Clinical Report of Wondfo 2019-nCoV Antigen Test (Lateral Flow Method)

I. Abstract

The clinical trial used 2019-nCoV Antigen Test (Colloidal Gold Method) manufactured by Guangzhou Wondfo Biotech Co., Ltd. as evaluation reagent together with marketed nucleic acid reagent as reference reagent for simultaneous detection of 2019-nCoV antigen and nucleic acid, through performing joint emergency clinical evaluation to objectively evaluate the clinical performance of different reagents.

Sponsor: Guangzhou Wondfo Biotech Co., Ltd

Product Name: Wondfo 2019-nCoV Antigen Test (Lateral Flow Method)

Lot number: W19600801

Evaluation Site: Laboratório Integral - Hospital Vera Cruz (Brazil)

Nucleic acid reagent：Celer Sansure Kit de Detecção por PCR em Tempo Real para SARS-CoV-2

Trial Date: December 2020

II. Result and Analysis

Totally 58 samples are enrolled for statistical analysis, including 13 COVID-19 confirmed cases and 45 excluded cases.

2.1 Completion Status

From the table below, 58 valid cases are enrolled for comparative test, which includes 13 COVID-19 confirmed cases and 45 excluded cases. The distribution of Ct value of target gene is shown as below:

Table 1. Number of valid samples actually enrolled and completed test in the comparative test

Clinical institution	Number of actually enrolled cases		Total
	COVID-19 confirmed cases (13)	COVID-19 excluded cases	

					(45)	
	Nucleic acid positive (25)				Nucleic acid negative (45)	
	0<Ct≤25	25<Ct≤30	30<Ct≤35	Ct>35	/	
Total	5	5	1	2	45	58

2.2 Overall Comparative Analysis of Test Result between Evaluation Reagent and Marketed Nucleic Acid Reagent

58 samples are enrolled in comparative test for statistical analysis. According to the test result of marketed nucleic acid reagent, group all enrolled cases into 2019-nCoV nucleic acid positive and 2019-nCoV nucleic acid negative, and they are compared with the test result of evaluation reagent for analysis.

Table 2. Comparative analysis of test result for evaluation reagent

Result of evaluation reagent	Result of marketed nucleic acid reagent		Total
	Positive	Negative	
Positive	12	0	12
Negative	1	45	46
Total	13	45	58

Positive percent agreement:	92.31%	95%CI:	66.69%~98.63%
Negative percent agreement:	100%	95%CI:	92.13%~100.00%
Overall percent agreement:	98.28%	95%CI:	90.86%~99.70%

From above result, there are 57 cases whose test result of evaluation reagent are consistent with nucleic acid test result, of which 12 nucleic acid positive cases are antigen positive, 45 nucleic acid negative cases are antigen negative. There is 1 case are of inconsistent result, of which 1 nucleic acid positive cases is antigen negative.

Through statistical analysis, compared with nucleic acid test result, for evaluation reagent, positive percent agreement is 92.31% (95%CI: 66.69%, 98.63%), negative

percent agreement is 100.00% (95%CI: 92.13%, 100.00%), overall percent agreement is 98.28% (95%CI: 90.86%, 99.70%), and the Kappa value is 0.950. Negative percent agreement and overall agreement rate of test results between evaluation reagent and nucleic acid reagent are good.

Table 3. Inconsistent test result between evaluation reagent and nucleic acid reagent

No.	Patient basic information						Test results								Remarks
								Wondfo RDT		PCR					
	Patient code	Age	Date of onset of symptoms	Duration of onset (day)	Confirmed/Excluded	Specimen type	Specimen collection date	Testing date	RDT results	Testing date	PCR results	Ct value			
												Ct FAM	Ct Rox	Internal Control	
33	5.1.2e	42	No Data	No Data	C	NP	2020/12/17	2020/12/17	Negative	2020/12/17	Positive	Undet	37.29	26.38	

Analysis for inconsistency: For 1 inconsistent sample, of which 1 nucleic acid positive cases is antigen negative. Based on the distribution of Ct value of inconsistent samples, considering that the viral load may affect the detection effect of the antigen, the following is a stratified analysis of the sample according to the Ct value of the target gene of nucleic acid detection.

