Final Technical Report

for

Research Project

Longitudinal follow-up of ongoing Multi-Centric COVID-19, Population-Based Age-Stratified Sero - Epidemiological Prospective Study under WHO Unity protocol in India, Including Cell-mediated Immune Response Post infection/Vaccination: Phase 2 (2022-2023)

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Disclaimer

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Technical Report

December 2023

A multi-centric Novel Coronavirus (COVID-19) population-based age-stratified sero-epidemiological study (in urban, rural and tribal communities) at selected centers in India: A Prospective Cohort study

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Study Title

A multi-centric novel coronavirus (COVID-19) population-based age-stratified sero-epidemiological study (in urban, rural and tribal communities) at selected centers in India: A prospective cohort study.

Introduction

COVID-19 was designated as a global public health emergency by the WHO and was declared a pandemic. The specific epidemiological and serologic characteristics of the new pathogen, such as its transmissibility and virulence, remain unclear. [1] The number of reported cases and actual infection rates differ due to the lack of accessible and reliable diagnostic tests, as well as a large number of asymptomatic cases. [2,3] It was crucial to promptly identify infected individuals and interrupt the transmission chain to control the spread of COVID-19. [4] To obtain more accurate estimates of infection prevalence in the population,

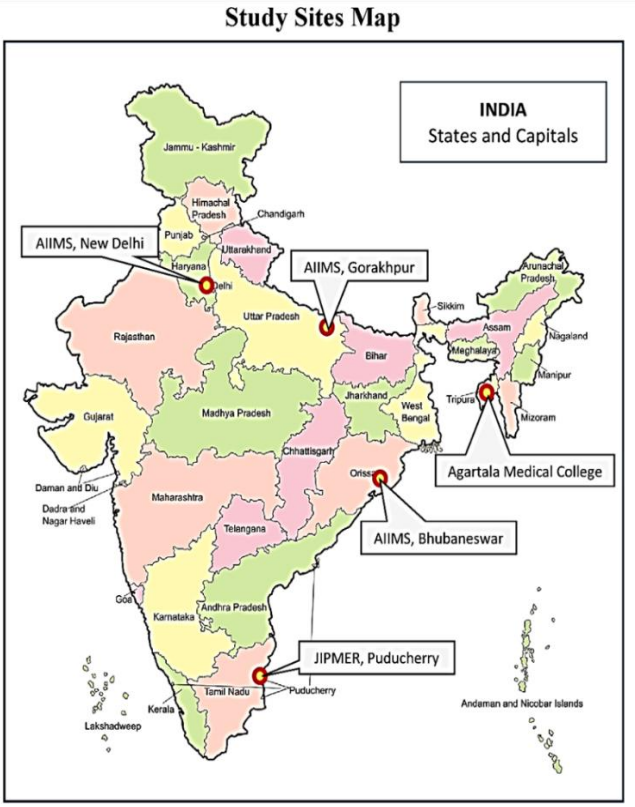


Figure 1: Geographical location of the study sites in India

seroprevalence studies were necessary, which involve assessing the presence of antibodies through surveys. [5] It was important to identify groups that are disproportionately affected and monitor progress towards achieving herd immunity. Understanding the characteristics and impact of COVID-19 at the country level is vital for future planning and management of the pandemic, including the effective deployment of vaccines. This comprehensive study was designed to capture variations in seroprevalence based on different geographic settings, population densities, and existing data from urban, rural, and tribal areas with varying infection rates. The objective of this seroprevalence study was to investigate the extent of infection in the general population by determining the proportion of individuals testing positive for antibodies.

Objectives

Following were the objectives for this sero-epidemiological investigation:

1. Longitudinal follow up of registered cohort of ten thousand individuals at various sites across India in urban, rural, and tribal population.
2. To contribute to an improved understanding of antibody kinetics following COVID-19 infection/ vaccination
    a. To study the longitudinal pattern of antibody level following vaccination and natural infection)
    b. In a subset - evaluation of SARS CoV-2 NAbs for understanding immunity to COVID- 19 and protective immunity among the recipients of COVID-19 vaccines vs natural infection.
3. To compare the cellular immunity among SARS CoV-2 antibody positive and negative participants; to compare the cellular immunity among symptomatic and asymptomatic participants of past COVID-19 infection
4. Establishment of longitudinal sera repository to store sera for answering further questions in future among symptomatic and asymptomatic, Vaccinated and Unvaccinated, urban vs rural vs tribal populations to study biomarkers of future need.

