

1 **Repeated population-based surveys of antibodies against SARS-**
2 **CoV-2 in Southern Brazil**

3
4 Mariângela F Silveira, Aluísio J D Barros, Bernardo L Horta, Lúcia C Pellanda, Odir A
5 Dellagostin, Claudio J Struchiner, Marcelo N Burattini, Andréia R M Valim, Evelise M
6 Berlezi, Jeovany M Mesa, Maria Letícia R Ikeda, Marília A Mesenburg, Marina
7 Mantesso, Marinel M Dall'Agnol, Raqueli A Bittencourt, Fernando P Hartwig, Ana M B
8 Menezes, Fernando C Barros, Pedro C Hallal*[#], Cesar G Victora[#]

9
10 (#) Joint last authors

11
12 **Universidade Federal de Pelotas, Brazil** (Dr. Pedro C Hallal, Prof. Aluísio J D Barros,
13 Prof. Bernardo L Horta, Prof. Mariângela F Silveira, Prof. Odir A Dellagostin, Dr.
14 Fernando P Hartwig, Prof. Ana B Menezes, Prof. Fernando C Barros, Prof. Cesar G
15 Victora), **Fundação Universidade Federal de Ciências de Saúde de Porto Alegre, Brazil**
16 (Prof. Lúcia C Pellanda, Profa Marília A Mesenburg), **Fundação Getúlio Vargas and**
17 **Universidade do Estado do Rio de Janeiro, Brazil** (Prof. Claudio J Struchiner),
18 **Universidade de São Paulo, Brazil and Universidade Federal de São Paulo, Brazil**
19 (Prof. Marcelo N Burattini), **Universidade de Santa Cruz do Sul, Brazil** (Profa. Andréia R
20 M Valim), **Universidade de Ijuí, Brazil** (Profa Evelise M Berlezi), **IMED - Passo Fundo,**
21 **Brazil**, (Prof. Jeovany M Mesa), **Universidade do Vale do Rio dos Sinos, Brazil** (Profa.
22 Maria Letícia R Ikeda), **Universidade de Caxias do Sul, Brazil** (Profa. Marina Mantesso),
23 **Universidade Federal de Santa Maria, Brazil** (Profa. Marinel M Dall'Agnol), **Secretaria**
24 **Municipal de Saúde de Uruguaiana, Brazil** (Raqueli A Bittencourt).

25
26 **Correspondence to:** Pedro C Hallal, Marechal Deodoro 1160 – 96020-220 - Pelotas, RS,
27 Brazil – Phone (fax): +55 53 3284-1300 - Email: prchallal@gmail.com

28
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34

35 **ABSTRACT**

36

37 Population based data on COVID-19 are urgently needed for informing policy decisions,
38 yet few such studies are available anywhere, as most surveys rely on self-selected
39 volunteers. In the Brazilian State of Rio Grande do Sul (population 11.3 million), we are
40 carrying out fortnightly household surveys in nine of the largest cities. Multi-stage
41 probability sampling was used in each city to select 500 households, within which one
42 resident was randomly chosen for testing. The Wondfo lateral flow rapid test for
43 detecting antibodies against SARS-CoV-2 has been validated in four different settings,
44 including our own, with pooled estimates of sensitivity (84.8%, 95% CI 81.4%;87.8%)
45 and specificity (99.0%, 95% CI 97.8%;99.7%), which are within the acceptable range for
46 epidemiological studies. In the first wave of the study (April 11-13), 4,188 subjects
47 were tested, of whom two were positive (0.0477%; 95% confidence interval (CI)
48 0.0058%;0.1724%). In the second round (Apr 25-27) there were six positive subjects
49 (0.1333%; 95% CI 0.0489%;0.2900%). We also tested family members of positive index
50 cases, and nine out of 19 had positive results. Testing of reported COVID-19 cases
51 according to RT-PCR confirmed that the test was highly sensitive under field
52 conditions. The epidemic is at an early stage in the State, as the first case was reported
53 on Feb 28, and by Apr 30, 50 deaths were registered. Strict lockdown measures were
54 implemented in mid-March, and our results suggest that compliance was high, with full
55 or near full compliance rates of 79.4% in the first and 71.7% in the second round. As far
56 as we know, this is the only large population anywhere undergoing regular household
57 serological surveys for COVID-19. The results show that the epidemic is at an early
58 phase, and findings from the next rounds will allow us to document time trends and
59 propose Public Health measures.

