Protocol

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National sero-surveillance to monitor the trend of SARS-CoV-2 infection transmission in India: Protocol for community-based surveillance

Muthusamy Santhosh Kumar[1], Tarun Bhatnagar[1], Ponnaiah Manickam[†], V. Saravana Kumar[2], Kiran Rade[5], Naman Shah[13], Shashi Kant[6], Giridhara R. Babu[8], Sanjay Zodpey[9], C.P. Girish Kumar[†], Jeromie Wesley Vivian Thangaraj[†], Pranab Chatterjee[10], Suman Kanungo[12], Ravindra Mohan Pandey[7], Manoj Murhekar[†], Sujeet K. Singh[11], Swarup Sarkar[10], J.P. Muliyil[3], Raman Gangakhedkar[4] & D.C.S. Reddy[14]

[1]ICMR School of Public Health, [2]Division of Epidemiology & Bio-Statistics, [†]ICMR-National Institute of Epidemiology, Chennai, [3]Independent Consultant, Vellore, Tamil Nadu, [4]Division of Epidemiology & Communicable Diseases, Indian Council of Medical Research, [5]WHO Country Office for India, [6]Centre for Community Medicine, [7]Department of Biostatistics, All India Institute of Medical Sciences, [8]Indian Institute of Public Health-Bengaluru, [9]Indian Institute of Public Health-Delhi, Public Health Foundation of India, [10]Translational Global Health Policy Research Cell (Department of Health Research), Indian Council of Medical Research, New Delhi, [11]National Centre for Disease Control, Delhi, [12]ICMR-National Institute of Cholera & Enteric Diseases, Kolkata, West Bengal, [13]Jan Swasthya Sahyog, Bilaspur, Chhattisgarh & [14]Independent Consultant, Lucknow, Uttar Pradesh, India

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Conducting population-based serosurveillance for severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) will estimate and monitor the trend of infection in the adult general population, determine the socio-demographic risk factors and delineate the geographical spread of the infection. For this purpose, a serial cross-sectional survey would be conducted with a sample size of 24,000 distributed equally across four strata of districts categorized on the basis of the incidence of reported cases of COVID-19. Sixty districts will be included in the survey. Simultaneously, the survey will be done in 10 high-burden hotspot cities. ELISA-based antibody tests would be used. Data collection will be done using a mobile-based application. Prevalence from the group of districts in each of the four strata will be pooled to estimate the population prevalence of COVID-19 infection, and similarly for the hotspot cities, after adjusting for demographic characteristics and antibody test performance. The total number of reported cases in the districts and hotspot cities will be adjusted using this seroprevalence to estimate the expected number of infected individuals in the area. Such serosurveys repeated at regular intervals can also guide containment measures in respective areas. State-specific context of disease burden, priorities and resources should guide the use of multifarious surveillance options for the current COVID-19 epidemic.

Key words Antibody - COVID-19 - hotspot - SARS-CoV-2 - serial cross-sectional - seroprevalence - surveillance - trend

Coronavirus disease 2019 (COVID-19) has emerged as a pandemic, and the infection due to SARS-CoV-2 has now spread to more than 200 countries[1]. Surveillance systems form the foundation stone of active case finding, testing and contact tracing, which are the key components of the public health response to this novel, emerging infectious disease[2]. There is uncertainty about the true proportion of patients who remain asymptomatic or pre-symptomatic at a given time. As per the WHO-China Joint Monitoring Mission Report, and an analysis of 21 published reports, anywhere between 5 and 80 per cent of SARS-CoV-2-infected patients have been noted to be asymptomatic[3,4]. Facility-based surveillance efforts are likely to miss mild and asymptomatic cases. Through household-targeted, antibody-based serologic testing, we can minimize the biases of referral and selective testing affecting laboratory-based surveillance, generate evidence on the role of asymptomatic infection in driving transmission and estimate the extent of infection in a given population.

The WHO global research map for COVID-19 and others recommend population-level seroepidemiological studies to generate data on the levels of infection in populations and recommend containment measures accordingly[5,6]. In order to achieve this, a protocol for conducting such population-based seroepidemiological investigation for COVID-19 has been proposed by the WHO[7]. The WHO suggests three possibilities to conduct the seroepidemiological investigation: cross-sectional investigation, most apt after the peak transmission is established; repeated cross-sectional investigation in the same geographic area (but not necessarily the same individuals each time) to establish trends in an evolving pandemic; and longitudinal cohort study with serial sampling of the same individuals. Establishing cohorts during a pandemic being resource intensive, and India being in the early stages of the pandemic, the second option is the most appropriate choice to guide public health response.

