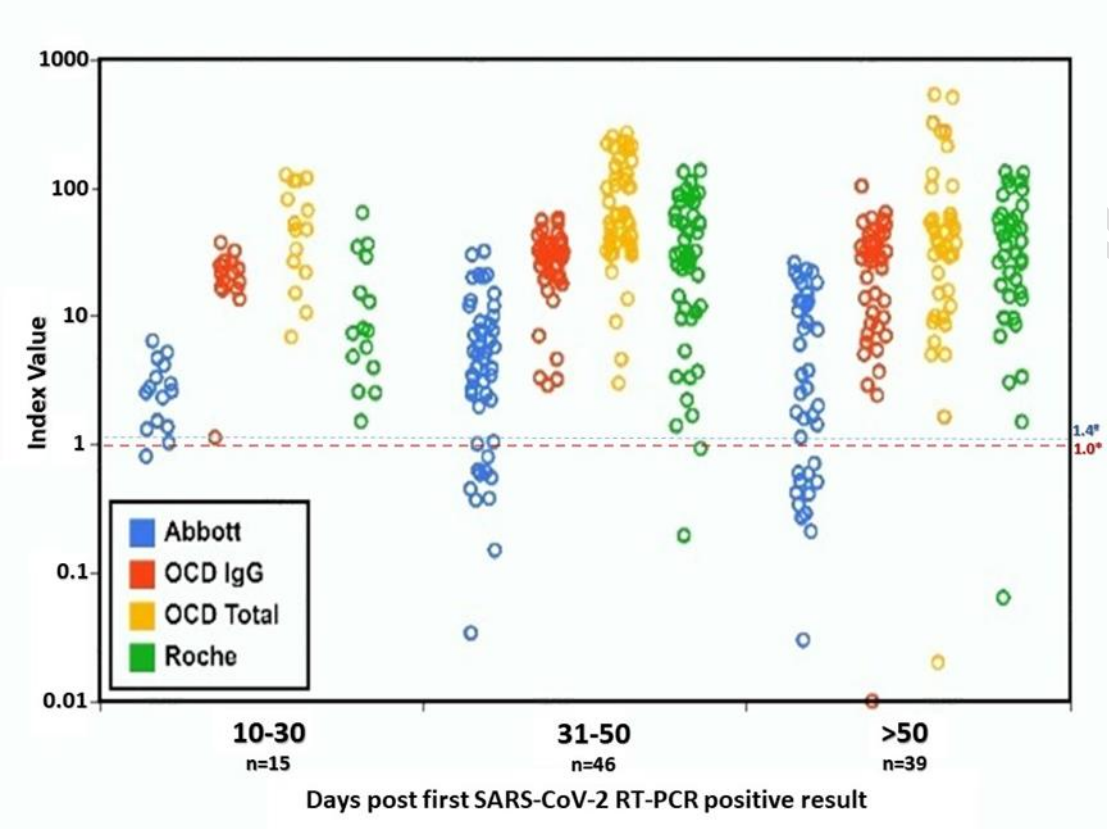
Characteristic	Know positive (infected) HCWs N (%)	Known negatives (asymptomatic) HCWs N (%)
Total Numbers	**100**	**505**
Sex		
Male	76 (76)	307(60.8)
Female	24 (24)	198 (39.2)
Age, median (range) years	34 (21-53)	35 (20-64)
Staff category		
Doctors	14 (14)	101 (20)
Nurses	23 (23)	136 (26.9)
Lab technician/other 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)	-

3.2 Specificity, sensitivity and agreement of the 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 (Figure II).

Figure II: 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



Intra-assay sensitivity for each assay was 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 PCR-positive test, while Roche had sensitivity 100 % at 10-30 days, 93.48 % at 31-50 days and 96.43 % at >50 days post PCR-positive test. Ortho showed 100% sensitivity at 10-30 days and 31-50 days and 99 % at >50 days post PCR-positive test. One case tested negative by all 4 assays, was 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 explained by several factors, including mild disease, transient antibody response only, 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, 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]. Sensitivity of the rapid immunochromatographic test 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 III).