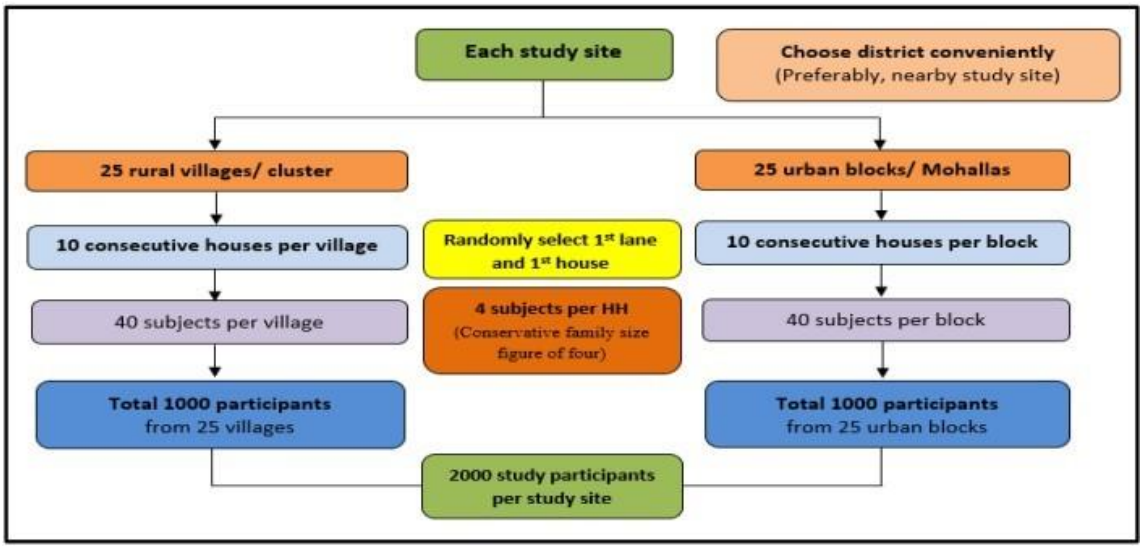
Methodology

A population-based, age-stratified prospective study was planned and conducted across five selected sites in India. The sites are urban and rural field practice areas of tertiary care medical colleges located in Delhi, Bhubaneswar, Gorakhpur, Puducherry and Agartala (Figure 1). Whereas in Agartala, tribal population in place of urban population was chosen to capture heterogeneity across the study sites. At each study site, 1000 participants were selected aggregating a total of 10000. Participants were selected as per the algorithm in Figure2.

The study team visited the selected households and obtained written consent and details, trained phlebotomists in the study team collected venous blood from each participant. From the serum, detection of SARS-CoV-2-specific IgG antibodies was performed. For the first 3 rounds, WANTAI SARS-CoV-2 total assay was used which is a qualitative type of assay and for the 4thround KANTARO SARS-CoV-2 IgG kit is being used which is a semi quantitative

assay. For 5th, 6th and 7th round Roche Elecsys Anti SARS CoV-2 immunoassay was used for qualitative detection of antibodies including IgG to SARS CoV-2.

Figure 2: Participant Recruitment algorithm



Ethical Clearance was taken from AIIMS Institutional Review Board (IRB) and site-specific IRB before the start of the study. **(Ref. No- IEC-857/03.12.2021)**

Progress of the study

Under WHO Unity protocol, a cohort of 10,000 individuals was constructed for population-based, age- stratified sero-epidemiological study for COVID-19 virus infection from five selected states covering rural, urban, and tribal areas. We planned to continue serial sampling of the same individuals who were recruited in Phase 1 of the study (Nov.2020 – Dec 2021), and were followed up longitudinally for six months (Phase 2). Then later, for the next18 months phase (July 2022 onwards – Dec 2023), we continued serial sampling of the same individuals who gave consent to be a part of the Phase 2 (initial six months) longitudinal follow-up study.

For objective 1:

There have been 7 rounds of sample collection and all have been completed. The details of timeline and status for sample collection are mentioned in **Table 1 and 2.**

The sample processing for all the 7 rounds is completed. The summarized analysis of the samples collected for 7 rounds has been completed. A detailed analysis will be done later.

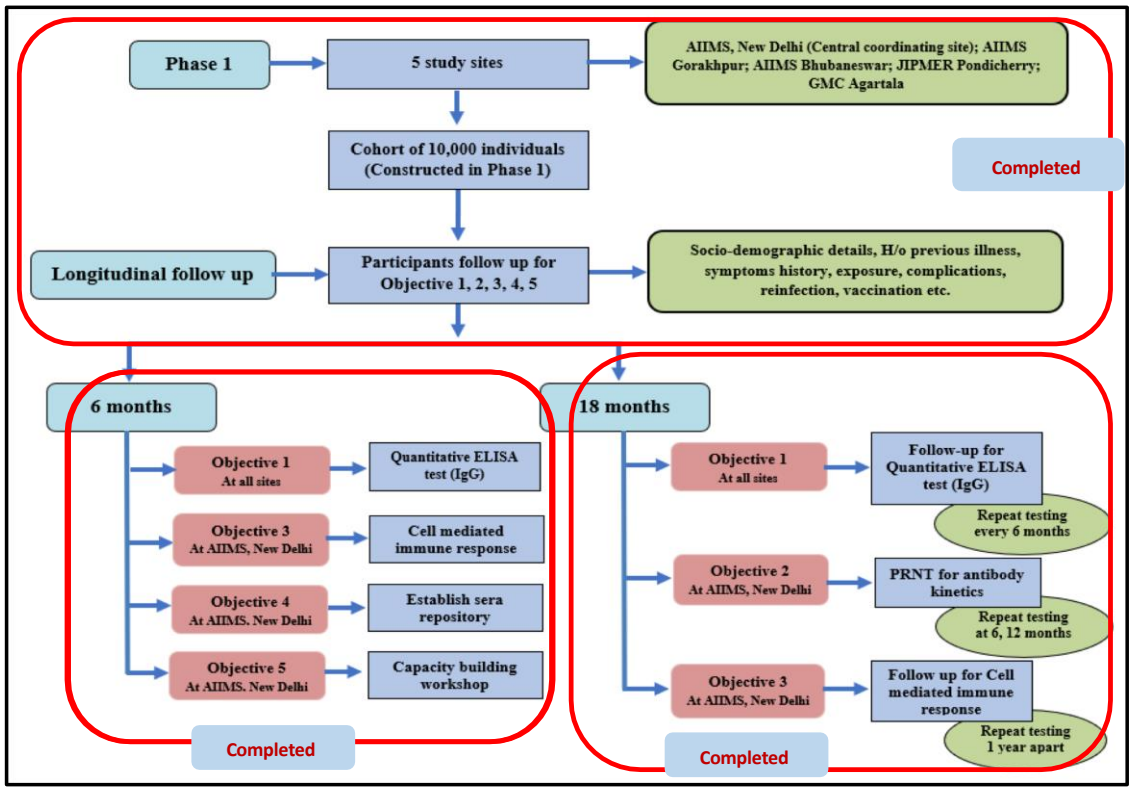


Fig 3: Longitudinal follow up case investigation algorithm

For objective 2:

For the continued understanding of the antibody kinetics, we included 100 participants having documented evidence of confirmed SARS-CoV-2 infection within last 2 months either by RTPCR or rapid antigen test. PRNT test was performed for estimation of SARS- CoV-2 neutralizing antibodies (Nabs) to help us understand the immunity to COVID-19 and protective immunity among the recipients of COVID-19 vaccines vs natural infection. We followed the enrolled participants two times more. Sampling for PRNT for antibody kinetics and sample processing is completed and summary is given in **Table 7**.