The Indian Council of Medical Research (ICMR), therefore, proposes to establish a community-based district-level serosurveillance system to monitor the transmission of SARS-CoV-2 infection in the general population. The initial survey would serve as a baseline to determine the seroprevalence of SARS-CoV-2 infection in the community and in high-burden cities as well, while the subsequent rounds would help to monitor the trends of infection in the community. This information will also guide the strategy for making decisions related to lockdown options at a district level. The objectives of this serosurveillance are to estimate and monitor the trend of seroprevalence for SARS-CoV-2 infection in the general population and high-burden cities, determine the socio-demographic risk factors for SARS-CoV-2 infection and delineate the geographical spread of the infection in the general population and hotspot cities.

Proposed Protocol

This serosurveillance is designed as a repeated cross-sectional survey of adults aged 18 yr or more.

Sampling strategy: The districts will be categorized into four strata according to the reported COVID-19 cases per million population (zero, low: 0.1-4.7, medium: 4.8-10 and high: >10), as per data from the ICMR testing laboratory reporting portal (*https://cvanalytics.icmr.org.in/*). Fifteen districts from each stratum will be selected randomly for a total of 60 districts. In addition, the top 10 cities reporting the highest number of cases in the country will be included, to be considered as hotspots. The proposed sampling strategy considers the operational feasibility of the survey.

Sample size: For the first round of serosurvey, with the assumption of one per cent seropositivity, relative precision of 40 per cent, confidence interval of 95 per cent and design effect of 2.5, there is a need to enrol 5,929 (rounded to 6000) individuals in each of the four strata, amounting to 24,000 overall. For this purpose, 400 individuals will be allocated to each of the 60 districts. Within each of these 60 districts, 40 individuals will be included for the survey from 10 clusters (villages in rural areas and wards in urban areas). The sample size for subsequent rounds will be adjusted (to account for better precision and acceptable confidence interval) after analyzing the results from the first round of the survey.

Sampling method: In the selected districts, the 2011 census-projected population list of villages and wards will be used to select 10 clusters. A cluster is a village in rural areas and a ward in urban areas. Clusters will be randomly selected in numbers as per the proportion of rural : urban population. Four random locations will be selected in each of the identified village/ward. The survey team will visit houses in the selected clusters (village/ward) in each of the four random locations as the centre point from where they will select 10 households sequentially. One individual will

Table. Schema for selection of individuals from selected household in the cluster

Number of adult females in household	Number of adults in the household			
	1	2	3	4 or more
0	Male	Youngest male	Youngest male	Oldest male
1	Female	Female	Oldest male	Female
2		Oldest female	Male	Oldest male
3			Youngest female	Older male
4 or more				Oldest female

be selected from each selected household as per the schema in the Table.

With the above-mentioned method for individual selection from each household in a cluster, 400 individuals will be enrolled in each district (10 clusters × 4 random starting points × 10 households from each point × 1 adult from each house).

Hotspot cities: With the assumption of five per cent seropositivity, relative precision of 20 per cent, confidence interval of 95 per cent and design effect of 2.5, there is a need to enrol 4,554 (rounded to 5000) individuals. For this purpose, 500 individuals will be allocated to each of the top 10 cities reporting the highest number of cases. Within each of these cities, 100 individuals will be included for the survey from five randomly selected clusters (containment zones within the city).

Four random locations will be selected in each of the identified containment zones. The survey team will visit houses in the selected containment zones in each of the four random locations as the centre point from where they will select 25 households sequentially. One individual will be selected from each selected household as per the schema in the Table.

With the above-mentioned method for individual selection from each household in a containment zone, 500 individuals will be enrolled in each hotspot city (5 containment zones × 4 random starting points × 25 households from each point × 1 adult from each house).

Study procedures: The study team will visit the randomly selected households and brief them about the survey objectives and process involved. After obtaining written consent, information on basic demographic details, exposure history to laboratory-confirmed COVID-19 cases, symptoms suggestive of COVID-19 in the preceding one month and clinical history will be recorded. All data will be entered in an ODK application on mobile phones by the survey teams. When there is unavailability of eligible individual in a household, the team will move to the next household and complete the survey until the required number of households are visited. Trained phlebotomists in each of the survey team will collect 3-5 ml of venous blood from each participant. Serum will be separated after centrifugation in a local health facility and transported to the laboratories in designated ICMR institutes. Detection of SARS-CoV-2-specific IgG antibodies will be performed using an ELISA-based test as per the specified optical density (OD) cut-off value.

