Time interval between onset of symptoms and COVID-19 testing in Brazilian state capitals, August 2020*

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Abstract

Objective: To analyze notifications of flu-like syndrome according to the time interval between onset of symptoms and testing for COVID-19. Methods: This was a cross-sectional study using records of flu-like syndrome cases containing results of COVID-19 diagnostic tests in the Brazilian state capitals and Federal District, held on the e-SUS Notifica system, from March 1st, 2020 to August 18th, 2020. The time interval between symptom onset and testing was compared using the ANOVA test, classifying it according to test adequacy/timeliness. Results: Taking 1,942,514 notifications, average time between symptom onset and testing was 10.2 days (±17.1). Among those tested, females (55.1%), people aged 20-39 years (43.8%), and the Southeast region of Brazil (43.0%) predominated. 58.8% of IgM ELISA tests were performed at an adequate time while 68.0% of rapid antigen tests were not performed at an adequate time. Conclusion: Inadequacy was found between symptom onset and time taken to test for COVID-19 in the Brazilian regions.

Keywords: Coronavirus Infections; Laboratory Test; Serologic Tests; Public Health Surveillance; Cross-Sectional Studies.

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Introduction

COVID-19, a respiratory disease caused by the novel coronavirus, SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2), was declared to be a pandemic by the World Health Organization (WHO) at the beginning of March 2020.1

Test indication, use and interpretation need to be done adequately in order to ensure greater result reliability and avoid wastage and increased costs of the procedure.5 Notwithstanding, there is divergence in the literature as to the best time for diagnostic testing for COVID-19 after symptom onset.

In view of these considerations, the findings of this study can provide information for performing testing in health services within the recommended time limits, thus favoring more reliable results, in addition to enhancing health surveillance actions for COVID-19 detection and control, thereby reducing the number of new cases of the disease and associated costs.

The objective of this study was to analyze flu-like syndrome notifications according to the interval of time elapsing between onset of symptoms and testing for COVID-19.

Methods

This was a cross-sectional study based on records of tests used to diagnose COVID-19 in all the Brazilian state capitals and Federal District.

In March 2020, when COVID-19 began to spread in Brazil, the country began logistic operations to provide supplies and qualified personnel in order to perform diagnostic tests of the disease and, based on the mapping of the results, implement response actions.6 In that context, there was diversity among the laboratory network with regard to timing and type of test used, depending on their availability in the Brazilian states, both in Public Health services and also in Supplementary Health services.

According to the diagnostic test panel available on the Health Ministry website, 15,150,356 tests have been performed in Brazil, of which 7,132,276 (47.1%) were RT-PCR and 8,018,080 (52.9%) were rapid tests, resulting in public expenditure of BRL 384,331,533.7

It should be noted that it is obligatory to notify the Ministry of Health of all results of all diagnostic tests to detect COVID-19, whether they be performed by public or private laboratories, university laboratories or any other laboratories in the national territory. Notification is compulsory for the results of all diagnostic tests performed, whether they are positive, negative, inconclusive or correlated, regardless of the method used, and notification should be done within 24 hours with effect from the result being ready, by recording and transmitting this information via the National Health Data Network. Notification is the responsibility of health service managers and those in charge of the
respective laboratories and is supervised by local health service management. Failure to notify is a breach of health regulations.  

This study included notifications of suspected COVID-19 cases in all the Brazilian state capitals and Federal District. Information on the variables listed below was retrieved from these records:

a) Type of COVID-19 test
- Reverse transcription polymerase chain reaction (RT-PCR); rapid test for detecting antibodies (antibody rapid test); rapid test for detecting antigens (antigen rapid test); enzyme immunoassay for detecting IgM antibodies (enzyme immunoassay – IgM ELISA); and electrochemiluminescence immunoassay for detecting IgG antibodies (Electrochemiluminescence immunoassay – IgG ECLIA).

b) Date of symptom onset (day, month and year).

c) Date COVID-19 test performed (day, month and year).

d) Federative Unit of residence, used to classify cases participating in the study by region of residence.

e) Age (in completed years: 0-9; 10-19; 20-39; 40-59; 60 or over).

f) Sex (female; male; undefined).

The data were retrieved from the Health Ministry website on Flu-like Symptom Notifications (https://opendatasus.saude.gov.br/dataset/casos-nacionais), the purpose of which is to provide the flu-like syndrome epidemiological database by incorporating the e-SUS Notifica system, which has been in force since March 2020. The data we used were retrieved on August 18th 2020. All records from March 1st to August 18th 2020 were included in the study with no restrictions.