60 **INTRODUCTION**

61

62 Despite calls for population-based data on COVID-19,¹ there have been remarkably
63 few household seroprevalence surveys anywhere, and none in Latin America.² In Rio
64 Grande do Sul, the southernmost state in Brazil (population 11.3 million), the first case
65 of COVID-19 was diagnosed on February 29, 2020. As of April 30, 1,466 confirmed
66 cases (129 per 1,000,000 inhabitants) and 50 deaths had been reported
67 (<http://ti.saude.rs.gov.br/covid19/>). It is important to remark that in the state, as also
68 in Brazil, only persons with moderate to severe symptoms had been tested, using PCR
69 to detect SARS-Cov-2. The state and most municipal governments issued strong social
70 distancing policies in mid-March, including closures of schools, shops and services,
71 except for businesses deemed to be essential.

72

73 Other than studies based on convenience samples, such as individuals who
74 volunteered to be tested, supermarket customers, or blood donors, there are few
75 general population sample surveys in the literature. In a national study in Iceland³,
76 one of the three groups of participants was recruited through random sampling of the
77 population, but only about one third of those invited were tested. In this group, 13 of
78 2283 persons tested positive (0.6%; 95% CI 0.3;0.9) in quantitative real-time
79 polymerase-chain-reaction (qRT-PCR) assays. A national household study in Austria
80 used random sampling to invite households to participate. Of the households
81 contacted, 77% or 2,197 declared their willingness to participate, and 1,541 persons
82 were successfully tested. Assuming two persons per household, those tested
83 correspond to about 30% of the intended sample. The study found a prevalence of
84 0.33% (95% CI 0.12;0.76%) using qRT-PCR, or 5 individuals out of those tested.⁴
85 Smaller studies in hot spots for COVID-19 showed prevalence of 14% the German city
86 of Gangelst⁵ and 3% in the Italian village of Vò.⁶

87

88 As expected, studies based on volunteers found higher prevalence, as was the case for
89 the first study in Iceland (0.9%)³, the population screening in South Korea (2.1%)⁷ and
90 two studies in California: prevalence of 1.5% in Santa Clara county⁸ and 4.1% in Los
91 Angeles County.

92 <http://publichealth.lacounty.gov/phcommon/public/media/mediapubhpdetail.cfm?prid=2328>

93

94 Starting on April 11-13, we set out to test the presence of antibodies against SARS-
95 CoV-2 in population-based samples of 500 individuals in each of nine sentinel cities in
96 the state, with a total sample of 4,500. The same methodology was used in a second
97 round in the same cities on April 25-27, and subsequent rounds are planned to take
98 place every two weeks in order to monitor how the pandemic is evolving.

99

100

101 **METHODS**

102

103 The state of Rio Grande do Sul is divided by the National Institute of Geography and
104 Statistics in eight intermediary regions (Figure 1). The main city in each region was
105 selected for the study. In the main metropolitan region, we selected the State Capital,
106 Porto Alegre, and Canoas, the second largest city in the metropolitan area. Populations
107 ranged from 78,915 in Ijuí to 1,409,351 in Porto Alegre
108 (<https://cidades.ibge.gov.br/brasil/rs/panorama>).

109

110 **Figure 1. Location of the nine sentinel cities.**



111

112

113 We used multistage sampling to select 50 census tracts with probability proportionate
114 to size in each sentinel city, and 10 households at random in each tract based on
115 census listings updated in 2019. All household members were listed at the beginning of
116 the visit, and one individual was randomly selected through an app used for data
117 collection. The survey waves took place on April 10-12 and 25-27.

118

119 The Statewide sample of 4,500 individuals allows estimating a prevalence levels of 3%
120 and 10% with margins of error of 0.5 and 1.0 percent points, respectively.

121

122 In the first wave, interviewers had listings of 35 households in each tract. Any refusals
123 at household level led to selection of the next household in the list, and so on until 10
124 households were included. In the second wave, field workers went to the house visited
125 in the first wave, and then selected the tenth household to its right. In case of refusal,
126 the next household to the right side was selected. In the case of acceptance at the
127 household level but the index individual refused to provide a sample, a second
128 member was selected. If this person also refused, the field workers moved on to the
129 next household in the list.

