Evaluation of the Prevalence of SARS-CoV-2 Infection in Inhabitants of the City of São Paulo, Brazil: A Pilot Study.

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INTRODUCTION AND JUSTIFICATION

The SARS-CoV-2 virus, which causes the disease called COVID-19, has been spreading in the city of São Paulo since the end of February 2020 and has led government authorities to implement social isolation measures. Due to a large number of asymptomatically or oligosymptomatically infected people, and the small number of laboratory tests available, the actual percentage of infected people in the general population is still unknown.

It is anticipated that infected people become totally or partially immune, i.e., resistant to the virus, and therefore, knowing the percentage of the population that is no longer susceptible to the SARS-CoV-2 virus is essential for subsidizing public policies for control and combating the COVID-19 pandemic. Knowing and monitoring the fraction of the population already infected throughout the epidemic period is fundamental, so the authorities can make decisions about when and how to relax social distancing measures.

The objective of this pilot project is to determine the regions most affected – the proportion of the population already infected – by the novel coronavirus in the municipality of São Paulo (MSP), Brazil. Therefore, questions about symptoms will be taken, blood samples from a representative sample of the population living in the western, central, and southeastern regions of the MSP will be collected, and the presence of specific antibodies against SARS-CoV-2 will be detected. This approach will make it possible to estimate the percentage of the population that has already been infected, produced antibodies, and become

totally or partially immune to the virus. This epidemiological survey may be repeated periodically to determine the growth in the number of immune people over time.

Serological tests will be performed using the currently available methodology for detecting antibodies against SARS-CoV-2. However, as they are recently developed tests, their accuracy needs to be evaluated in different contexts and compared with possible new tests that are being developed. As such, the collected serum samples will be stored for future testing according to the description on the Free, and Informed Consent Form (FICF) that is found in Annex I. Participants will be informed of the results of their exams.

If this pilot project is successful, it may be extended to the entire city of São Paulo and other cities in the country. This information will be essential for providing government agencies with the information necessary to organize the relaxing of social isolation and resuming economic activity.

RESEARCH OBJECTIVE

The objective is to estimate the percentage of people infected with SARS-CoV-2 residing in the western, central, and southeastern regions of the MSP, the regions most affected by the epidemic according to official data released on 17/04/2020.

A household survey will be conducted, and venous blood samples will be collected to ascertain immunity to SARS-CoV-2 using the laboratory test for the detection of specific IgM and IgG antibodies against the virus to achieve this goal. Households in the neighborhoods of the MSP – Morumbi, Jardim Paulista, Bela Vista, Água Rasa, Belém, Pari – will be selected and invited to participate in this study.

METHODOLOGY

1. Study framework and target population

A cross-sectional, population-based study was proposed, whose target population was individuals aged 18 years or older, on the date of data collection, residing in permanent private households, located within six MSP neighborhoods. These neighborhoods were selected considering official data from the Municipal Department of Health released on 4/17/2020, where numbers of confirmed COVID-19 cases and numbers of confirmed or suspected deaths by COVID-19 were reported by neighborhood.

The criteria adopted for inclusion in the study were; the three neighborhoods with the highest number of confirmed cases per 100,000 inhabitants (Morumbi, Bela Vista, and Jardim Paulista) and the three neighborhoods with the highest number of confirmed or suspected deaths per 100,000 inhabitants (Pari, Belém and Água Rasa).

To compose the study population, we adopted the following criteria:

Inclusion criteria: people 18 years of age or older, on the date of data collection, living in permanent private homes within the six neighborhoods listed above, who consent to participate in the study will be eligible.

Non-inclusion criteria: people with no legal capacity will not be included in the survey.

2. Sampling plan

The target population corresponds to the population aged 18 or over at the time of the interview, living in permanent private homes in the neighborhoods of Morumbi (west region), Jardim Paulista (west region), Bela Vista (central region), Água Rasa, Belém, and Pari (all three from the southeast region).