The spreadsheets for each region of Brazil were compiled and stored on a .dta format database. This database was later transferred to the Stata version 15 computer program to clean the data and prepare them for analysis. Missing data resulting from lack of information or failure to fill in fields at the time of notification were not taken into consideration when analyzing the variable corresponding to them, i.e. the measurements of position and dispersion and statistical significance only take into consideration the data available for each variable of interest.

The time elapsed between the date of symptom onset and the date the COVID-19 test was performed was calculated in days, according to the difference between the two dates; later the averages and standard deviations for these data were obtained for the ‘sex’, ‘age range’ and ‘region of the country’ variable categories. A one-factor ANOVA test (F test) was used to investigate the presence of statistically significant average differences between three or more groups.

The time interval between onset of symptoms and the test being performed was classified as adequate or inadequate. An adequate interval was considered to be: 3-7 days for RT-PCR; ≥8 days for the antibody rapid test; 2-7 days for the antigen rapid test; ≥8 days for enzyme immunoassay (IgM ELISA); and ≥8 days for electrochemiluminescence immunoassay (IgG ECLIA).

We calculated the adequacy and inadequacy frequencies for each type of COVID-19 test included in the study. Following this, we calculated the average and standard deviation for the intervals of time between symptom onset and the tests being performed according to the adequacy classification. In addition, subgroup analysis was performed for the time elapsed between symptom onset and the COVID-19 tests being performed, taking a maximum interval of 30 days, using a one-factor ANOVA test (F test). The 30-day period was adopted because the majority of COVID-19 tests were found in this interval.

Distribution of COVID-19 testing by region was investigated by calculating adequacy and inadequacy frequencies and analyzed using Pearson’s chi-square test. A 5% significance level was used in all the analyses. The results were organized and presented in tables and graphs.

### Results

In the period from March 1st to August 18th 2020, 2,420,904 suspected COVID-19 cases were notified on the e-SUS Notifica system for all the state capitals and Federal District. Of these notifications, 1,942,514 (80.2%) had information on the type of COVID-19 test performed, and 1,798,327 (92.6%) of these notifications had a test performance date and a symptom onset date, thus enabling the time interval between the two dates to be calculated.

The time elapsed between onset of symptoms and COVID-19 testing was 10.2 days on average, with ±17.1 standard deviation (SD). Tests were performed predominantly among females (55.1%), people in
Time elapsed before testing for COVID-19

Table 1 – Distribution of COVID-19 diagnostic tests performed in the Brazilian state capitals and Federal District, among flu-like syndrome notifications (N=2,420,904), by sex, age range and region of the country, Brazil, March 1st – August 18th 2020

<table>
<thead>
<tr>
<th>Variables</th>
<th>n</th>
<th>%</th>
<th>Days between onset of symptoms and testing*</th>
<th>Average ± SD</th>
<th>p-value^c</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex (n=2,420,255)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>1,065,598</td>
<td>44.0</td>
<td>9.8±16.6</td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Female</td>
<td>1,332,150</td>
<td>55.1</td>
<td>10.5±17.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Undefined</td>
<td>22,507</td>
<td>0.9</td>
<td>19.5±28.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Age range (years) (n=2,420,904)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-9</td>
<td>72,772</td>
<td>3.0</td>
<td>8.3±14.3</td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>10-19</td>
<td>105,158</td>
<td>4.3</td>
<td>8.5±14.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20-39</td>
<td>1,060,340</td>
<td>43.8</td>
<td>9.6±16.2</td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>40-59</td>
<td>892,130</td>
<td>36.9</td>
<td>11.0±18.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥60</td>
<td>290,504</td>
<td>12.0</td>
<td>11.0±18.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Region (n=2,420,904)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>North</td>
<td>329,900</td>
<td>13.6</td>
<td>14.5±20.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Northeast</td>
<td>692,457</td>
<td>28.6</td>
<td>10.7±17.9</td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Midwest</td>
<td>293,099</td>
<td>12.1</td>
<td>6.0±9.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>South</td>
<td>65,007</td>
<td>2.7</td>
<td>4.9±11.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Southeast</td>
<td>1,040,441</td>
<td>43.0</td>
<td>10.1±17.1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a) Records were included which had information on the time elapsed between onset of symptoms and testing.
b) SD: standard deviation.
c) One-factor ANOVA – F test.