Objective 3:

To compare cell-mediated immune responses, we continued the follow-up of the selected subset of 150 individuals. Serial sampling of the same selected 150 subjects was done 1 year apart i.e., in the month of April – June 2023 from the baseline sample (Sample 1), and see its presence even in absence of antibodies to understand the role of cell-mediated immunity in Covid 19. The sample processing for cell mediated immune response test is completed and the summary is given in **table 8.**

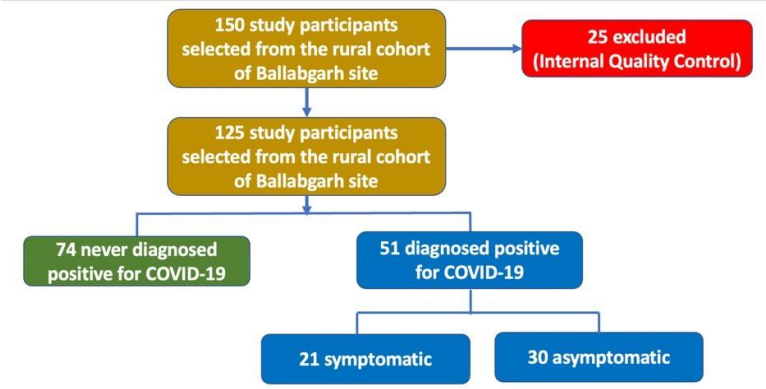


Fig 4: Algorithm for the selection of study participants

For objective 4:

Sera collection and storage was continued in this phase of the study. The sera samples collected across all the study sites were transported to the central coordinating site and have been stored properly following all SOPs. This will help in answering further questions in the future.

For this purpose, we kept two deep freezers and a dedicated lab technician to look for the sera samples. The sera samples were labeled with waterproof taping, and it was ensured that all the SOPs are followed for sera preservation.