The expected sample size is 500 people. This number will allow a prevalence greater than 4% to be estimated with coefficients of variation below 30%, taking into account the complex delineating effects (deff=2). The maximum sampling error (semi-amplitude of the confidence interval), corresponding to the 50% prevalence will be 6.2, indicating a 95% confidence interval ($CI_{95\%}$) of 43.8% - 56.2%.

Sampling errors and coefficients of variation by prevalence, for a sample of 500 individuals

Prevalence	Sampling Error[1]	Coefficient of Variation[2]
50	6.2	6.3
40	6.1	7.7
30	5.7	9.7
20	5.0	12.6
10	3.7	19.0
8	3.4	21.4
6	2.9	25.0
5	2.7	27.6
4	2.4	31.0

Considering that the sample may suffer a loss of approximately 30% due to the possibility of having households in which the research is not conducted (vacant,

closed, refusal), 720 households will be selected in the drawing

We will use probabilistic sampling for those selected to be tested, by clusters, in three stages: census sector, household, and resident.

In the first stage, 72 census sectors will be drawn by systematic sampling with probability proportional to size, with the number of households being used as a measure of size, according to the 2010 IBGE Census. For the drawing, the sectors will be arranged by neighborhood to obtain an implicit stratification by neighborhood.

In the second stage, ten private households will be selected by systematic drawing in each census sector in the sample, from the list of households obtained by "laying out the sector." This activity will establish a complete and updated register of every household, occupied or not, that exists within the sectors.

In the third selection stage, one resident will be drawn. In this stage, every resident eligible for the interview (aged 18 or over) will be listed, and one of them will be randomly drawn using an internal program on a tablet. People with no legal capacity will not be included in the survey. Here, it should be noted that those residents of the chosen household who wish to take the test administered by

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1 The sampling error (d) was calculated using the algebraic expression: $n = \frac{p(1-p)}{(d/z)^2} \cdot deff$, where z = 1.96 is the normal curve value corresponding to a 95% confidence interval, deff=2 the delineating effect, and n=500.

2 The prevalence variation coefficient (cv(p)) was calculated by: $n = \frac{(1-p)/p}{cv(p)^2} . deff$

the research team must understand the risks and benefits of the test and sign the FICF (Annex Ib, Cohabitant), that are described in the same forms for the research participants.

The sampling fraction corresponding to the sampling process described above is:

$$f = \frac{72 \cdot M_i}{M} \cdot \frac{10}{M_i'} \cdot \frac{1}{M_{ij}} = \frac{720}{M} \cdot \frac{M_i}{M_i' \cdot M_{ij}} \ ,$$

in which M_i and M are the numbers of households existing in the sector *i* and in the total study area as according to data from the 2010 census, and M_i' is the updated number of households in the sector *i* obtained in the "plotting out of the sector," and M_{ij} is the number of residents in the household *ij*.

The switching of randomly selected sampling units (sectors, households, or residents) has not been considered during this study. If the interview is not conducted because the selected household was unable to be visited or the selected resident was unavailable for interviewing/testing, the corresponding situation will be recorded. The percentages of non-response from households and residents and the reasons why will be recorded by the census sector.

In order to reduce the percentages of non-response, three visits are scheduled at different times and days to each household in the sample. With this same objective, other strategies will be adopted, i.e., distribution of pamphlets (Annex III) during the "plotting of the sector," media disclosure, making telephone calls, and sending printed and signed letters (Annex IV) to randomly selected households.

3. Data collection

Data collection will be performed through face-to-face home interviews, using mobile devices. The questionnaire can be found in Annex II. In addition to the interviews, venous blood samples will be collected for laboratory analysis to detect antibodies against the SARS-CoV-2 virus.

Before the interview, the selected respondent will be requested to sign the FICF. The signature of the term will only be obtained after the selected participant is sufficiently informed of all the possible benefits, risks, and procedures that will be carried out, and provided all the information pertinent to the research as established in the Brazilian Norms and Guidelines that regulate the research involving human beings, including Resolutions 466/12 and 510/16 of Brazil's National Health Council.

Residents over 18 years of age who live with the research participant (the

selected individual) who wish to take the test to know their serological status regarding SARS-CoV-2 infection will be attended to by the research team. The same data collection procedures adopted for the randomly selected household will be adopted. The coinhabitants' test results will not be included in the seroprevalence estimate calculations, as earlier described in the probabilistic sampling methodology.