In Table 2 it can be seen that enzyme immunoassay – IgM ELISA – was the test most performed within an adequate time interval (58.8%), when compared to the other tests. On the other hand, 68.0% of antigen rapid tests were not performed within an adequate time interval, representing the highest frequency of

Table 2 – Adequacy of COVID-19 diagnostic testing in relation to time between onset of symptoms and testing, among flu-like syndrome notifications (N=1,798,327), in the Brazilian state capitals and Federal District, Brazil, March 1st – August 18th 2020

<table>
<thead>
<tr>
<th>Type of test</th>
<th>Adequate time interval^a</th>
<th>Inadequate time interval</th>
<th>Total n</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>Average ± SD</td>
</tr>
<tr>
<td>RT-PCR</td>
<td>438,552</td>
<td>52.1</td>
<td>4.6±1.37</td>
</tr>
<tr>
<td>Antibody RT</td>
<td>442,290</td>
<td>53.4</td>
<td>25.6±25.6</td>
</tr>
<tr>
<td>Antigen RT</td>
<td>30,795</td>
<td>32.0</td>
<td>4.7±1.74</td>
</tr>
<tr>
<td>IgM ELISA</td>
<td>9,770</td>
<td>58.8</td>
<td>20.2±20.02</td>
</tr>
<tr>
<td>IgG ECLIA</td>
<td>8,048</td>
<td>48.3</td>
<td>35.7±31.32</td>
</tr>
</tbody>
</table>

a) Adequate time interval for each test: 3 - 7 days for reverse transcription polymerase chain reaction (RT-PCR); ≥8 days for antibody rapid test; 2 - 7 days for antigen rapid test; ≥8 days for enzyme immunoassay (IgM ELISA); and ≥8 days for electrochemiluminescence immunoassay (IgG ECLIA).
b) SD: standard deviation.
non-conformity with recommended testing periods out of all the types of tests performed.

Figure 1 shows the frequency distribution of tests performed within the 30-day interval between onset of symptoms and testing. The majority of RT-PCRs, antibody rapid tests and enzyme immunoassays – IgM ELISA – can be seen to have been performed within an adequate time interval, although a considerable part of the tests were performed later than recommended. The majority of antigen rapid tests and electrochemiluminescence immunoassays – IgG ECLIA – were not performed within an adequate time interval.
Table 3 – Adequacy of COVID-19 diagnostic testing in relation to time between onset of symptoms and testing, among flu-like syndrome notifications, by region of the country, Brazil, 2020

<table>
<thead>
<tr>
<th>Tests</th>
<th>North</th>
<th>Northeast</th>
<th>Midwest</th>
<th>South</th>
<th>Southeast</th>
<th>p-value</th>
<th>a</th>
<th>b</th>
</tr>
</thead>
<tbody>
<tr>
<td>RT-PCR</td>
<td>M</td>
<td>6.8</td>
<td>5.5</td>
<td>4.7</td>
<td>3.3</td>
<td>8.3</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>56.3</td>
<td>58.4</td>
<td>54.4</td>
<td>33.9</td>
<td>49.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antibody RT</td>
<td>M</td>
<td>17.7</td>
<td>14.8</td>
<td>7.2</td>
<td>6.5</td>
<td>18.0</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>65.9</td>
<td>55.9</td>
<td>33.9</td>
<td>23.5</td>
<td>51.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antigen RT</td>
<td>M</td>
<td>13.9</td>
<td>8.1</td>
<td>6.7</td>
<td>5.9</td>
<td>9.4</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>16.7</td>
<td>41.0</td>
<td>36.2</td>
<td>19.0</td>
<td>34.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ELISA</td>
<td>M</td>
<td>14.4</td>
<td>8.8</td>
<td>9.8</td>
<td>2.7</td>
<td>27.3</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>44.7</td>
<td>55.2</td>
<td>56.2</td>
<td>9.4</td>
<td>75.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ECLIA</td>
<td>M</td>
<td>22.7</td>
<td>16.0</td>
<td>4.5</td>
<td>4.2</td>
<td>32.4</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>53.3</td>
<td>47.4</td>
<td>20.4</td>
<td>19.9</td>
<td>71.8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Legend:
RT-PCR: reverse transcription polymerase chain reaction.
Antibody RT: rapid test to detect antibodies.
Antigen RT: rapid test to detect antigens.
ELISA: enzyme immunoassay – IgM ELISA.
ECLIA: electrochemiluminescence immunoassay – IgG ECLIA.
a) Average in days.
b) Percentage of tests performed in an adequate time interval. Adequate time interval for each test: 3 - 7 days for the reverse transcription polymerase chain reaction test (RT-PCR); ≥8 days for the antibody rapid test; 2 - 7 days for the antigen rapid test; ≥8 days for enzyme immunoassay (IgM ELISA); and ≥8 days for electrochemiluminescence immunoassay (IgG ECLIA).
c) P-value refers to association between proportion of adequate tests and Brazilian region, calculated using Pearson’s chi-square test.

