TECHNICAL-SCIENTIFIC REPORT

RESULTS - RESEARCH PROJECT

Accuracy of the Rapid Rapid Antigen Test Celer Hightop Covid-19: comparison with RT-PCR

Project approved by the Research Ethics Committee (CEP)
of the Federal University of Ouro Preto (UFOP)

Ouro Preto, MG, October 18, 2020

1 OBJECTIVES

1.1 General objective

Evaluate the accuracy of the Rapid Rapid Hightop Covid-19 antigen test by comparing it with the RT-PCR test for SARS-CoV-2.

1.2Specific objectives

- Perform the rapid antigen test "Celer Hightop Covid-19 Ag Test" in patients suspected of Covid-19.
- Perform, simultaneously, the RT-PCR test for SARS-CoV-2 in the same patients.
- Evaluate the sensitivity and specificity of rapid antigen testing compared to RT-PCR.
- Evaluate the positive and negative predictive value, and the accuracy of the rapid antigen test compared to RT-PCR.

2 METHODOLOGY:

2.1 Design and area of study

A cross-sectional study was conducted with patients from the Municipal Health Department of the cities of Nepomuceno (MG), Perdões (MG) and Campo Belo (MG) who presented suspected symptoms of Covid-19 on September 19 and 20, 2020.

Centrally trained health professionals simultaneously collected samples for rapid SARS-CoV-2 antigen testing and RT-PCR SARS-CoV-2 test in patients with suspected symptoms. Every study was conducted in physical spaces of the Secretariats

Municipal Health Authorities of the respective cities. Before any procedure, the research participant was informed of theobjectives of the project, as well as all the procedures that would be performed. After this information, in case of agreement, the research participant and/or his/her guardian signed the Free and Informed Consent Form (TCLE).

2.2 Study and sampling population

The research considered as study population the patients of the Municipal Health Departments of Nepomuceno (MG), Perdões (MG) and Campo Belo (MG).

2.3 Inclusion criteria

- Be an outpatient of the Municipal Health Department of one of the 3 cities mentioned above.
- Be a suspected case of active SARS-CoV-2 infection, i.e. having presenting, with a start date within the last 15 days, at least minus two (2) of the following symptoms: chills;; conjunctivitis;; runny nose;; diarrhea; difficulty breathing (dyspnea);; headache;; pain of throat;; fever (above 37.8°C);; hemoptysis;; myalgia/arthralgia;; Nausea or vomiting;; loss of smell;; loss of taste;; Cough.
- Be contact of a case identified as POSITIVE RT-PCR in this study, even if asymptomatic. Contact has been defined as being at less than 2.0 meters of an infected person, regardless of the weather duration of the contact, and this contactis 48 hours before the symptoms of the infected person.

2.4 Exclusion criteria

- Find yourself outside the inclusion criteria.
- Not to agree to participate in the research after explaining the objectives of the as well as all the procedures that will be carried out.

2.5 Sample collection

With the logistical support of the Health Departments, health professionals, trained centrally, performed nasopharine swab samples from patients to perform both virological tests (antigen test and RT-PCR) and venous blood collection (serum) was also performed for seroconversion analysis in those patients in whom there is disagreement between antigen test and RT-PCR.

2.6 Rapid antigen test

With a sample from nasoarcheal swab, a rapid immunochromatographic test of SARS-CoV-2 antigen was performed that identifies the presence in the sars-cov-2 protein sample (Celer Hightop Covid-19 Ag Test, Celer Biotechnology S/A, Brazil). Soon after collection, the patient's nasophayngeal swab was inserted into a mini-tube with extracting solution (which extracts the viral proteins inthe swab) and three drops of this extractor solution were placed on an immunochromatographic tape (rapid test cassette) and the final result was read after 15 minutes. The result was considered positive if in the testline a band appeared with intensidade 2, 3, 4 or 5;; the result was considered undetermined in case of intensity 1;; the result was negative if no band appeared on the test line (Figure 1).

Figure 1. Celer Hightop Covid- Antigen Test 19. Intensity bands 1 are undetermined. Intensity bands 2, 3, 4 or 5 are positive.