4. Collection of blood samples

In addition to the interview, a venous blood sample will be collected from the respondent selected to participate in the research to check for the presence of antibodies to SARS-COV-2. The collection will be performed by a trained health professional who will accompany the interviewer during the approach to the households. The team of health professionals will be made available by Grupo Fleury.

Although the interview is scheduled to take place with respondents who agree to participate in every stage of the study, dropouts can occur in the blood sample collection phase, even after the commitment and signing of the informed consent form.

Contact information (address and telephone number) of the study participants will be obtained while conducting the interviews so that the result of the test performed will be available later. This personal information will be under the sole responsibility of the research coordinator.

In the case of coinhabitants in the household of the selected individual wishing to perform the test, the same measures will be adopted, including obtaining contact information so that individual and confidential communication of the test result is made.

5. Laboratory testing

The blood analysis for the detection of IgM and IgG antibodies will be performed in Grupo Fleury laboratories according to the manufacturer's guidelines using the CLIA methodology (chemiluminescence), MAGLUMI 2000 PLUS equipment, "MAGLUMI IgM 2019-nCoV (CLIA) kits," and "MAGLUMI IgG 2019-nCoV (CLIA)," all from Snibe Diagnostic.

In order to perform the tests, the study participants' serum samples will be mixed and incubated with buffer solution and magnetic microspheres coated with IgM monoclonal antibodies subtypes and human magnetic microspheres, forming immunocomplexes. After magnetic-field-induced precipitation, the supernatant is decanted and washed. Then, ABEI (non-enzymatic nanomolecule) labeling with anti-IgM and anti-IgG human antibodies is performed, followed by incubation to form complexes. After magnetic-field-induced precipitation, the supernatant is decanted and washed. Subsequently, *starters 1+2* are added to initiate a chemiluminescence reaction. The light signal

is then measured by a photomultiplier as relative light units (RLUs), which are proportional to the concentration of anti-SARS-CoV-2 IgM and IgG present in the sample.

The results are calculated automatically using calibration curves. Values equal to or greater than 1.00 AU/mL are considered reactive, and results below this value are non-reactive.

6. Data analysis

The data obtained in the research should be weighted since the individuals in the sample will be selected with unequal probabilities, and these differences should be compensated. The weights will be given by inverting the sampling fractions used in each selection stage.

The prevalence of SARS-CoV-2 virus infection will be studied using the proportional estimates and the respective 95% confidence intervals. The estimates will be obtained, taking into account the complex aspects of the sampling plan (drawing of clusters, stratification, and classification).

7. Quality control

All research will be governed by ethical standards of the Brazilian Association of Research Companies (ABEP) and the World Research Association (ESOMAR). The procedures are also following the international quality standard in Market Research and Opinion of ISO 20.252 and the international standard of Quality Management ISO 9001.

- The interviews will be conducted by a team of properly trained, supervised, and identified interviewers.

- All interviewers and health professionals will go to the field with the appropriate Personal Protective Equipment, following the guidelines listed in the Standardized Operating Procedure released by the Brazilian Ministry of Health. This is for the protection of research participants and minimizes the risk of coronavirus infection, as well as to protect professionals who will collect data and biological materials from individuals who agree to participate in the research.

- At least 20% of the interviewers' material will be verified.

- 100% of the questionnaires will be submitted to an electronic consistency test to verify the coherence of the answers.

8. Ethical aspects

The project will be developed based on information and examinations of a sample of the population residing in the western, central, and southeastern regions of the MSP. When invited to participate in the study, they will be duly informed by the research team that participation is voluntary and confidential, the purpose of the research, and the venous blood collection by puncture, including its risks. Potential risks and benefits related to participating in the research are explained in the FICF and commented on in the “final considerations” of this document.

Participants will also be informed about their freedom to refuse or withdraw their consent at any stage of the research without causing any type of penalty. The FICF also includes information about the participant’s right to full and free assistance, for as long as necessary, in case of harm resulting from participating in this research; in addition, you will be entitled to legally established indemnities in case of personal injury directly caused by the procedures proposed in the study.