Table 3 shows the average times between appearance of the first symptoms and testing for COVID-19, according to the Brazilian regions. There were statistically significant differences (p < 0.001) in the distributions of tests being performed within an adequate time interval between the country’s five regions for all five types of tests recorded on e-SUS Notifica. The average time interval for RT-PCR tests varied between 3.3 and 8.3 days, and the majority of these tests were performed within an adequate time limit, achieving over 50% in the North, Northeast and Midwest regions. With regard to chromatographic immunoassay tests, the average time interval for the antibody rapid test was 6.5 to 18 days, with the Northern region having the highest proportion of this type of test performed in an adequate time interval (65.9%); in turn the average time interval for the antigen rapid test was 5.9 to 13.9 days, with the Northeast having the greatest distribution of adequate time intervals (41.0%). With regard to immunological tests (serology), the most adequate time interval was found in the Southeast region, for both IgM ELISA (75.1%) and for IgG ECLIA (71.8%); the average number of days elapsed between first COVID-19 symptoms and performance of immunobiological tests in all regions of the country varied between 2.7 and 27.3 days for IgM ELISA, and between 4.2 and 32.4 days for IgG ECLIA. It is noteworthy that out of all the tests analyzed, the highest proportion of inadequate time intervals was found in the Southern region (over 65.0%).

Discussion

This study identified that average time between onset of symptoms and testing for COVID-19 in the Brazilian state capitals and Federal District was 10.2 days. The IgM ELISA test was performed within the recommended time for more than half of the cases. On the other hand, the time interval for the antigen rapid was inadequate in the majority of cases, which is possibly prejudicial to its accuracy.

The SARS-CoV-2 tests were performed predominantly on samples from females, young adults and people living in the Southeast region. Two studies of cases notified in China, one covering the period December 11th 2019 to January 29th 2020, and the other from December 8th 2019 to February 10th 2020, had similar results which found that the majority of confirmed COVID-19 cases were comprised of younger people.

Other studies conducted in February 2020 in the large Chinese cities of Wuhan and Shanghai found fewer notifications among children, who tested less, perhaps because schools were closed early and there having been school holidays during this period.11-15

According to the e-SUS Notifica records analyzed in this study, RT-PCR and the antibody rapid tests were
the tests most performed in Brazil. This fact may have arisen from the Health Ministry’s efforts to comply with the WHO recommendation to increase the number of COVID-19 diagnosis tests. However, only around 50% of each of these tests was performed within an adequate time interval.

According to a study which assessed viral load courses using RT-PCR with samples from mouth and nasopharyngeal swab, sputum, feces, blood and urine samples from nine hospitalized cases discovered on January 27th 2020 in Munich, Germany, RT-PCR identifies the viral RNA of SARS-CoV-2 through nasopharyngeal secretion samples and should be performed between the third and seventh day of symptoms, in order to ensure greater precision of the method and to reduce false-negative results. This occurs because SARS-CoV-2 infection begins in the lungs and not in the upper respiratory tract.

A study involving 173 people with COVID-19 admitted to the Shenzen Hospital in China between January 11th and February 9th 2020, identified that after the seventh day RT-PCR positivity begins to fall, falling as much as 45% between the 15th and 39th day after symptom onset. In our study we found that RT-PCR tests were performed on approximately half the notified cases within the recommended time period of 5 to 7 days, in particular in the Northeast, North e Midwest regions.

The antigen rapid test can be used for diagnosis in the acute phase of the disease (2nd to 7th day following symptom onset) when molecular tests are not available; or when a molecular test result is negative, in the case of incorrect sample collection or collection outside the acute phase. In Belgium, between April 6th and 21st 2020, an assessment was made of antigen rapid test performance in relation to RT-PCR for 148 nasopharyngeal swabs samples, with 106 positive RT-PCR results – 32 of which were detected by the rapid antigen test, showing overall sensitivity of 30.2%. All the samples detected as being positive for COVID-19 by the antigen rapid test also had positive RT-PCR results.