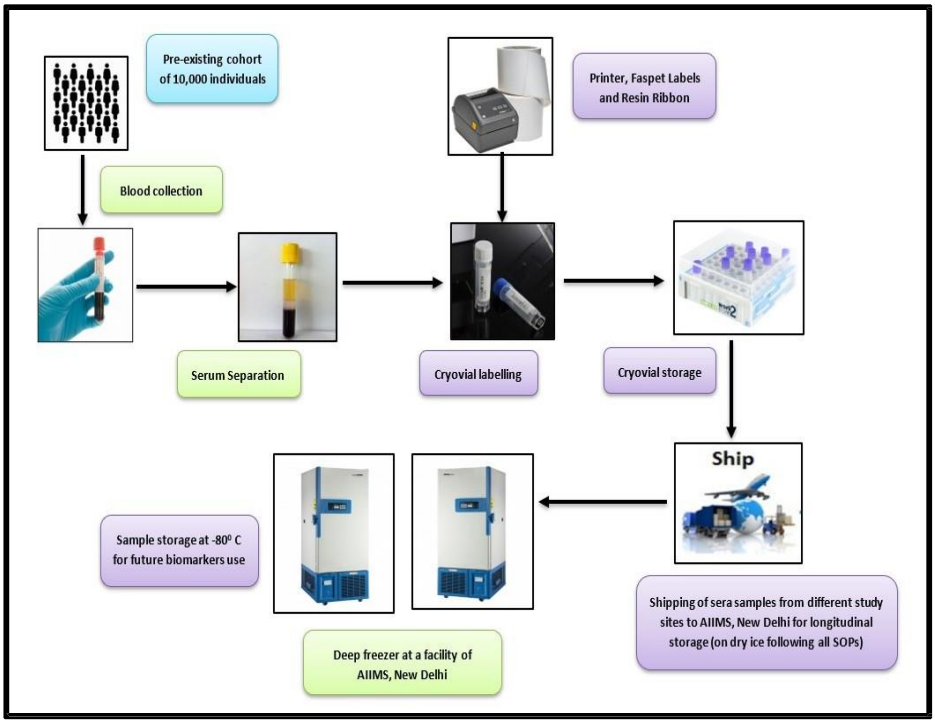


Fig. 5: Establishment of longitudinal sera repository

Figure 6: Deep fridge for sample repository in AIIMS

Table 1: Site wise timeline of sample collection for 7 rounds

Study Site	Area	Round One	Round Two	Round Three	Round Four	Round Five	Round Six	Round Seven
AIIMS, New Delhi	Rural	March 3 – June 10, 2021	May 27 – Oct 18, 2021	August 26 – Dec 31, 2021	April 26 – June 30, 2022	Oct 12 – Oct 21, 2022	March 28 - April 9, 2023	Oct 17-Oct 25,2023
	Urban	March 15 – July 31, 2021	June 18 – Oct 26, 2021	Sept 28 – Dec 11, 2021	April 20 – June 30, 2022	Oct 17 – Nov 16, 2022	March 16 – April 8, 2023	Oct 9-Oct 13,2023
AIIMS, Bhubaneswar	Rural	March 22 – May 7, 2021	July 9 – August 11, 2021	Oct 11 – Dec 19, 2021	April 22 – July 30, 2022	Dec 10 – Dec 17, 2022	June 21 – July 11, 2023	Dec 19 - Dec 23,2023
	Urban	June 7 – June 30, 2021	Sept 6 – Oct 11, 2021	Oct 12, 2021 – Jan 14, 2022	April 21 – July 08, 2022	Dec 19, 2022 – Jan 25, 2023	April 14 – June 13, 2023	Nov 24 – Nov 29, 2023
AIIMS, Gorakhpur	Rural	April 22 – June 29, 2021	July 24 – Oct 4, 2021	Oct 12 – Dec 19, 2021	April 05 – July 07, 2022	Nov 12, 2022 – Feb 28, 2023	April 13 – Aug 10, 2023	Nov 6 – Dec 12, 2023
	Urban	July 1 – August 18, 2021	Oct 5 – Nov 27, 2021	Dec 18, 2021 – Jan 10, 2022	May 18 – July 12, 2022	Nov 27, 2022 – Feb 22, 2023	May – 3 Aug – 16, 2023	Nov 22 - Dec 29 , 2023
Agartala Medical College	Rural	26 March – June 1, 2021	July 12 – Dec 10, 2021	Sept 20 – Dec 31, 2021	April 12 – June 30, 2022	Nov 3 – Dec 31, 2022	April 24, 2023 – July 12, 2023	Nov 14 – Dec 20 , 2023
	Tribal	17 June – August 7, 2021	Sept 14 – Nov 29, 2021	Dec 15 – Dec 31, 2021	April 11 – June 30, 2022	Oct 19, 2022 – Jan 6, 2023	April 11, 2023 - July 14, 2023	Oct 4 – Dec 20 , 2023
JIPMER, Pondicherry	Rural	June 9 – July 30, 2021	Sept 1 – Nov 23, 2021	Oct 12 – Dec 27, 2021	April 22 – June 20, 2022	Nov 6, 2022 – Jan 25, 2023	May 14 – June 22, 2023	Nov 4 – Dec 11 , 2023
	Urban	June 10 – July 23, 2021	Sept 2 – Nov 27, 2021	Oct 12 – Dec 28, 2021	April 23 – June 30, 2022	Nov 13, 2022 – Jan 14, 2023	May 16 – June 16, 2023	Nov -7 Dec 14 , 2023

Table 1 shows the timeline of sample collection of each site for the 7 rounds of sample collection that has been completed till now. The 7th round of sample collection was completed before 31st December 2023 at all the sites.

Table 2: Number of participants according to the study sites, rounds and areas

Study site	Area	Round 1 n (%)		Round 2 n (%)		Round 3 n (%)		Round 4 n (%)		Round 5 n (%)		Round 6 n (%)		Round 7 n (%)	
		Total	Area wise	Total	Area wise	Total	Area wise	Total	Area wise	Total	Area wise	Total	Area wise	Total	Area wise
AIIMS, New Delhi	Urban	2060 (100)	1001 (100%)	1569 (76.2%)	741 (74%)	1399 (67.9%)	686 (68.5%)	978 (47.5%)	447 (44.7%)	940 (45.6%)	437 (43.6%)	719 (34.9%)	323 (32.3%)	536 (26%)	258 (25.8%)
	Rural		1059 (100%)		828 (78.2%)		713 (67.3%)		531 (50.1%)		503 (47.5%)		396 (37.4%)		278 (26.2%)
AIIMS, Bhubaneswar	Urban	2000(100)	1000 (100.0%)	1704 (85.2%)	820 (82%)	1473 (73.6%)	699 (70%)	1138 (56.8%)	522 (52.2%)	1045 (52.2%)	501 (50.1)	1038 (51.9%)	537 (53.7%)	687 (34.4%)	330 (33%)
	Rural		1000 (100.0%)		884 (88.4%)		774 (77.4%)		616 (61.6%)		544 (54.4%)		501 (50.1%)		357 (35.7%)
AIIMS, Gorakhpur	Urban	2010(100)	1002 (100%)	1151 (57.3%)	553 (55.2%)	938 (46.6%)	436 (43.5%)	1029 (51.2%)	512 (51.1%)	1030 (51.2%)	505 (50.4%)	1043 (51.8%)	515 (51.9%)	806 (40.1%)	388 (38.7%)
	Rural		1008 (100%)		598 (59.3%)		502 (50%)		517 (51.3%)		525 (52%)		528 (52.4%)		418 (41.5%)
Agartala Government Medical College	Tribal	2000(100)	1339 (100%)	857 (42.8%)	483 (36%)	1097 (54.8%)	673 (50.3%)	577 (28.8%)	385 (28.7%)	608 (30.4%)	401 (30%)	520 (26%)	335 (25%)	257 (12.8%)	171 (12.7%)
	Rural		661 (100%)		374 (56.6%)		424 (64.1%)		192 (29%)		207 (31.3%)		185 (28%)		86 (13%)
JIPMER, Pondicherry	Urban	2040(100)	1020 (100%)	1222 (59.9%)	601 (58.9%)	669 (32.8%)	346 (34%)	774 (37.9%)	356 (35%)	775 (38.0%)	338 (33.1%)	601 (29.4%)	252 (24.7%)	454 (22.2%)	188 (18.4%)
	Rural		1020 (100.0%)		621 (60.8%)		323 (31.6%)		418 (41%)		437 (42.8%)		349 (34.2%)		266 (26%)
Total	Urban	10110	4023 (100%)	6503 (64.3%)	2715 (67.5%)	5576 (55.1%)	2167 (53.8%)	4496 (44.5%)	1837 (45.6%)	4398 (43.5%)	1781 (40.5%)	3921 (38.8%)	1627 (40.4%)	2740 (27.1%)	1164 (28.9%)
	Rural		4748 (100%)		3305 (69.6%)		2736 (57.6%)		2274 (47.9%)		2216 (50.4%)		1959 (41.2%)		1405 (29.6%)
	Tribal		1339 (100%)		483 (36%)		673 (50.2%)		385 (28.7%)		401 (9.1%)		335 (25%)		171 (12.7%)

A total of 10110 subjects were recruited and samples collected at the start of the study from total five sites. Out of the total population enrolled at the beginning of the study, overall 2740 (27%) were retained for the 7^{th} round of sample collection. More participants were retained in rural areas than in urban.