2.7 RT--PCR

Immediately after sample collection for rapid antigen testing, another nasophayngeal swab sample from the same patient was collected in viral transport (Celer Sansure Reagent for Sample Transport and Storage, Sansure Biotech Inc., China) within 24 hours to the Immunopathology Laboratory of the Biological Sciences Research Center (NUPEB) of UFOP. After receiving the sample, the Laboratory processed and released the result of the RT-PCR (Celer Sansure Pcr Detection Kit in Tempo Real forSARS-CoV-2, Sansure Biotech Inc., China) within 24 hours.

2.8 Serum collection for analysis of patients with discordant results between antigen test and RT-PCR

Just before the collection of nasovial swab samples, 3ml of venous blood were collected from each participant for serum extraction and storage of the same at -80°C in the biorepository of the Immunopathology Laboratory of the Biological Sciences Research Center (Nupeb) of

Ufop. Patients with discordant results between antigen test and RT-PCR were followed up, to re-collect serum for evaluation of seroconversion d andantibodies against SARS-CoV-2 after 25 days. For this analysis, 2 antibody kits were used: 1. Total antibodies (IgM+IgG) from manufacturer Wondfo (Guangzhou Wondfo Biotech Co. Ltd., China);; 2. IgM and IgG antibodies separated from hightop manufacturer (Qingdao Hightop Biotech Co. Ltd., China).

2.9 Data analysis

The main results of this study are sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy of the rapid antigen test "Celer Hightop Covid-19 Ag Test" compared to RT-PCR in patients with suspected Covid-19.

3 RESULTS:

A total of 126 patients were included in the study. Of these, 90 (71.4%) had symptoms on set in the last 15 days, and 36 (28.6%) they had nosymptoms in the last 15 days, but were contacts of symptomatic cases confirmed by RT-PCR in this study. The most common symptoms reported by patients in the last 15 days were: headache (70/90: 77.7%), cough (59/90: 65.5%), myalgia and/or arthralgia (49/90: 54.4%), diarrhea (45/90: 50.0%), color iza (38/90: 42.2%), sore throat (32/90: 35.5%), loss of smell and/or taste (30/90: 33.3%), fever (27/90: 30.0%), and dyspnea (26/90: 28.8%).

Of the 126 patients, 11 tested positive for SARS-CoV-2. Of these 11, 9 had a positive result in the antigen test and 2 had an indeterminate result. If we consider the indeterminate result

in the antigen test in the same category as "negative result", the sensitivity of the test is 81.81% and the specificity is 100% (Table 1).

Table 1. Contingency table 2 x 2 for evaluation of the diagnostic performance of the Covid-19 Celer Hightop rapid antigen test compared to RT-PCR

		RT--PCR		
		Positive	Negative	Total
Test Antigen	Positive	9	0	9
	Negative or Indeterminate	2	115	117
	Total	11	115	126

Sensitivity = 81.81% (9/11)
Specificity = 100.00% (115/115)
Positive predictive value = 100.00% (9/9)
Negative predictive value = 98.29% (115/117)
Accuracy= 98.41% (9+115/126)

Of the 126 patients, 9 had a positive result in the antigen test, 109 negative results, and 8 had an indeterminate result. Of these 8 patients with indeterminate results, 2 had positive RT-PCR, while 6 had negative RT-PCR. All 8 patients were followed up and, after 25 days, serum was collected for seroconversion evaluation for SARS-CoV-2 using 2 antibody kits (Wondfo and Hightop). Only the 2 patients who had also been POSITIVE RT-PCR serum converted into both antibody kits, and the outros 6 patients (who had NEGATIVE RT-PCR) did not serum convert into any of the 2 antibody kits.

If the 8 patients with indeterminate antigen results are removed from the 2x2 contingency table and only the remaining 118 patients are included inthe clinical performance evaluation, both the sensitivity and specificity of the antigen test would be 100% (Table 2).

Table 2. Contingency table 2 x 2 for evaluation of the diagnostic performance of rapid left of Covid-19 Celer Hightop antigen compared to RT-PCR, without considering the 8 patients with indeterminate result* in the antigen test

		RT--PCR		
		Positive	Negative	Total
Test Antigen	Positive	9	0	9
	Negative	0	109	109
	Total	9	109	118

Sensitivity = 100.00% (9/9)
Specificity = 100.00% (109/109)
Positive predictive value = 100.00% (9/9)
Negative predictive value = 100.00% (109/109)
Accuracy= 100.00% (9+109/118)
*Of the 8 patients with undetermined antigen test results, 2 were positive RT-PCR and seroconverted after 25 days. The remaining 6 were RT-PCR negative and non-seroconverted after 25 days.