Biological material and information will only be collected after signing the FICF form (Annex I). All individuals tested in the field will have their contact information registered so that they can receive their test results. Each participant will have an identification number in the research, and the identification data will be in the exclusive possession of the project coordinator for the sole purpose of informing each participant of the laboratory test result. The data will be analyzed together, coded, without any possibility of compromising the identity of the participant.

The coinhabitants of the selected individual who wanted to and performed the test, despite not being part of the study sample, will also receive an identification number to preserve the confidentiality of the data. Their results will be communicated individually, following the same precautions described above for the research participants.

The biological safety measures recommended by Brazil’s Ministry of Health will be taken to minimize the risk of contaminating the research participants with SARS-Cov-2, as well as to protect field workers acting in the collection of data and material. They are described in the “AnexoV_ Mobile_Cornoa_Service_Flow”) document sent to CONEP.

The project and the FICF will be entered into the CEP/CONEP system by the Grupo Fleury researcher, and fieldwork will only start after its approval.

The project will be partially financed by Instituto Semeia and Grupo Fleury. The project has no commercial ends and aims to bring information to government agencies.

The study participants and researchers listed above will receive no payment of any kind.

8.1 **About sample storage and creating a biorepository (biobank)**

Bearing in mind that even the traditionally designed enzymatic serological tests that are already in use, like the one that will be used in this study (chemiluminescence), may have limited sensitivity when applied to individuals who have presented more discrete clinical forms of the infection. It is prudent that the collected samples are preserved in adequate conditions (frozen at -20º C) so that they can be evaluated when, and if, new serological tests are available. There would be no need to collect new samples when using this method. More accurate information could be generated to complement the data obtained in the present study and provide comparable results of this study for future research with the same objective. These stocked samples will not be subjected to any other type of evaluation other than determining the presence of antibodies against the SARS-CoV-2 virus. Only tests whose future research has shown that they can show greater sensitivity for individuals with more discreet clinical forms will be used. The samples will be discarded after being stored for one year or, if necessary, the CEP/CONEP system will request the appropriate consent to extend this storage time. Fleury has adequate storage conditions inside its facilities, in compliance with the “SPONSOR’S INFRASTRUCTURE AND RESPONSIBILITY STATEMENT” already annexed to this process. Execution timeline

The estimated duration of this research project is four weeks, according to the following schedule:

Activities	**Weeks ***			
	1	**2**	**3**	**4**
Ethical approval	X			
Organization of fieldwork	X	X		
Data collection		X		
Data analysis		X	X	
Preparation of final project report and scientific work		X	X	X

* Expected start date: 4/29/2020

EXPECTED RESULT

The expected result is to achieve a proportional estimate of the inhabitants of the studied regions that have already been infected with SARS-CoV-2 since the introduction of the virus in the MSP up to the date of the survey.

FINAL CONSIDERATIONS

We believe that this project is critical for monitoring the COVID-19 pandemic and that, if this pilot is successful, it can be extended to other areas of the city of São Paulo and of Brazil. Other countries like Italy, Germany, England, and the United States have implemented similar projects.

The project has a low risk for the people involved. These risks involve venipuncture for sample collection that can generate bruising or phlebitis at the puncture site or some transitory mark on the skin where the collection was made. The participant will also receive a visit from an interviewer and a health professional who will collect their blood. These professionals will be properly attired with a face shield, mask, disposable long sleeve apron, foot protection, and disposable gloves. They will follow all the procedures recommended by the Brazilian Ministry of Health so that the risk of contamination is minimal.

The precautions above are already adopted in the home collection procedures carried out by Grupo Fleury's teams and are detailed in the document sent as "AnexoV_Mobile_Cornoa_Service_Flow."

The test will determine the presence of antibodies against the SARS-CoV-2 virus, and the result will be reported to the participant. The participant, as well as the coinhabitants of their home who wished to take the test, will receive an email with a login and password to access their results online and will also receive the results of the exam by standard mail. In addition, the communication channel with the researcher responsible for the study will be available, as described in the FICF.