Table 3: Site wise and overall seroprevalence among the study participants

Site	Round One	Round Two	Round Three	Round Four	Round Five	Round Six	Round Seven
Delhi	66.6%	94.6%	95.8%	97.6%	99.7%	99.9%	100%
Bhubaneswar	64.5%	83.9%	87.2%	81.5%	99.8%	99.9%	100%
Gorakhpur	92%	94.9%	96.4%	93%	99.6%	100%	100%
Agartala	63%	90.8%	93.3%	91.2%	99.8%	99.8%	100%
Pondicherry	84.2%	91.2%	94.5%	98.2%	100%	100%	100%
Total	74.1%	90.7%	92.9%	91.8%	99.7%	99.9%	100%

Table 3 shows that the overall seroprevalence of COVID-19 across the five sites during 7 rounds. The overall seroprevalence of COVID-19 was 74% at the start of the study. With subsequent rounds the seroprevalence across Delhi and Pondicherry has shown an increasing trend over the 7 rounds while for Bhubaneswar, Gorakhpur and Agartala sites there was a decrease in the trend during round 4 and then further increased in subsequent rounds. The seroprevalence has been observed to be 100% by the end of round 7 at all the sites.

Table 4:Site wise seroprevalence according to gender

Study Site	Sex	Round One	Round Two	Round Three	Round Four	Round Five	Round Six	Round Seven
AIIMS, New Delhi	Male	588/930 (63.2%)	633/684 (92.5%)	540/575 (93.8%)	386/395 (97.7%)	358/360 (99.4%)	284/285 (99.6%)	186/186 (100%)
	Female	785/1130 (69.7%)	852/885 (96.3%)	800/824 (97.2%)	569/583 (97.5%)	579/580 (99.8%)	434/434 (100%)	350/350 (100%)
AIIMS, Bhubaneswar	Male	596/926 (64.4%)	643/779 (82.4%)	551/653 (84.3%)	395/490 (80.1%)	434/436 (99.5%)	458/459 (99.8%)	308/308 (100%)
	Female	687/1074 (63.9%)	788/925 (85.1%)	735/820 (89.6%)	532/647 (81.9%)	609/609 (100.0%)	579/579 (100%)	379/379 (100%)
AIIMS, Gorakhpur	Male	955/1036 (92.2%)	526/555 (94.9%)	404/422 (96.0%)	445/477 (90.5%)	495/497 (99.6%)	515/515 (100%)	390/390 (100%)
	Female	890/974 (91.4%)	567/596 (95.1%)	500/516 (96.6%)	513/553 (92.7%)	531/533 (99.6%)	528/528 (100%)	416/416 (100%)
Agartala Government Medical College	Male	508/803 (63.3%)	265/297 (89.3%)	365/395 (92.3%)	179/191 (93.7%)	192/193 (99.5%)	164/165 (99.4%)	87/87 (100%)
	Female	746/1197 (62.3%)	513/560 (94.9%)	659/702 (93.6%)	347/386 (89.8%)	415/415 (100%)	355/355 (100%)	170/170 (100%)
JIPMER, Pondicherry	Male	697/834 (83.6%)	400/443 (89.9%)	205/222 (92.2%)	266/273 (97.4%)	289/289 (100%)	231/231 (100%)	180/180 (100%)
	Female	1022/1206 (84.7%)	715/779 (91.8%)	428/447 (95.7%)	494/501 (98.6%)	486/486 (100%)	370/370 (100%)	274/274 (100%)
Total	Male	3344/4529 (73.8%)	2473/2767 (89.4%)	2065/2265 (91.1%)	1671/1826 (91.5%)	1768/1775 (99.6%)	1652/1655 (99.8%)	1151/1151 (100%)
	Female	4130/5581 (74.0%)	3424/3736 (91.6%)	3121/3309 (94.3%)	2455/2670 (91.9%)	2620/2623 (99.9%)	2266/2266 (100%)	1589/1589 (100%)

Table 4 shows that both male and female participants reported a similar seroprevalence of 100% at the end of round 7 across all the sites.