4 DISCUSSION AND CONCLUSION:

In the sample evaluated in this study, the rapid antigen test "Celer Hightop Covid-19 Ag Test" demonstrated excellent sensitivity and specificity. It is noteworthy that the sample was composed of outpatients (outside the hospital environment) and mostly suspected symptomatic patients, that is,patients who were most likely infected by other viruses that can generate simil air symptoms toCovid-19, such as rhinovirus, adenovirus, other coronaviruses, influenza, parainfluenza, respiratory syticial virus, among others. That is, the test showed excellent performance in a sample that can benefit

availability of rapid antigen tests. Hospitalized patients usually have access to RT-PCR testing and, therefore, were not the focus of this study.

In relation to patients with indeterminate result in the antigen test, our data suggest that there is 2 possibility for diagnostic elucidation: an RT-PCR test may be performed to confirm the case, or the patient may be followed up to evaluate soroconversion for SARS-CoV-2.

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SARS-CoV-2 Antigen Rapid Test

Testing Report

1. Sample type

Nasopharyngeal swab

2. Clinical trial reagents

Name	Factory	Storage Conditions	Remarks
SARS-CoV-2 antigen rapid test (Immunochromatography)	Qingdao Hightop Biotech Co., Ltd	4～30℃	Assessment reagent
Novel coronavirus 2019- nCoV nucleic acid detection kit (fluorescence PCR method)	The Beijing Genomics Institute	-20℃	Nucleic acid contrast reagent

3.Basis for interpretation of clinical trial results【Technical Guidelines for Laboratory Testing of Pneumonia Infected by Novel Coronavirus(Sixth Edition)】

Remarks: To be consistent with the sample processing of nucleic acid contrast reagents, the samples tested by the assessment reagents are actually tested after being diluted 1 times.

500 μL of sample extract was added according to the instructions, actually 1000 μL of sample extract was added.

Result judgment

Negative: without Ct value or Ct value ≥ 40.

Positive: If the Ct value < 37, it can be reported as positive.

Gray area: If the Ct value is between 37-40, it is recommended to repeat the experiment. If the Ct value < 40 and the amplification curve has obvious peaks, the sample is judged as positive, otherwise it is negative.

Note: If a commercial kit is used, the results should be judged according to the instructions provided by the manufacturer.

4.Clinical trial results(excluding nucleic acid gray area CT value 37~39)

SARS-CoV-2 Antigen Rapid Test	Comparator Method		Total
	Positive	Negative	
Positive	30	1	31
Negative	3	101	104
Total	33	102	135

Sensitivity＝30/(30+3)×100%=90.91%，95%CI (76.43，96.86)
Specificity＝101/(101+1)×100%=99.02%，95%CI (94.66，99.83)
Total consistent=(30+101)/(30+3+1+101)×100%=97.04%，95%CI (92.63，98.84)

Note: There are 44 cases with CT value 37~39 in the gray area of nucleic acid. The assessment reagents were positive in 16 cases and negative in 28 cases.

5.Detailed data：

Negative and positive areas	Nucleic acid CT value	Number of cases	Test results of HIGHTOP	Remarks
Nucleic acid positive	19	2	All positive	Coincide with nucleic acid results
	20	2	All positive	Coincide with nucleic acid results
	21	0	/	/
	22	2	All positive	Coincide with nucleic acid results
	23	1	positive	Coincide with nucleic acid results
	24	1	positive	Coincide with nucleic acid results
	25	2	All positive	Coincide with nucleic acid results
	26	1	positive	Coincide with nucleic acid results
	27	4	All positive	Coincide with nucleic acid results
	28	2	All positive	Coincide with nucleic acid results
	29	3	All positive	Coincide with nucleic acid results
	30	2	All positive	Coincide with nucleic acid results
	31	3	All positive	Coincide with nucleic acid results
	32	2	All positive	Coincide with nucleic acid results
	33	1	positive	Coincide with nucleic acid results
	34	1	positive	Coincide with nucleic acid results

	35	2	1 positive cases, 1 negative cases	*1
	36	2	2 negative cases	*1
Nucleic acid grayscale	37	18	8 positive cases, 10 negative cases	Uncertain nucleic acid test results
	38	16	6 positive cases, 10 negative cases	Uncertain nucleic acid test results
	39	10	2 positive case, 8 negative cases	Uncertain nucleic acid test results
Nucleic acid negative	40	37	1 positive cases, 36 negative cases	*2
	41	15	All negative	Coincide with nucleic acid results
	42	8	All negative	Coincide with nucleic acid results
	43	10	All negative	Coincide with nucleic acid results
	44	15	All negative	Coincide with nucleic acid results
	45	6	All negative	Coincide with nucleic acid results
	46	6	All negative	Coincide with nucleic acid results
	47	5	All negative	Coincide with nucleic acid results

*1 Because the sample was diluted, the coincidence rate may be higher if you follow the instructions.