130

131 Prevalence of antibodies was assessed with a rapid test using finger prick blood
132 samples - the WONDFO SARS-CoV-2 Antibody Test (Wondfo Biotech Co., Guangzhou,
133 China). This test detects immunoglobulins of both IgG and IgM isotypes specific to
134 SARS-CoV-2 antigens in a lateral flow assay. The capture reagent consists of an
135 unspecified viral antigen immobilized at a defined position on a nitrocellulose
136 membrane. Following the introduction of the sample, a solution containing labelled
137 detector anti-immunoglobulin monoclonal antibodies is added. If the test is valid, a
138 control line appears on the kit's window. If this line is not visible, the test is deemed
139 inconclusive, which is very uncommon. A positive result is triggered by binding of the
140 detector antibody to any serum immunoglobulins immobilized on the viral antigen,
141 and is visible as a second colored line. Two drops of blood from a pinprick are sufficient
142 to detect the presence of antibody.

143 Four independent validation studies are available for the rapid test. Its sensitivity and
144 specificity are 86.4% and 99.6% according to the manufacturer, using samples
145 collected from 361 confirmed cases and 235 negative
146 controls([https://en.wondfo.com.cn/product/wondfo-sars-cov-2-antibody-test-lateral-
147 flow-method-2/](https://en.wondfo.com.cn/product/wondfo-sars-cov-2-antibody-test-lateral-flow-method-2/)). The tests were purchased in bulk by the Brazilian government, being
148 earmarked for use in population surveys and surveillance programs. An initial
149 validation study was carried out by the National Institute for Quality Control in Health
150 (INCQS, Oswaldo Cruz Foundation, RJ, Brazil) using 18 qRT-PCR positive and 77
151 negative serum samples. The reported sensitivity was 100.0% (95% confidence interval
152 (CI) 81.5;100.0%), while specificity was 98.7% (95% CI 93.0;100.9%). Recently,
153 Whitman and colleagues⁹ evaluated 10 different lateral flow assays using as
154 specimens plasma or serum samples from symptomatic SARSCoV-2 RT-PCR-positive
155 individuals and 108 pre-COVID-19 negative controls. Sensitivity of the Wondfo test was
156 81.5% (95% CI 70.0-90.1%) among 65 patients with a positive RT-PCR 11 or more days
157 before the test, and specificity was 99.1% (95% CI 94.9;100.0%). Of the 10 tests
158 studied, the Wondfo test was one of the two lateral flow tests with the best
159 performance. Lastly, we carried out our own validation study, based on 83 volunteers
160 with a positive qRT-PCR result 10 days or more before the rapid test. This analysis
161 showed a sensitivity of 77.1% (95% CI 66.6;85.6%). We also analysed 100 sera samples
162 collected in 2012 from participants of the 1982 Pelotas (Brazil) Birth Cohort Study¹⁰

163 and found 98 negative results, yielding a specificity estimate of 98.0% (95% CI
164 93.0;99.8%). By pooling the results from the four separate validations studies,
165 weighted by sample sizes, sensitivity is estimated at 84.8% (95% CI 81.4%;87.8%) and
166 specificity at 99.0% (95% CI 97.8%;99.7%).

167

168 Participants answered short questionnaires including sociodemographic information
169 (sex, age, schooling and skin color), COVID-19-related symptoms, use of health
170 services, compliance with social distancing measures and use of masks. Field workers
171 used tablets or smartphones to record the full interviews, register all answers, and
172 photograph the test results. All positive or inconclusive tests were read by a second
173 observer, as well as 20% of the negative tests. If the index subject in a household had a
174 positive result, all other family members were invited to be tested.

175

176 Interviewers were tested and found to be negative for the virus, and were provided
177 with individual protection equipment that was discarded after visiting each home.
178 Ethical approval was obtained from the Brazilian's National Ethics Committee (process
179 number 30415520.2.0000.5313), with written informed consent from all participants.
180 Positive cases were reported to the statewide COVID-19 surveillance system. The study
181 protocol was published prior to the first wave of data collection.¹¹ Data will become
182 publicly available upon request from the corresponding author 30 days after publication.

183

184 In the analyses reported in the body of this article, we analyzed the surveys as if they
185 included simple random samples from the population, using the exact binomial
186 method for confidence intervals. We calculated absolute (in percent points) and
187 relative differences between the two survey waves regarding the prevalence of
188 infection. P-values were calculated using Cochran's Q heterogeneity test, implemented
189 as fixed-effects meta-regression, which also yielded confidence intervals for the
190 differences. More complex analyses with allowances for the sampling design,
191 population weights and corrections for the specificity and sensitivity of the rapid test,
192 are included in the web annex. All analyses were performed using R version 3.6.1
193 (<https://www.r-project.org/>). The "metafor" package was used to compare the
194 prevalence between surveys.