Analysis plan: The seroprevalence of SARS-CoV-2 infection will be estimated in different rounds of serosurveys. The trend of seropositivity will also be looked at to monitor the community-level transmission. Pooled seroprevalence from the group of districts for each of the four strata will be used to estimate the population prevalence of COVID-19 infection in the country after adjusting for population and antibody test characteristics. Similar analysis will be done separately for the hotspot cities. A sensitivity analysis will be done to determine the influence of antibody test kit performance on prevalence estimates[7]. Gaps in testing, missing convalescent cases on real-time reverse transcription (RT) PCR-based assay and undetected asymptomatic or mild symptomatic infections can influence the reported number of cases. The infection-to-case ratio will be calculated to account for the same.

Ethical considerations: Interviews will happen in a place as per the convenience of the participants to ensure privacy. All data will be stored securely under the investigator's responsibility, with a focus on ensuring the participant's confidentiality. The final report will be based on aggregate data without any

identifying information. Electronic case report forms (eCRFs) will be used to collect data. A database with electronic tracking, password-restricted access and audit trail, with time and date stamps on data entry and edits, will be used.

State Health Department and District authorities will be actively engaged to ensure smooth operationalization of the surveillance teams and also to reduce any stigma arising for the residents due to their participation in this surveillance at the community level. The survey will be conducted primarily as a public health surveillance activity in the context of epidemic disease. In addition, written consent for sampling will be taken from the participants. Given the unknown implications of immunity in the presence of the antibodies, the potential of false-positive and false-negative cases, the participants will be explained about their test results. A participant information sheet will be provided to each household, which will provide details of these limitations. Approval for the protocol will be obtained from the ICMR COVID-19 National Ethics Committee.

Discussion

Many of the COVID-19 pandemic-affected countries have initiated large-scale sero-surveys to quantify the actual burden of COVID-19[8,9]. Use of serologic tests for identifying COVID-19 antibodies has been reported from several countries. Rapid tests were used in Finland[10] and the USA[11]; lateral flow immunoassays were used in the USA[12,13] and ELISA-based tests were used in France[14], Germany[15], Scotland[16] and the USA[17]. Drive-through testing in the USA[11], school-based testing in France[14], testing in retirement homes in Sweden[18], representative community screening in Germany[15], volunteer screening in Italy[19] and community-wide screening using various strategies in the USA[12,13,20-22], represent the various strategies for undertaking community-based testing. Positivity rates in population-based community screening ranged from 0.5 per cent in San Miguel County[20], to 14 per cent in Gangelt, Germany[15], and New York, USA[22]. A higher positivity rate obtained following the representative recruitment of participants, such as the effort in New York, where over 3000 participants were tested across 40 locations in 19 counties, could indicate the extent of population that has been exposed to SARS-CoV-2[22].

A nationally representative serosurvey with multistage sampling design will be useful to identify the most affected areas and provide crucial data to develop epidemiological models to predict the trajectory of COVID-19[8]. Instead of waiting for the end of the epidemic, repeated serosurveys carried out at regular intervals can be a useful tool to monitor the epidemic precisely[23]. In addition to the ongoing surveillance, testing, contact tracing and isolation, India had announced a nationwide lockdown on March 24, 2020, for 21 days, which has been extended in four phases. Hence, establishing sentinel serosurveillance in the general population, and hotspots in particular, integrated with district routine surveillance activities, can guide public health measures and easing of restrictions in a timely manner[24].

The surveillance will be directly monitored by the apex scientific working group on epidemiology and surveillance established for COVID-19 by the ICMR. District administration and survey teams will ensure privacy and confidentiality of the data collected during the surveys. COVID-19 response protocol as per the local situation will be followed under the guidance of respective State government and district administration.

The population-based seroepidemiological studies will help us to determine the burden of COVID-19 infection at the community level and monitor the trends in the transmission of SARS-CoV-2 infection. The survey findings will be useful to guide in designing and implementing appropriate containment measures. Subsequent rounds of this survey will depend on the results from the first round and the ensuing epidemic situation in the country.

This generic protocol provides a plan of action to implement serosurveillance for COVID-19 in the country. Given the socio-economic, geographic and cultural diversity of India, various surveillance strategies can be customized to fit the regional priorities, resources and disease burden. People-centric approaches to surveillance should be prioritized, focusing on the need to save lives and protect the vulnerable. A bouquet of choices from the available surveillance plans for active or past SARS-CoV-2 infection (house-to-house surveillance; stratified, risk-based surveillance; sentinel surveillance; ports-of-entry surveillance and contact-tracing-related surveillance) could be provided to States or local health and administrative authorities, with the option to choose the alternative which is most acceptable considering the local contexts.

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For correspondence: Dr Manoj V. Murhekar, ICMR-National Institute of Epidemiology, R127, TNHB, Ayapakkam, Chennai 600 007, Tamil Nadu, India
e-mail: mmurhekar@gmail.com