Table 5:Site wise seroprevalence according to study area

Study Site	Study area	Round One	Round Two	Round Three	Round Four	Round Five	Round Six	Round Seven
AIIMS, New Delhi	Rural	624/1059 (58.9%)	764/828 (92.2%)	665/713 (93.2%)	509/531 (95.8%)	501/503 (99.6%)	395/396 (99.7%)	278/278 (100%)
	Urban	749/1001 (74.8%)	721/741 (97.3%)	675/686 (98.4%)	446/447 (99.8%)	436/437 (99.7%)	323/323 (100%)	258/258 (100%)
AIIMS, Bhubaneswar	Rural	551/1000 (55.1%)	759/884 (85.8%)	718/774 (92.8%)	530/616 (86%)	544/544 (100.0%)	500/500 (100%)	357/357 (100%)
	Urban	732/1000 (73.2%)	669/820 (81.5%)	568/699 (81.3%)	397/521 (76.1%)	499/501 (99.6%)	537/538 (99.8%)	330/330 (100%)
AIIMS, Gorakhpur	Rural	897/1008 (88.9%)	556/598 (93.4%)	478/502 (95.2%)	453/517 (89.1%)	522/525 (99.4%)	530/530 (100%)	418/418 (100%)
	Urban	948/1002 (94.6%)	537/553 (97.2%)	426/436 (97.7%)	505/513 (94.1%)	505/505 (100%)	513/513 (100%)	388/388 (100%)
Agartala Government Medical College	Rural	361/661 (54.6%)	341/374 (91.2)	405/424 (95.5%)	183/192 (95.3%)	207/207 (100%)	185/185 (100%)	86/86 (100%)
	Tribal	893/1339 (66.7%)	437/483 (90.3%)	619/673 (91.9%)	343/385 (89%)	400/401 (99.7%)	334/335 (99.7%)	171/171 (100%)
JIPMER, Pondicherry	Rural	870/1020 (85.3%)	568/621 (91.5%)	308/323 (95.3%)	412/418 (95.5%)	437/437 (100.0%)	349/349 (100%)	266/266 (100%)
	Urban	849/1020 (83.2%)	547/600 (90.8%)	324/346 (93.6%)	348/356 (97.7%)	338/338 (100.0%)	252/252 (100%)	188/188 (100%)
Total	Rural	3303/4748 (69.6%)	2991/3305 (90.4%)	2574/2736 (94.1%)	2087/2270 (91.9%)	2211/2216 (99.7%)	1959/1960 (99.9%)	1405/1405 (100%)
	Urban	3278/4023 (81.5%)	2474/2715 (91.1%)	1993/2168 (91.9%)	1696/1837 (91.3%)	1778/1781 (99.8%)	1625/1626 (99.9%)	1164/1164 (100%)
	Tribal	893/1339 (66.7%)	437/483 (90.4%)	619/673 (91.9%)	343/385 (89%)	400/401 (99.7%)	334/335 (99.7%)	171/171 (100%)

Table 5 shows the seroprevalence was higher in Urban area (81.5%) as compared to rural and tribal areas at the start of the study during round 1and has reached 100% in urban, rural and tribal population.

Table 6: Site wise seroprevalence among <18 years and ≥18 years

Study Site	Age category	Round One	Round Two	Round Three	Round Four	Round Five	Round Six	Round Seven
AIIMS, New Delhi	<18 years	183/281 (65.1%)	203/223 (91%)	165/185 (89.2%)	97/103 (94.1%)	108/109 (99%)	76/76 (100%)	31/31 (100%)
	≥18 years	1190/1779 (66.9%)	1282/1346 (95.2%)	1175/1214 (96.8%)	858/875 (98%)	829/831 (99.7%)	643/643 (100%)	505/505 (100%)
AIIMS, Bhubaneswar	<18 years	176/320 (55%)	219/283 (77.3%)	198/244 (81.1%)	129/172 (75%)	147/147 (100.0%)	150/150 (100%)	84/84 (100%)
	≥18 years	1114/1680 (66.3%)	1212/1421 (85.3%)	1088/1229 (88.5%)	799/966 (82.7%)	896/898 (99.7%)	887/888 (99.9%)	603/603 (100%)
AIIMS, Gorakhpur	<18 years	285/339 (84%)	163/191 (85.3%)	148/165 (89.7%)	172/201 (85.6%)	188/190 (98.9%)	134/134 (100%)	129/129 (100%)
	≥18 years	1566/1671 (93.7%)	930/960 (96.9%)	756/773 (97.8%)	785/828 (94.8%)	839/840 (99.8%)	909/909 (100%)	677/677 (100%)
Agartala Government Medical College	<18 years	108/230 (49.9%)	58/75 (77.3%)	95/112 (84.8%)	31/34 (91.2%)	40/40 (100%)	39/39 (100%)	13/13 (100%)
	≥18 years	1153/1770 (65.1%)	720/782 (92%)	929/985 (94.3%)	495/543 (91.2%)	567/568 (99.8%)	480/481 (99.8%)	244/244 (100%)
JIPMER, Pondicherry	<18 years	286/372 (76.9%)	173/218 (79.3%)	73/92 (79.3%)	109/115 (94.8%)	100/100 (100%)	117/117 (100%)	77/77 (100%)
	≥18 years	1433/1668 (85.9%)	942/1004 (93.8%)	559/577 (96.8%)	651/659 (98.8%)	675/675 (100%)	484/484 (100%)	377/377 (100%)
Total	<18 years	1038/1542 (67.3%)	816/990 (82.4%)	679/798 (85%)	538/625 (86%)	583/586 (99.5%)	516/516 (100%)	377/377 (100%)
	≥18 years	6456/8568 (75.3%)	5086/5513 (92.2%)	4507/4778 (94.3%)	3588/3871 (92.7%)	3806/3812 (99.8%)	3403/3405 (99.9%)	2406/2406 (100%)

Among the participants aged<18 years and ≥18 years, the total prevalence was reported to be 100% by end of round 7.