195

196 **RESULTS**

197

198 Out of the planned 4,500 interviews, it was possible to test 4,188 individuals in the first
199 round. The number of tests carried out included 500 in each of five cities (Pelotas,
200 Passo Fundo, Santa Cruz, Caxias), 396 in Porto Alegre, 332 in Canoas, 499 in
201 Urugaiana and 461 in Santa Maria. In the last three cities, the desired sample size was
202 not completed due to logistic difficulties resulting from the need to complete the
203 survey in a 3-day period. Refusals, requiring the selection of the next household in the
204 census tract listing, ranged from 5.4% in Ijuí to 26.9% in Santa Maria, with a median of
205 17.9% in the nine cities. In the second round, it was possible to obtain 500 interviews
206 in each of the nine cities.

207

208 Table 1 shows the characteristics of individuals who provided blood samples. Both
209 samples were similar in terms of sex, age, skin color and schooling distributions.
210 Although the nine sentinel cities are not representative of the state as a whole, the
211 comparison shows what the samples had higher proportions of women and of older
212 persons than the state as a whole. Young children were particularly underrepresented.
213 Up-to-date information on schooling is not available for the State.

214

215 Of the 4,188 individuals tested in the first round, 10 had inconclusive results and only
216 two (0.0477%; 95% confidence interval (CI) 0.0058%;0.1724%) tested positive, one
217 each in the cities of Pelotas and Urugaiana. In the second round, there were two
218 inconclusive results and six positive subjects (0.1333%; 95% CI 0.0489%;0.2900%). The
219 absolute prevalence difference between the second and first waves was equal 0.086
220 percent point (95% CI -0.400;0.211; P=0.181), and the ratio was equal to 2.793 (95% CI
221 0.564;13.831, P=0.208).

222

223 Given the small numbers of subjects who tested positive, we focus the presentation on
224 unadjusted results. The web annex provides results from more complex analyses, all of
225 which produced results that are highly comparable to those reported here.

226

227 Regarding social distancing measures, 20.6% of respondents reported leaving home on
228 a daily basis, 58.3% leaving home occasionally for essential activities, and 21.1%
229 staying at home all the time in the first phase, and 28.3%, 53.4% and 18.3%,
230 respectively, in the second phase.

231

232 The households of the eight positive cases in the two phases included other 20
233 residents. Of these, 19 were tested; the rapid test showed nine positive, eight
234 negative, and two inconclusive results. One positive case lived alone. Among the other
235 seven, four had at least another positive individual in their families.

236

237 **Table 1. Sociodemographic characteristics of the two samples in nine cities and of**
 238 **the State population.**
 239

	ROUND 1	ROUND 2	STATE POPULATION ^{12,13}
SEX	%	%	%
Male	41.7	40.5	48.7
Female	58.3	59.5	51.3
AGE			
0-9	3.6	2.6	12.3
10-19	5.4	5.1	12.6
20-29	12.2	11.4	15.1
30-39	15.3	16.9	15.1
40-49	15.5	14.6	13.3
50-59	17.9	17.9	12.9
60-69	16.4	17.9	10.2
70-79	9.4	10.2	5.7
80+	4.3	3.4	2.9
SKIN COLOR			
White	76.6	75.9	81.5
Brown	15.8	16.2	13.0
Black	6.5	6.6	5.2
Other	1.1	1.3	0.3
EDUCATION			
Primary or less	37.4	34.1	Not available
Secondary	29.8	31.8	
University or higher	32.8	34.1	

240

241

242

243 As an additional check on how the rapid test performed under field work conditions,
 244 we conducted two separate assessments. The first was during the validation study in
 245 Porto Alegre, where 83 RT-PCR positive individuals were tested in the field using the
 246 rapid test. As described in the Methods section, 64 of these had positive results with
 247 the rapid test. Second, a more limited assessment entailed asking the coordinators of
 248 field work in different cities whether they were aware of any RT-PCR positive
 249 individuals in their communities. Four persons were identified and tested, all of whom
 250 had positive results in the rapid test.

251

252

253 **DISCUSSION**

254

255 This is the first report on repeated population-based surveys for the detection of SARS-
256 CoV-2 antibodies. With two weeks interval we were able to perform antibody tests on
257 representative samples in nine sentinel cities in Rio Grande do Sul State in Southern
258 Brazil.