2.3 Analysis of Comparative Test Result for High Viral Load

Samples are stratified and analyzed based on the Ct value of the target gene of the nucleic acid reagent. Nucleic acid positive samples are analyzed with Ct=25 and Ct=30 as the Cutoff values of high viral load.

2.3.1 Take Ct=25 as the Cutoff Value of High Viral Load for Analysis

(1) Analysis of Comparative Test Result for High Viral Load

Table 4. Analysis of comparative test result for evaluation reagent

Result of evaluation reagent	Result of marketed nucleic acid reagent		Total
	Positive		
Positive	5		10
Negative	0		0
Total	5		10
Positive percent agreement:	100.00%	95%CI:	56.55%～100.00%

When taking Ct=25 as the Cutoff value of high viral load for analysis, there are 10 high viral load samples whose Ct value are ≤25 for the target gene of the nucleic acid reagent, among them, 5 samples are of consistent result for nucleic acid and antigen, which are nucleic acid positive samples with positive antigen results.

Positive percent agreement of evaluation reagent for detecting high viral load samples is 100% (95CI: 56.55%, 100.00%).

(2) Analysis of Comparative Test Result for Low Viral Load

Table 5. Analysis of comparative test result for evaluation reagent

Result of evaluation reagent	Result of marketed nucleic acid		Total
	Positive		
Positive	7		7
Negative	1		1
Total	8		8
Positive percent agreement:	87.50%	95%CI:	52.91%-97.76%

From above result, when taking Ct=25 as the Cutoff value of high viral load for analysis, there are 8 low viral load samples whose Ct value is >25 for the target gene of the nucleic acid reagent. Among them, 7 samples are of consistent result for nucleic acid and antigen, which are nucleic acid positive samples with positive antigen results. There is 1 inconsistent sample, which are nucleic acid positive samples with negative antigen result.

Positive percent agreement of evaluation reagent for detecting low viral load samples is 87.50% (95%CI: 52.91%, 97.76%).

2.3.2 Take Ct=30 as the Cutoff Value of High Viral Load for Analysis

(1) Analysis of Comparative Test Result for High Viral Load

Table 6. Analysis of comparative test result for evaluation reagent

Result of valuation reagent	Result of marketed nucleic acid		Total
	Positive		
Positive	10		10
Negative	0		0
Total	10		10
Positive percent agreement:	100.00%	95%CI:	72.25%~100.00%

From above result, when taking Ct=30 as the Cutoff value of high viral load for analysis, there are 10 high viral load samples whose Ct value is ≤30 for target gene of nucleic acid reagent. Among them, 10 samples are of consistent result for nucleic acid and antigen, which are nucleic acid positive samples with positive antigen results. There is no inconsistent sample.

Positive percent agreement of evaluation reagent for detecting high viral load samples is 100.00% (95%CI; 72.25%, 100.00%).

(2) Analysis of Comparative Test Result for Low Viral Load

Table 7. Analysis of comparative test result for evaluation reagent

Result of evaluation reagent	Result of marketed nucleic acid		Total
	Positive		
Positive	2		2
Negative	1		1
Total	3		3
Positive percent agreement:	66.67%	95%CI:	20.77%~93.85%

When taking Ct=30 as the Cutoff value of high viral load for analysis, there are 3 low viral load samples whose Ct value is >30 for target gene of the nucleic acid reagent.

Among them, 2 samples are of consistent result for nucleic acid and antigen, which are nucleic acid positive samples with positive antigen results. There is 1 inconsistent sample, which are nucleic acid positive samples with negative antigen result.
Positive percent agreement of evaluation reagent for detecting high viral load samples is 66.67% (95%CI: 20.77%, 93.85%).