Table 7: Roundwise sample collection for PRNT and test result status

Round	Number of PRNT Samples collected	Number of samples Positive for PRNT n%)	Number of samples Negative for PRNTn(%)	Anti-RBD antibodytest positive n(%)	Number of participants Vaccinated n(%)	Median with IQR for PRNT50 Positive
Round 1	**100**	69 (69%)	31 (31%)	97 (97%)	22 (22%)	171 (67.5-772.5)
Round 2	**98**	72 (73.5%)	26 (26.5%)	90 (92%)	60 (61%)	114 (60.2-358)
Round 3	**91**	72 (79%)	19 (21%)	85 (93.4%)	63 (69%)	99.5 (54.5-254)
Round 4	**79**	76 (96.2%)	3 (3.8%)	78 (98.7%)	75 (95%)	304 (135-770)
Round 5	**67**	64 (95.5%)	3 (4.5%)	-	67 (100%)	299 (179.7-600)
Round 6	**54**	54 (100%)	0 (0%)	-	54 (100%)	243.3 (154.9-529.9)

100 participants with confirmed RT-PCR/ RAT were enrolled to see the antibody kinetics. The mean (S.D.) age of the participant was 37.0 (13.5) years. 64% of the participants were male and 36% were females. Majority (82%) of the participants belonged to rural area. By round 5, 100% of the participants had received vaccination againstCOVID-19.

The table shows, out of the total participants, 69% had neutralizing antibodies against SARS-CoV-2 at the start of study. By the end of round 6 all the participants had neutralizing antibodies against SARS-CoV-2 who continued. 99% of participants had antibodies against SARS CoV 2 (when tested on Anti RBD) and 96% had neutralizing antibodies levels against the same by the end of round 4. 100% of participants had antibodies against SARS CoV 2 (when tested on CLIA) and neutralizing antibodies levels against the same by the end of round 6.

The median (IQR) PRNT50 titre shows a decreasing trend till round 3 and then increased in round 4 which may be due to reinfection.

Table 8: The SARS-CoV-2 specific IFN-γ levels among participants according to age group, gender and diagnosis status

	Round 1		Round 2	
Age	**N**	**SFU/million cells (median with IQR)**	**N**	**SFU/million cells (median with IQR)**
14-18y	4	209 (22.5 – 360.5)	6	140(78-166)
19-60y	98	77 (4 – 371.5)	96	192 (78-296)
>60y	23	88(0-220)	24	102(6-280)
Total	**125**		**126**	
Sex				
Male	65	118 (18-383)	67	166 (36-272)
Female	60	48(0-289)	59	180 (98-308)
Total	**125**		**126**	
CMI response positive	98	136 (46.5-392)	108	210 (111-308.5)

A subset of approximately 150 individuals from the selected study site was enrolled. Follow up and serial sampling of the selected individuals was done 1 year apart i.e., at 0 and 12 months. Due to internal quality control criteria, 25 participants were excluded for round 1. Almost equal proportions of both genders were recruited (52% males and 48% females). Nearly all of the participants enrolled had received vaccination.

Out of the 125 samples tested for anti-SARS-CoV-2 T cell-mediated IFN-γ response, 78.4% (n=98/125) samples were positive for round 1 and 86% (n=108/126) were positive for 2nd round.

The median of SARS-CoV-2 specific T cells was higher (209 SFU/million cells) in participants aged 14-18 years during round 1 while median was higher (192 SFU/million cells) in the age group of 19-60 years as compared to other age groups in round 2. The median of SARS-CoV-2 specific T cells was higher (210 SFU/million cells) by the round 2 as compared to the first round (136 SFU/million cells).

Investigator's meetings organized under the study

Four investigators meetings were organized to impart training, discuss the progress of the study at each site and also dissemination of the data analyzed in collaboration with WHO. This was a multicentric study which was carried out at five selected centres across the country and all the sites participated in the trainings and workshops organized.

The first Inauguration Meeting and Training Program was held on virtual platform on 8th February 2021. This meeting was attended by all site leaders, Co-Investigators and their teams. The main coordinating site was AIIMS, New Delhi. The four working sites were- Agartala Govt. Medical College, Agartala, AIIMS, Gorakhpur, AIIMS, Bhubaneshwar and JIPMER, Puducherry.

The Second Investigator's meeting and capacity building workshop was held from 19th-20th May 2022 at Radisson Blu Plaza, Delhi Airport, New Delhi. It was attended by WHO Representative to India-Dr Roderico H. Ofrin, Dr Manju Rani,Regional Advisor (Research and Innovation)SEARO, WHO, Dr Tran Minh Nhu Nguyen, Team Leader- Health Security and Emergency Response, WHO India,Dr Mohammad Ahmad, National Professional Officer, Research WHO-India, , Ex Director AIIMS- Dr Randeep Guleria, Director THSTI- Dr Pramod Garg,. Site leaders from four working sites-Agartala, Bhubaneswar, Delhi, Gorakhpur and Pondicherry, site leaders from new sites, namely Andaman, Arunachal, Kochi and Srinagar, team from THSTI, Faridabad.

The Third Investigator's meeting was held from 23rd-24thNovember 2022 at The Umrao, New Delhi. It was attended by all four working sites and main coordinating site AIIMS-Delhi for the review and progress of study along with THSTI team.

The fourth and final Investigators meeting was held on 27th-28th November, 2023 at Habitat World, India Habitat Centre, New Delhi. The meeting was attended by Deputy Country Head, WHO-India-Ms. Payden, Director AIIMS-Dr M. Srinivas, Dr Mohammad Ahmad - National Professional Officer Research, WHO-India. Dr Manisha R. Madkaikar, Scientist G & Director of ICMR-National Institute of Immuno hematology (NIIH) and ICMR-Center for Research, Management and Care of Hemoglobinopathies (CRMCH), Dr Madhu Mohanty, Scientist F, Deputy Director, ICMR-National Institute of Virology-Mumbai Unit. A separate panel discussion among microbiologist about future action on "Sera bank, sample storage and need for new biomarkers" was organized to discuss future course of action on biomarkers. It

was attended by all the working sites alongwith the microbiologists from each site and team from THSTI, Faridabad.