Table III: 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%)

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

Table IV: Concordances of 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%

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 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 SARSCoV-2 on both 2 chemiluminescent assays.

Male HCW showed higher adjusted sero-positivity and relative risk as compared to female HCW and difference in rates was statistically significant (P = 0.0004). HCW amongst high risk group had a positivity rate 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 V).

Table V: Overall distribution detectable SARS Cov-2 antibodies in known negatives (i.e. asymptomatic) healthy frontline HCW with relative risk of Infection in different group of care givers.

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 technician	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	2.208	1.42 to 3.43	**0.0004**

	198 (28)			
Direct patient care giving	369 (54)	0.57	0.39 - 0.83	**0.0034**
employees versus non-	vs			
patient facing employees	136 (35)			
Nurses versus other direct	136 (15)	0.66	0.37 - 1.15	0.15
patient care giving	vs			
employees	233 (39)			
Housekeeping versus	51 (31)	8.40	5.35 - 13.20	**< 0.0001**
other direct care giving	vs			
employees	318 (23)			

Pre-planned comparisons were done amongst high-risk plus intermediate risk versus low-risk setting (direct care givers versus non-patient facing employees). Nurses (being at higher risk owing to longer period of direct patient contact) versus all others; housekeeping versus other direct patient facing employees. Difference in rates was statistically significant (P = 0.0034) in group not facing patients directly versus direct patient care giving 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 care giving 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 to 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). Around 29.1% (89/189) of seropositive HCW had never been diagnosed with COVID-19 infection. Among the rRT-PCR negative and seropositive HCW on taking detailed history, 18 of them reported very vague symptoms like fatigue or mild

headache lasting for less than a day, however none of them had history of fever, cough, sore throat, gastrointestinal symptoms or anosmia.

4 Discussion

This study presents a head-to-head comparison of four 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 [8,9] 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 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 the 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 IV). Our findings indicate that majority of 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 optimised 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 sensitivity of each immunoassay is variable depending on the time of onset [13, 14, 15, 16]. Our sensitivity data show that Roche out performs Abbott through all time ranges. This could be due to the Roche Elecsys assay measuring total antibodies and the 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 declining phase in this group. We do not intend to suggest that the chosen antigen (N vs S RBD) affects the assay performance in general but instead, we propose that differences in performance seem to be associated with overall assay design (Total antibody versus only IgG) along with than choice of antigen and the most important, time of sample testing from the disease onset .

Though rapid immunochromatographic assays provide an easy solution for mass screening of population for establishing seroprevalence infection, a negative result should be followed up on an assay with higher sensitivity before labelling 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 prioritized high sensitivity at 96% and diagnostic specificity (≥99%) as our main criterion, since India has a low anti-SARS-CoV-2 seroprevalence (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 higher assigned cut-off value. For example, adjusting 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 the 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 a significantly higher seropositivity in 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 which 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 15 084 randomly selected adults (one per household), the weighted and adjusted seroprevalence was 7·1% (6·2–8·2). Findings of our study are similar to studies published from other countries who 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, their study on 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 most susceptible to acquire the infection from workplace [24]. In the COVID-19 pandemic, 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 care giving 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 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 trainings to re-inforce adherence to correct practices for their own 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]. 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. Sex-related difference in seroprevalence might be due to unknown underlying patterns of transmission or to different behaviour e.g., women might follow recommendations more carefully. Difference might also be of a biological origin if differences in immunological response or severity of disease between sexes exist. More research is needed to answer these questions.

5 Conclusion

Performance of immunoassays for SARS-CoV-2 antibody testing depend on overall assay design (Total antibody versus only IgG), choice of antigen and time of sample testing from the disease onset. 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. Seroprevalence study in the frontline HCWs of the institute showed that seroprevalence was high (17.6%) in HCWs compared to the community Housekeeping staff and waste handlers showed significantly higher positivity as compared to other groups of frontline HCW.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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