Although the antigen rapid test has several advantages, such as being easy and quick to perform, rapid result, low cost and no requirement for special equipment or skills, when compared with molecular techniques it appears that its low sensitivity means that the rapid antigen test should not be used on its own as a frontline test for COVID-19 diagnosis, given that it can lead to false-negative results. In addition, all the Brazilian regions achieved less than 50% in performing the antigen rapid test in an adequate time interval.

With regard to the IgM ELISA, rapid antibody and IgG ECLIA serological tests, these should be used with effect from the eighth day after symptom onset. However, a study conducted in China assessed 208 plasma samples from 82 confirmed COVID-19 cases and 58 suspected COVID-19 cases during the initial stage of the epidemic in January 2020, and found that average IgM conversion time was 5 days, while for IgG it was 14 days after symptom onset. That study also showed that combining the IgM ELISA and the RT-PCR test significantly increased COVID-19 detection (98.6%), compared to just testing with RT-PCR (51.8%). IgM ELISA and IgG ECLIA were performed within an adequate time interval in more than 70% of the Southeast region capital cities.

Furthermore, some studies indicate the need to conduct more research into serological test sensitivity after a period of 33 and 35 days following the onset of symptoms, based on data produced by the Minas Gerais Health department for the period from March 4th to June 22nd 2020. In our study, however, we opted to analyze time with effect from the eighth day as per Ministry of Health recommendations. Antibody rapid tests have become an option for the general population, for screening asymptomatic cases or people with mild to moderate symptoms who do not need to be hospitalized, in order to observe the immunity/recovery of confirmed cases. Antibody rapid tests are performed on venous or capillary blood or blood plasma samples and allow rapid, straightforward and highly sensitive diagnosis, these being important advantages to consider in view of the fact of COVID-19 being a new disease with symptoms very similar to those of influenza, as well as available quantities of RT-PCR tests being insufficient, given that they were made available at the beginning of the pandemic in particular for health workers and seriously ill cases. This may be why a larger amount of antibody rapid tests have been performed in Brazil.

A variety of different forms of rapid tests have been validated for use in Brazil: some only enable antibody detection with a positive or negative result, without specifying type, while others distinguish between IgM and IgG antibodies. It should be noted that serological tests have variable sensitivity and specificity, depending on the manufacturer. In general, the sensitivity of tests...
approved for use in Brazil is over 85%, while specificity is over 94%. Diagnostic tests with low sensitivity can result in greater probability of detecting false-negatives, thus interfering in the social isolation measures adopted, especially in the case of asymptomatic individuals, thus having a direct influence on virus transmissibility. 24

Diagnostic test safety and quality can be threatened by incorrect identification of people and/or samples collected, inadequate or insufficient sample collection, uncertain transportation and sample storage conditions (e.g.: prolonged transportation time and exposure to wounds), presence of interfering substances (e.g.: cell components, due to whole blood freezing and inadequate additives) and problems arising from sample preparation procedures, such as pipetting mistakes during manual preparation of the sample or aliquot, cross contamination or sample incompatibility. This can cause unnecessary investigation and, consequently, increase the financial burden on the health system and result in inadequate and slow health care, according to reviews conducted in Qatar and South Africa.25,26

The limitations of this study relate to recording of dates, in particular uncertainty as to symptom onset, and the high number of incomplete records on the e-SUS Notifica system, resulting in losses in the sample studied. However, we believe that these losses did not interfere in the results found.

Identification of time taken to perform tests can favor the drawing up of emergency plans, Public Health policies and training for health workers to efficiently carry out prevention actions to contain the COVID-19 pandemic. We suggest that future studies analyze the period elapsed between symptom onset and COVID-19 testing using a larger time interval.

Authors’ contributions

Albuquerque NLS, Florencio SSG, Fontenele MGM, Queiroz APO and Lima GA contributed equally in all stages of the study to data analysis and interpretation and drafting the contents of the manuscript. Figueiredo LM and Amorim SMC contributed to data analysis and reviewing the manuscript. Barbosa LP and Lima FET took part in the study concept, data analysis and guidance in all stages of the study, as well as reviewing the manuscript. All the authors have reviewed and approved the final version of the article.

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