III. Discussion and Conclusion

Test result of 2019-nCoV Antigen Test (Colloidal Gold Method) is summarized as follows:

3.1 Analysis of Comparative Test Result

(1) Completion Status

The clinical trial was carried out at Clinical Sit: Laboratório Integral - Hospital Vera Cruz. This site enrolled 58 samples (all are uninactivated nasopharyngeal swabs), and totally 58 samples are enrolled for statistical analysis, which includes 13 COVID-19 confirmed cases and 45 excluded cases.

(2) Overall Comparative Analysis of Test Result between Evaluation Reagent and Marketed Nucleic Acid Reagent

Totally 58 valid samples are enrolled in the comparative test, and the test results of the evaluation reagent are compared with the result of marketed nucleic acid reagent. There are 78 samples where the 2019-nCoV antigen results detected by evaluation reagent are consistent with the nucleic acid test result, of which 24 nucleic acid positive cases are antigen positive, 54 nucleic acid negative cases are antigen negative. There is 1 inconsistent sample, of which 1 nucleic acid positive cases is antigen negative.

According to statistical analysis, compared with the nucleic acid test result, the positive percent agreement of the evaluation reagent is 92.31% (95%CI: 66.69%, 98.63%), negative percent agreement is 100.00% (95%CI: 92.13%, 100.00%), overall percent agreement is 98.28% (95%CI: 90.86%, 99.70%), and the Kappa value is 0.950.

There is 1 inconsistent case, of which 1 nucleic acid positive cases is antigen negative. According to the distribution of Ct value of inconsistent cases, considering that the viral load may affect the detection effect of the antigen, the samples are stratified and

analyzed according to the Ct value of target gene of the of nucleic acid reagent.

Negative percent agreement and overall agreement rate of test result between evaluation reagent and nucleic acid test reagent are good. Considered that the viral load might affect the detection result of antigen, samples are stratified and analyzed based on the Ct value of target gene of nucleic acid reagent.

(3) Analysis of Comparative Test Result for High Viral Load

In this clinical trial, the samples are stratified and analyzed based on the Ct value of the target gene of nucleic acid reagent. The samples with positive nucleic acid result are analyzed with Ct=25 and Ct=30 as the Cutoff values for high viral load.

① Take Ct=25 as the Cutoff Value of High Viral Load for Analysis

When taking Ct=25 as the Cutoff value of high viral load for analysis, there are 5 high viral load samples whose Ct value is ≤25 for the target gene of the nucleic acid reagent. Among them, 5 nucleic acid positive samples are antigen positive. The positive percent agreement of evaluation reagent for detecting high viral load samples is 100% (95CI: 56.55%, 100.00%).

When taking Ct=25 as the Cutoff value of high viral load for analysis, there are 8 low viral load samples whose Ct value is >25 for target gene of nucleic acid reagent. Among them, 7 nucleic acid positive samples are antigen positive. The positive percent agreement of evaluation reagent for detecting low viral load samples is 87.50% (95%CI: 52.91%, 97.76%).

② Take Ct=30 as the Cutoff Value of High Viral Load for Analysis

When taking Ct=30 as the Cutoff value of high viral load for analysis, there are 10 high viral load samples whose Ct value is ≤30 for target gene of the nucleic acid reagent. Among them, 10 nucleic acid positive samples are antigen positive. The positive percent agreement of evaluation reagent for detecting high viral load samples is 100.00% (95%CI; 72.25%, 100.00%).

When taking Ct=30 as the Cutoff value of high viral load for analysis, there are 3 low viral load samples whose Ct value is >30 for target gene of the nucleic acid reagent.

Among them, 2 nucleic acid positive samples are antigen positive. The positive percent agreement of evaluation reagent for detecting low viral load samples is 66.67% (95%CI: 20.77%, 93.85%).