259

260 Based on reported death rates by April 30, 2020, Rio Grande do Sul is one of the six
261 states, out of 27, with the lowest mortality, of 4 per million, well below Rio de Janeiro
262 (46 per million) or Sao Paulo (49 per million). Amazonas state (92 per million) shows
263 the highest death rates. The national mortality rate is estimated at 26 per million.

264 (<https://covid.saude.gov.br/>)

265

266 Taking our present results at face value, there would be 477 cases per million
267 inhabitants (95% confidence interval 58-1,719 cases) in the first wave, compared to 62
268 reported cases per million, as of April 14. According to the results of the second wave
269 (April 25-27), there would be 1,333 cases per million inhabitants (95% CI 489;2900)
270 compared to 128 reported cases per million as of April 30. Additional estimates, taking
271 into account corrections for the sample design, population weighting and adjustment
272 for sensitivity and specificity, are provided in the web annex.

273

274 Important concerns have been issued about rapid serological tests, but these mostly
275 refer to their use in making clinical decisions,¹⁴ and on issuing “immunity passports”¹⁵
276 for individuals who are assumed to have developed immunity. Both of these
277 circumstances refer to individual-level diagnoses based on rapid tests. Use of rapid
278 tests for population-based estimates, and particularly for monitoring trends over time,
279 is a different issue for which rapid tests with less than perfect sensitivity and specificity
280 may be acceptable. The Wondfo lateral flow test used in our analyses underwent four
281 different validation studies, being able to correctly identify 5 out of every 6 RT-PCR
282 confirmed cases, and 99 out of 100 individuals without SARS-CoV-2 antibodies. Among
283 10 lateral flow tests recently assessed by Whitman and colleagues⁹, it was among the
284 two with the best performance. Our finding of positive results for 10 of 13 family
285 members of the six index individuals who tested positive confirms that the
286 performance of the rapid test was adequate under field conditions.

287

288 The limitations of our analyses include the restriction of the sample to sentinel cities
289 that jointly account for 31% of the state’s population, while smaller towns and rural
290 areas were not included. Second, antibody tests result in many false negatives for
291 recent infections, particularly within the first two weeks since contagion, and thus
292 prevalence reflects levels of infection a week or two prior to the survey, about 15 days
293 after the first case was reported in the state. The non-response rate at household

294 level, estimated at 17.9%, was low compared to other population-based studies,^{3,4} or
295 to studies based on volunteers. Our samples had fewer children than expected, which
296 was probably due to their reluctance to undergo a finger prick when randomly selected
297 within the household; in these cases, a second person was randomly selected and if
298 that person also refused the household was replaced.

299

300 Lastly, our results were at the lower range of the 95% confidence interval for the false
301 positive rate, which in the pooled estimate from four validation studies was estimated
302 at 1.0% (95% CI 0.3%;2.2%). In these studies, specificity was measured in frozen
303 samples. Whitman and colleagues, in their analyses of 10 lateral flow tests, observed
304 *“moderate-to-strong positive bands in several pre-COVID-19 blood donor specimens,*
305 *some of them positive by multiple assays, suggesting the possibility of non-specific*
306 *binding of plasma proteins, non-specific antibodies, or cross-reactivity with other*
307 *viruses.”*⁹ Our results on family clustering show that - out of the seven index cases who
308 lived alone - four had family members who also tested positive. These four individuals
309 are most likely true positives, thus suggesting that up to four of the remaining index
310 cases with positive results, out of 8,689 individuals, might represent false positive
311 results. The test’s specificity would then be equal to 99.95% (95% CI 99.88;99.99%).

312

313 Our finding of low prevalence is consistent with an early phase of the pandemic,
314 coupled with high compliance with social distancing measures, as confirmed by our
315 own results. Such a low prevalence level is compatible with other population-based
316 studies: 0.6% in Iceland³ and 0.3% in Austria,⁴ which is close to Northern Italy which
317 was strongly hit by the pandemic. One should note that in both studies about 2/3 of
318 those invited failed to participate, compared to our own non-response rate of 17.9%.
319 Our results are not comparable with those based on self-selected volunteers.

320

321 The surveys are being partly funded by the state and national governments of Brazil.
322 Survey results were disseminated, two days after the completion of data collection
323 round, in press briefings with the presence of the state governor, who is making use of
324 the information to guide stay-at-home and other policies. Results from the next rounds
325 of our study – planned for May 8-10 and 22-23 - will allow us to follow the dynamics of
326 the pandemic in the state, especially when social restriction measures are starting to
327 be relaxed in most municipalities.

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