The serological test made available (and detailed above in item 5 "laboratory testing") is approved by ANVISA (Brazilian Regulatory Health Agency), validated by Grupo Fleury, and made available regularly by the laboratory. However, they may have limited sensitivity when applied to individuals who have presented more discrete clinical forms of the infection. Participants, as well as coinhabitants in their household who wished to perform the test, will be informed of this limitation and instructed to continue following the recommendations of the Brazilian Ministry of Health and the Office of the São Paulo State Government regarding measures to protect the population.

The test result will inform the people who have taken the test if they have ever

had contact with the SARS-CoV-2 virus. This result is the only direct benefit to the participant, but the results may help health authorities to plan measures to control the epidemic in the city of São Paulo.

All data obtained will be made widely available after the study is completed and will be published in scientific journals.

REFERENCES

Kish, L. Survey Sampling. New York: Wiley, 1965.

Silva, N. N. Amostragem probabilística: um curso introdutório (3ª Ed). São Paulo: EDUSP, 2015.

United Nations. Designing Household Survey Samples: Practical Guidelines. New York: United Nations, 2005.

Cochran, W. G. Sampling techniques (3rd ed.). New York: John Wiley & Sons, 1977.

Jin Y, Wang M, Zuo Z, Fan C, Ye F, Cai Z, Wang Y, Cui H, Pan K, Xu A, Diagnostic value and dynamic variance of serum antibody in coronavirus disease 2019, International Journal of Infectious Diseases (2020), doi:https://doi.org/10.1016/j.ijid.2020.03.065

São Paulo, 26 de abril de 2020.

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Annex II – Participant Information Questionnaire

Household survey on the prevalence of infection by the SARS-CoV-2 virus in inhabitants of the western region of the municipality of São Paulo: a pilot study.

1. **Neighborhood: _____**
2. **Sector: _____ Household: _____ Resident: _____**

3. **Number of household residents: _____**

4. **Sex: (1) Male (2) Female**

5. **How old are you? _____years**

6. **What was the last grade you completed?**

   ____( ) grade ( ) year

   Level: ( ) K-8 ( ) high school ( ) college If college,

   did you complete the course? ( ) Yes ( ) No

7. **What is the highest grade completed in the residence?**

   ____( ) grade ( ) year

   Level ( ) K-8 ( ) high school ( ) college

   If college, was the course completed? ( ) Yes ( ) No

8. **How do you identify in terms of color or race?**

   (1) White (2) Two or more (3) Black (4) Asian (5) Indigenous

9. **Since <DAY OF THE WEEK> two weeks ago, have you had:**
   a. Fever? (1) Yes (2) No
      If yes, how many days ago did it start? _____

   b. Sore throat? (1) Yes (2) No
      If yes, how many days ago did it start? _____

   c. Cough (1) Yes (2) No
      If yes: Did the cough produce phlegm? (1) Yes (2) No
      If yes, how many days ago did it start? _____

   d. Have you had difficulty breathing? (1) Yes (2) No
      If yes: Was your nose stuffy? (1) Yes (2) No
      If yes, how many days ago did it start? _____

   e. Have you had diarrhea? (1) Yes (2) No
      If yes, how many days ago did it start? _____

   f. Have you had any vomiting? (1) Yes (2) No
      If yes, how many days ago did it start? _____

10. **Have you ever been diagnosed with the SARS CoV-2 or COVID-19**

infection?

(1) Yes (2) No

If so, when? _____

11. Do you do any work in the healthcare area?

(1) Yes (2) No

Annex I - Free and Informed Consent Form - participant

Annex Ib - Free and Informed Consent Term - cohabitant

Annex II – Participant Information Questionnaire

Annex III – Publicity material ("pamphlets") informing neighborhoods Examples of the research

Name of the document submitted: "ANNEXIII_Pamphlet"

Annex IV – Printed and signed letters to household residents drawn

Name of the document submitted: "ANNEXIV_Explanation_Letter"

Annex V – Mobile_Cornoa_Service_Protocol: Corona flow

Name of the document submitted:
"AnnexV_Mobile_Cornoa_Service_Flow"