3.2 Conclusion

In this clinical trial, the clinical performance data conforms to the current epidemic characteristics of the 2019-nCoV in my country. At the same time, taking Ct value =25 of nucleic acid reference reagent as the high-load Cutoff value, the antigen positive percent agreement of the evaluation reagent for the high viral load group with Ct≤25 is 100.00%. Antigen detection reagents is of certain clinical application value.

2019-nCoV Antigen Test (Colloidal Gold Method) produced by Guangzhou Wondfo Biotech Co., Ltd. is in line with the expected results of marketed nucleic acid reagent, which can meet the needs of clinical applications.

Annex I Clinical Data Sheet

Sponsor	**Guangzhou Wondfo Biotech Co., Ltd.**
Clinical Study Institution	**Laboratório Integral - Hospital Vera Cruz**
Responsible to Study	**Celer Biotecnologia SA - Kênia Magalhães CRBM3:5285**
Evaluation Product Name	**Wondfo 2019-nCoV Antigen Test (Lateral Flow Method)**

No.	Patient basic information						Test results								Remark
								Wondfo RDT		PCR					
	Patient code	Age	Date of onset of symptoms	Duration of onset (day)	Confirmed/ Excluded	Specimen type	Specimen collection date	Testing date	RDT results	Testing date	PCR results	Ct value			
												Ct FAM	Ct Rox	Internal Control	
1	5.1.2e	25	No Data	No Data	C	NP	2020/12/17	2020/12/17	Positive	2020/12/17	Positive	37.58	28.75	27.91	
2		32	No Data	No Data	C	NP	2020/12/17	2020/12/17	Positive	2020/12/17	Positive	36.13	27.40	27.18	
3		22	No Data	No Data	C	NP	2020/12/17	2020/12/17	Positive	2020/12/17	Positive	30.64	21.94	27.78	
4		37	No Data	No Data	C	NP	2020/12/17	2020/12/17	Positive	2020/12/17	Positive	35.58	27.47	28.58	
5		No Data	No Data	No Data	C	NP	2020/12/17	2020/12/17	Positive	2020/12/17	Positive	34.23	24.19	23.25	
6		No Data	No Data	No Data	C	NP	2020/12/17	2020/12/17	Positive	2020/12/17	Positive	35.77	25.62	24.55	
7		45	No Data	No Data	C	NP	2020/12/17	2020/12/17	Positive	2020/12/17	Positive	27.54	22.39	30.44	
8		No Data	No Data	No Data	C	NP	2020/12/17	2020/12/17	Positive	2020/12/17	Positive	Undet	35.78	25.29	
9		77	No Data	No Data	E	NP	2020/12/17	2020/12/17	Negative	2020/12/17	Negative	Undet	Undet	24.39	
10		No Data	No Data	No Data	C	NP	2020/12/17	2020/12/17	Positive	2020/12/17	Positive	Undet	31.02	26.74	
11		No Data	No Data	No Data	C	NP	2020/12/17	2020/12/17	Positive	2020/12/17	Positive	29.71	23.15	22.19	