Some of the photographs from the meetings have been attached as **Annexure 1**

Strength of the study

- Largest sero-epidemiological cohort in India
- Multi-centric study where five geographically distinct regions have been included
- Presence of rural, urban and tribal population
- Follow up and storage of serum sample of same individual from the early phase of COVID-19 pandemic and pre-vaccination period
- Measurement of the total antibody, neutralizing antibody and cell mediated immunity of the subset of the sample
- Established of latest cohort of all age group for COVID seroprevalence
- Establishment of sera bank for pre COVID and post COVID, Pre vaccination and post vaccination samples.
- Capacity building of five new states making it a total of ten states for large epidemiological study in India

Limitations of the study

- Due to limited resources, the understanding of antibody kinetics and to see cell mediated immunity response, participants could be included from only AIIMS, New Delhi site, and only in a small subset of population in the study.
- Recall bias in reporting of symptoms history, onset, etc., as we were collecting information in the past three months. So, we couldn't get the correct estimates of asymptomatic and symptomatic infections in the population.

Publications so far from this study:

1. Serological prevalence of SARS-CoV-2 antibody among children and young age group (between 2 and 17 years) in India: An interim result from a large multicentric population- based seroepidemiological study.

Citation: Misra P, Kant S, Guleria R, Rai SK, Kishore S, Baidya S, Singh AK, Chinnakali P, Medigeshi GR, Chaturvedi PK, Joshi HS, Mandal S, Sangral M, Yadav K, Bairwa M, Haldar P, Kardam P, Patil S, Jha S. Serological prevalence of SARS-CoV-2 antibody among children and young age group (between 2 and 17 years) in India: An interim result from a large multicentric population-based seroepidemiological study. J Family Med Prim Care. 2022 Jun;11(6):2816-2823. doi: 10.4103/jfmpc.jfmpc_2274_21. Epub 2022 Jun 30. PMID: 36119298; PMCID: PMC9480647.

2. Test concordance and diagnostic accuracy of three serological assays for detection of anti-SARS-CoV-2 antibody: result from a population-based sero-epidemiological study in Delhi.

Citation:Misra P, Kant S, Guleria R, Ahmad M, Mandal S, Chaturvedi PK, Medigeshi GR, Meena S, Rai SK, Rahman A, Sangral M, Yadav K, Bairwa M, Haldar P. Test concordance and diagnostic accuracy of three serological assays for detection of anti-SARS-CoV-2 antibody: result from a population-based sero-epidemiological study in Delhi. BMC Infect Dis. 2022 Dec 7;22(1):915. doi: 10.1186/s12879-022-07805-5. PMID: 36476336; PMCID: PMC9730588.

3. SARS-CoV-2 Serological Prevalence among General Population in India: A Short Summary from A Nationwide Sero-Epidemiological Study.

Citation:Puneet Misra, Suprakash Mandal, Shashi Kant, Randeep Guleria, Sanjay K Rai, Surekha Kishore, Subrata Baidya, Arvind Kumar Singh, Palanivel Chinnakali, Guruprasad R Medigeshi, Partha Haldar, Mohan Bairwa, Kapil Yadav. SARS-CoV-2 Serological Prevalence among General Population in India: A Short Summary from A Nationwide Sero- Epidemiological Study. Archives of Clinical and Medical Case Reports 6 (2022): 558-561.

4. Antibody Response to SARS-CoV-2 among COVID-19 Confirmed Cases and Correlates with Neutralizing Assay in a Subgroup of Patients in Delhi National Capital Region, India

Citation: Misra P, Kant S, Guleria R, Rai SK, Jaiswal A, Mandal S, Medigeshi GR, Ahmad M, Rahman A, Sangral M, Yadav K, Bairwa M, Haldar P, Kumar P. Antibody Response to

SARS-CoV-2 among COVID-19 Confirmed Cases and Correlates with Neutralizing Assay in a Subgroup of Patients in Delhi National Capital Region, India. Vaccines (Basel). 2022 Aug 14;10(8):1312. doi: 10.3390/vaccines10081312. PMID: 36016201; PMCID: PMC9412620.

5. Cell-Mediated Immunity (CMI) for SARS-CoV-2 Infection Among the General Population of North India: A Cross-Sectional Analysis From a Sub-sample of a Large Sero- Epidemiological Study.

Citation: Misra P, Garg PK, Awasthi A, Kant S, Rai SK, Ahmad M, Guleria R, Deori TJ, Mandal S, Jaiswal A, Gongal G, Vishwakarma S, Bairwa M, Kumar R, Haldar P, Binayke A. Cell-Mediated Immunity (CMI) for SARS-CoV-2 Infection Among the General Population of North India: A Cross-Sectional Analysis From a Sub-sample of a Large Sero- Epidemiological Study. Cureus. 2023 Nov 15;15(11):e48824. doi: 10.7759/cureus.48824. PMID: 38106811; PMCID: PMC10722242.

6. Anti-SARS-CoV-2 antibody kinetics up to 6 months of follow-up: Result from a nation- wide population-based, age stratified sero-epidemiological prospective cohort study in India

Citation: Misra P, Singh AK, Mishra B, Behera B, Patro BK, Medigeshi GR, Joshi HS, Ahmad M, Chaturvedi PK, Chinnakali P, Haldar P, Bairwa M, Kharya P, Dhodapkar R, Rath R, Guleria R, Rai SK, Kar SS, Kant S, Sarkar S, Baidya S, Meena S, Mandal S, Kishore S, Majumder T, Hada V. Anti-SARS-CoV-2 antibody kinetics up to 6 months of follow-up: Result from a nation-wide population-based, age stratified sero-epidemiological prospective cohort study in India. PLoS One. 2023 Dec 11;18(12):e0287807. doi: 10.1371/journal.pone.0287807. PMID: 38079384; PMCID: PMC10712