*2There was a case of nucleic acid negative in another hospital, but HIGHTOP antigen test was positive. Subsequent results of IgG and IgM verification were positive. So, false negative nucleic acid cannot be ruled out.

Statistical Table of Clinical Trial Date

Reagent name: SARS-CoV-2 Antigen Rapid Test

Manufacturer: Qingdao Hightop Biotech Co., Ltd.

Specification: 40 T/ box

Batch number: 200901

No.	Ct Value	PCR confirmation Result	No.	Ct Value	PCR confirmation Result
1	>40.00	-	34	>40.00	-
2	>40.00	-	35	28.09	+
3	28.63	+	36	>40.00	-
4	>40.00	-	37	>40.00	-
5	>40.00	-	38	>40.00	-
6	>40.00	-	39	>40.00	-
7	33.12	+	40	28.67	+
8	>40.00	-	41	>40.00	-
9	29.89	+	42	>40.00	-
10	>40.00	-	43	>40.00	-
11	>40.00	-	44	29.60	+
12	>40.00	-	45	23.76	+
13	29.37	+	46	>40.00	-
14	>40.00	-	47	>40.00	-
15	>40.00	-	48	>40.00	-
16	29.71	+	49	>40.00	-
17	>40.00	-	50	>40.00	-
18	28.24	+	51	29.89	+
19	>40.00	-	52	>40.00	-
20	>40.00	-	53	>40.00	-
21	28.79	+	54	>40.00	-
22	29.17	+	55	>40.00	-
23	>40.00	-	56	29.88	+
24	>40.00	-	57	>40.00	-
25	27.03	+	58	31.26	+
26	>40.00	-	59	>40.00	-
27	>40.00	-	60	>40.00	-
28	28.23	+	61	>40.00	-
29	>40.00	-	62	>40.00	-
30	33.02	+	63	29.12	+
31	>40.00	-	64	>40.00	-
32	>40.00	-	65	29.33	+
33	>40.00	-	66	25.16	+

67	>40.00	-	111	>40.00	-
68	>40.00	-	112	27.39	+
69	>40.00	-	113	>40.00	-
70	28.11	+	114	>40.00	-
71	>40.00	-	115	>40.00	-
72	>40.00	-	116	>40.00	-
73	>40.00	-	117	>40.00	-
74	>40.00	-	118	>40.00	-
75	>40.00	-	119	>40.00	-
76	>40.00	-	120	>40.00	-
77	34.01	+	121	>40.00	-
78	>40.00	-	122	>40.00	-
79	>40.00	-	123	>40.00	-
80	30.07	+	124	>40.00	-
81	>40.00	-	125	>40.00	-
82	29.64	+	126	21.58	+
83	>40.00	-	127	>40.00	-
84	>40.00	-	128	>40.00	-
85	>40.00	-	129	>40.00	-
86	31.35	+	130	>40.00	-
87	>40.00	-	131	>40.00	-
88	>40.00	-	132	>40.00	-
89	>40.00	-	133	>40.00	-
90	>40.00	-	134	>40.00	-
91	>40.00	-	135	26.88	+
92	30.62	+			
93	>40.00	-			
94	>40.00	-			
95	>40.00	-			
96	>40.00	-			
97	>40.00	-			
98	>40.00	-			
99	29.82	+			
100	>40.00	-			
101	>40.00	-			
102	>40.00	-			
103	27.25	+			
104	>40.00	-			
105	>40.00	-			
106	>40.00	-			
107	28.73	+			
108	>40.00	-			
109	>40.00	-			
110	>40.00	-			

The name of the PCR device:Novel Coronavirus (2019-nCoV) Nucleic Acid Diagnostic Kit (PCR-Fluorescence Probing)

The manufacturer of the PCR device: Sansure BioTech Inc.

Result of (-): Ct value >40 or Undetermined

Result of (+): Ct value ≤ 40