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No.	Patient basic information						Test results								Remark
	Patient code	Age	Date of onset of symptoms	Duration of onset (day)	Confirmed/ Excluded	Specimen type	Specimen collection date	Wondfo RDT		PCR					
								Testing date	RDT results	Testing date	PCR results	Ct value			
												Ct FAM	Ct Rox	Internal Control	
12	5.1.2e	No Data	No Data	No Data	C	NP	2020/12/17	2020/12/17	Positive	2020/12/17	Positive	28.86	19.29	26.72	
13		25	No Data	No Data	E	NP	2020/12/17	2020/12/17	Negative	2020/12/17	Negative	Undet	Undet	24.34	
14		25	No Data	No Data	E	NP	2020/12/17	2020/12/17	Negative	2020/12/17	Negative	Undet	Undet	26.96	
15		25	No Data	No Data	E	NP	2020/12/17	2020/12/17	Negative	2020/12/17	Negative	Undet	Undet	28.31	
16		25	No Data	No Data	E	NP	2020/12/17	2020/12/17	Negative	2020/12/17	Negative	Undet	Undet	27.25	
17		32	No Data	No Data	E	NP	2020/12/17	2020/12/17	Negative	2020/12/17	Negative	Undet	Undet	21.10	
18		24	No Data	No Data	E	NP	2020/12/17	2020/12/17	Negative	2020/12/17	Negative	Undet	Undet	26.38	
19		30	No Data	No Data	E	NP	2020/12/17	2020/12/17	Negative	2020/12/17	Negative	Undet	Undet	25.57	
20		23	No Data	No Data	E	NP	2020/12/17	2020/12/17	Negative	2020/12/17	Negative	Undet	Undet	25.17	
21		25	No Data	No Data	E	NP	2020/12/17	2020/12/17	Negative	2020/12/17	Negative	Undet	Undet	20.85	
22		25	No Data	No Data	E	NP	2020/12/17	2020/12/17	Negative	2020/12/17	Negative	Undet	Undet	25.22	
23		36	No Data	No Data	E	NP	2020/12/17	2020/12/17	Negative	2020/12/17	Negative	Undet	Undet	31.02	
24		No Data	No Data	No Data	E	NP	2020/12/17	2020/12/17	Negative	2020/12/17	Negative	Undet	Undet	24.39	

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	Patient code	Age	Date of onset of symptoms	Duration of onset (day)	Confirmed/ Excluded	Specimen type	Specimen collection date	Wondfo RDT		PCR					
								Testing date	RDT results	Testing date	PCR results	Ct value			
												Ct FAM	Ct Rox	Internal Control	
25	5.1.2e	No Data	No Data	No Data	E	NP	2020/12/17	2020/12/17	Negative	2020/12/17	Negative	Undet	Undet	27.89	
26		41	No Data	No Data	E	NP	2020/12/17	2020/12/17	Negative	2020/12/17	Negative	Undet	Undet	33.12	
27		No Data	No Data	No Data	E	NP	2020/12/17	2020/12/17	Negative	2020/12/17	Negative	Undet	Undet	25.22	
28		No Data	No Data	No Data	E	NP	2020/12/17	2020/12/17	Negative	2020/12/17	Negative	Undet	Undet	28.92	
29		35	No Data	No Data	E	NP	2020/12/17	2020/12/17	Negative	2020/12/17	Negative	Undet	Undet	24.73	
30		48	No Data	No Data	E	NP	2020/12/17	2020/12/17	Negative	2020/12/17	Negative	Undet	Undet	28.07	
31		No Data	No Data	No Data	E	NP	2020/12/17	2020/12/17	Negative	2020/12/17	Negative	Undet	Undet	23.33	
32		No Data	No Data	No Data	E	NP	2020/12/17	2020/12/17	Negative	2020/12/17	Negative	Undet	Undet	28.90	
33		42	No Data	No Data	C	NP	2020/12/17	2020/12/17	Negative	2020/12/17	Positive	Undet	37.29	26.38	
34		No Data	No Data	No Data	E	NP	2020/12/17	2020/12/17	Negative	2020/12/17	Negative	Undet	33.72	27.50	
35		27	No Data	No Data	E	NP	2020/12/17	2020/12/17	Negative	2020/12/17	Negative	Undet	Undet	23.81	
36		29	No Data	No Data	E	NP	2020/12/17	2020/12/17	Negative	2020/12/17	Negative	Undet	Undet	24.25	

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	Patient code	Age	Date of onset of symptoms	Duration of onset (day)	Confirmed/ Excluded	Specimen type	Specimen collection date	Wondfo RDT		PCR					
								Testing date	RDT results	Testing date	PCR results	Ct value			
												Ct FAM	Ct Rox	Internal Control	
37	5.1.2e	No Data	No Data	No Data	E	NP	2020/12/17	2020/12/17	Negative	2020/12/17	Negative	Undet	Undet	28.34	
38		No Data	No Data	No Data	E	NP	2020/12/17	2020/12/17	Negative	2020/12/17	Negative	Undet	Undet	29.63	
39		32	No Data	No Data	E	NP	2020/12/17	2020/12/17	Negative	2020/12/17	Negative	Undet	Undet	22.39	
40		No Data	No Data	No Data	E	NP	2020/12/17	2020/12/17	Negative	2020/12/17	Negative	Undet	Undet	29.84	
41		No Data	No Data	No Data	E	NP	2020/12/17	2020/12/17	Negative	2020/12/17	Negative	Undet	Undet	26.85	
42		40	No Data	No Data	E	NP	2020/12/17	2020/12/17	Negative	2020/12/17	Negative	Undet	Undet	20.44	
43		40	No Data	No Data	E	NP	2020/12/17	2020/12/17	Negative	2020/12/17	Negative	Undet	Undet	29.11	
44		No Data	No Data	No Data	E	NP	2020/12/17	2020/12/17	Negative	2020/12/17	Negative	Undet	Undet	26.45	
45		49	No Data	No Data	E	NP	2020/12/17	2020/12/17	Negative	2020/12/17	Negative	Undet	Undet	25.61	
46		26	No Data	No Data	E	NP	2020/12/17	2020/12/17	Negative	2020/12/17	Negative	Undet	Undet	30.02	
47		52	No Data	No Data	E	NP	2020/12/17	2020/12/17	Negative	2020/12/17	Negative	Undet	Undet	26.44	
48		54	No Data	No Data	E	NP	2020/12/17	2020/12/17	Negative	2020/12/17	Negative	Undet	Undet	26.19	

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	Patient code	Age	Date of onset of symptoms	Duration of onset (day)	Confirmed/ Excluded	Specimen type	Specimen collection date	Wondfo RDT		PCR		Ct value			
								Testing date	RDT results	Testing date	PCR results	Ct FAM	Ct Rox	Internal Control	
49	5.1.2e	80	No Data	No Data	E	NP	2020/12/17	2020/12/17	Negative	2020/12/17	Negative	Undet	Undet	27.06	
50		81	No Data	No Data	E	NP	2020/12/17	2020/12/17	Negative	2020/12/17	Negative	Undet	Undet	27.55	
51		24	No Data	No Data	E	NP	2020/12/17	2020/12/17	Negative	2020/12/17	Negative	Undet	Undet	29.04	
52		No Data	No Data	No Data	E	NP	2020/12/17	2020/12/17	Negative	2020/12/17	Negative	Undet	Undet	33.19	
53		No Data	No Data	No Data	E	NP	2020/12/17	2020/12/17	Negative	2020/12/17	Negative	Undet	Undet	29.38	
54		No Data	No Data	No Data	E	NP	2020/12/17	2020/12/17	Negative	2020/12/17	Negative	Undet	Undet	24.44	
55		32	No Data	No Data	E	NP	2020/12/17	2020/12/17	Negative	2020/12/17	Negative	Undet	Undet	29.08	
56		29	No Data	No Data	E	NP	2020/12/17	2020/12/17	Negative	2020/12/17	Negative	Undet	Undet	24.34	
57		No Data	No Data	No Data	E	NP	2020/12/17	2020/12/17	Negative	2020/12/17	Negative	Undet	Undet	27.27	
58		No Data	No Data	No Data	C	NP	2020/12/17	2020/12/17	Positive	2020/12/17	Positive	36.05	28.81	26.68	

Evaluation site: Laboratório Integral - Hospital Vera Cruz

Principal evaluator:

Signature:

Date: