Clinical Evaluation Review of Sona COVID-19 Rapid Antigen Lateral Flow Assay

Executive Summary

To assist in containing the spread of the SARS-CoV-2 virus, it is now globally recognised that rapid antigen tests (RDT's) have an important role to play in identifying undiscovered cases that would typically be missed if a rapid testing strategy were not put in place.

Sona's COVID-19 LFA rapid antigen test is set to play a major role is this battle. A recent clinical evaluation demonstrated that this test identified the presence of the SARS-CoV-2 virus in 33 of 39 RT-PCR positive patient samples (Sensitivity; 85%) and ruled out its presence in 54 of 60 negative RT-PCR patient samples (Specificity 90%).

Infection within 0-8 days of symptom onset and/or low Ct counts have both been associated with an increased risk of infectiousness of COVID-19. An increase in test sensitivity in these patient subgroups was observed. The Sona LFA identified 17/18 RT-PCR positive patient samples that presented symptoms within 0-8 days (94% Sensitivity) and 9/9 RT-PCR positive patients with low Ct counts (<25) (100% sensitivity)

These findings provide evidence that the Sona COVID-19 rapid antigen test can be a useful aid in screening programmes to identify subjects with a high risk of transmission of clinical COVID-19.

Introduction

Coronavirus Disease 2019 (COVID-19) is a severe respiratory illness caused by the SARS-CoV-2 Coronavirus. The first documented cases of COVID-19 were reported in Wuhan City, China in December 2019. The WHO declared that COVID-19 was a pandemic on March 11, 2020, and human infection has spread globally, with hundreds of thousands of confirmed infections and deaths.

The incubation period for COVID-19, which is the time between exposure to the virus (becoming infected) and symptom onset, is on average 5-6 days, however, it can be up to 14 days. The symptoms of COVID-19 are similar to other viral respiratory diseases and include fever, cough, shortness of breath and loss of taste or smell[1-3.]

There are a number of reports of asymptomatic laboratory-confirmed cases of infected people with COVID-19 who do not develop symptoms. The risk comes from asymptomatically infected individuals due to their ability to propagate and spread the virus which could affect existing infection control measures. Unrecognized cases of COVID-19 infections that are in the general community can be a source for virus transmission to healthy people[4,5.] This could be one reason for the rapid increase in clinical cases of the COVID-19.

The current testing climate relies on a RT-PCR technique that uses viral RNA that requires a dissolution of the viral envelope to expose that RNA. Its limitations are time to result, with many people having to wait up to 48hrs or more for a diagnosis, as well as the associated high costs and need for centralised lab processing facilities and health care professionals. There is now globally a recognized need for rapid point of care diagnostic tests for COVID-19 that can deliver a predictable and reliable result within a short time period.[6,7]

Study Objective

The **Sona Nanotech COVID-19 Lateral Flow Assay** is an immunochromatographic assay for the qualitative detection of the spike protein antigen from SARS-CoV-2 in nasopharyngeal (NP) swab specimens from individuals who are suspected of COVID-19 by their healthcare provider. The assay is intended for professional and laboratory use.

The main objective of this study was to evaluate the performance of COVID-19 Lateral Flow Assay and determine the clinical relevance of this test in comparison to an EUA FDA authorized RT-PCR test.

Principles of the Test

The **Sona Nanotech COVID-19 Lateral Flow Assay** employs lateral flow technology in a sandwich design to detect spike protein antigen from SARS-CoV and SARS-CoV2. The test allows for the detection of SARS-CoV and SARS-CoV-2. The test detects both viruses but does not differentiate between them.

A nasopharyngeal swab is placed in a microfuge tube containing reagent solution which extracts the sample from the swab tip. After extraction, the sample is applied to the test cartridge using a Dual Bulb Fixed Volume Pipette® where any SARS-CoV-2 spike protein antigens in the specimen will react with the reagents in the test cartridge.

If the specimen contains SARS-CoV-2 spike protein antigens, a grey-to-blue Test Line along with a blue procedural Control Line will appear on the test cartridge indicating a positive result. If SARS-CoV-2 spike protein antigens are not present, or are present at very low levels, only the blue procedural Control Line will appear.

Testing Methods

The clinical evaluation was undertaken in Saudi Arabia by SaudiVax was conducted at the point of care in a hospital ward setting and walk in clinic setting. The study was conducted over a period of 6 days, across 2 sites by 4 different operators.

Site 1 was Ohud Hospital in Madinah comprising of 8 wards, 20 beds per ward, an ICU center and Children's ICU centre. The hospital has a dedicated COVID ward. All patients tested at the Madinah site were suspected of COVID-19 by their healthcare provider and had severe enough symptoms to be admitted to a dedicated COVID ward of the hospital.

Site 1 conducted 31 and 28 tests across 2 different days respectively with a total of 59 tests conducted.

Site 2 was a COVID walk-in centre located at King Fahd Medical Research Centre, King Abdulaziz University, Jeddah. All patients tested at the Jeddah site were asymptomatic but with a known positive contact or required testing for inter-county travel purposes.

Site 2 conducted 5, 9, 11 and 15 tests across 4 different days respectively with a total of 40 tests conducted

Two nasopharyngeal swabs were taken from each individual patient. The initial swab was used for the primary route of care for the patient and sent for assessment using RT-PCR. A second swab was then taken for use with the Sona test device.

Each specimen was verified against an EAU approved RT-PCR method comprising of the PowerChek 2019-nCoV Real-time PCR Kit on the Roche light cycler 480.

Discordant tests results were examined to determine if there were operational issues with obtaining the patient sample due to patient adherence and/or discomfort during the second swab procedure. Such observations were made during the collection process by the trained health care professional.

The study was conducted under IRB approval: IRB number KSA: H-02-J-002; Project number: 1303. Patient confidentiality was ensured, and data was used only for the research purposes.

Summary of Performance Characteristics

Table 1: Patient Demographics:

Sex	
Male	**Female**
65%	35%

Age	**Sona COVID19 LFA Rapid Antigen Test**		
	Total #	**Positive**	**% Prevalence**
<5 years	0	0	N/A
6 to 25 years	3	1	1%
26 to 59 years	74	23	23%
≥ 60 years	22	15	15%

The clinical assessment shows that the Sona COVID-19 lateral flow assay fulfills the criteria as defined by the FDA for EUA approval of ≥80% sensitivity.

The overall test Sensitivity (PPA) was determined to be 85%, (33/39), and overall test specificity (NPA) was determined to be 90% (54/60) utilising 99 patient samples from symptomatic (59) and asymptomatic (40) patients.

Table 2: Summary Performance Results

Sona Results	RT-PCR		Total
	+	-	
+	33	6	39
-	6	54	60
Total	39	60	99
Sensitivity (PPA)			**84.6% (72.6 – 96.6)**
Specificity (NPA)			**90.0% (82.1 -97.6)**
Correlation (OPA)			**87.9%**

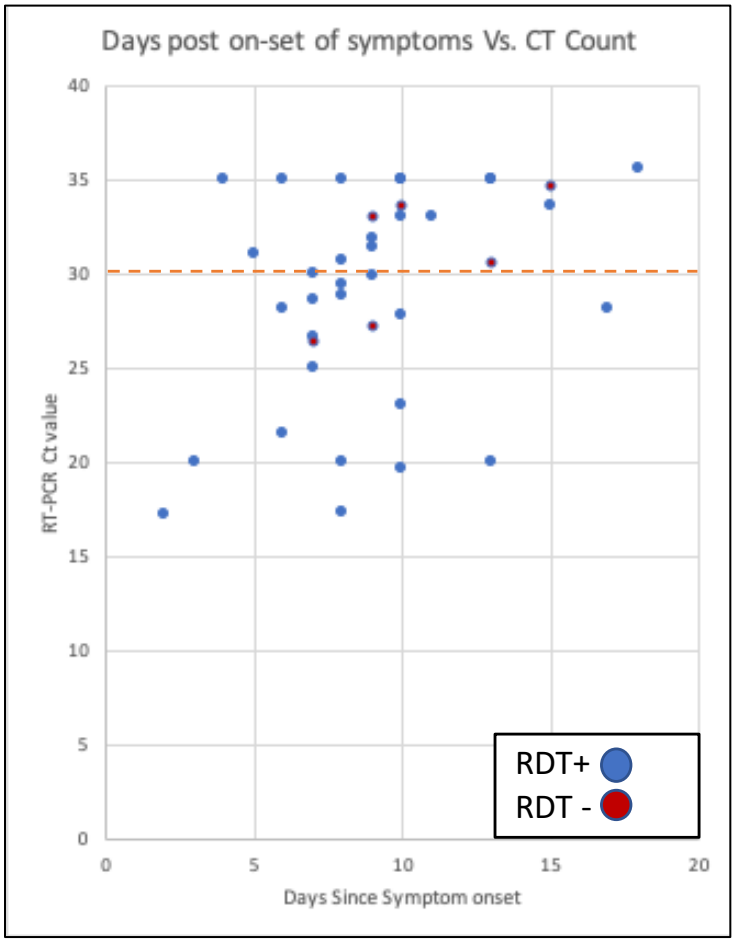
Infection within 0-8 days of symptom onset and/or low Ct counts have both been associated with an increased risk of infectiousness of COVID-19. Symptoms of infection and viral loads are present in patients in the early stages of the disease, with viral loads at levels detectable by rapid antigen test during this phase. As viral loads decrease, sensitivity of tests starts to diminish.

In this subgroup, The Sona LFA identified 17/18 RT-PCR positive patient samples that presented symptoms within 0-8 days (94% Sensitivity). As days from symptom onset increases, sensitivity levels decrease.

Table 3: Positive Results Stratified by Days Since Symptom Onset (sensitivity)

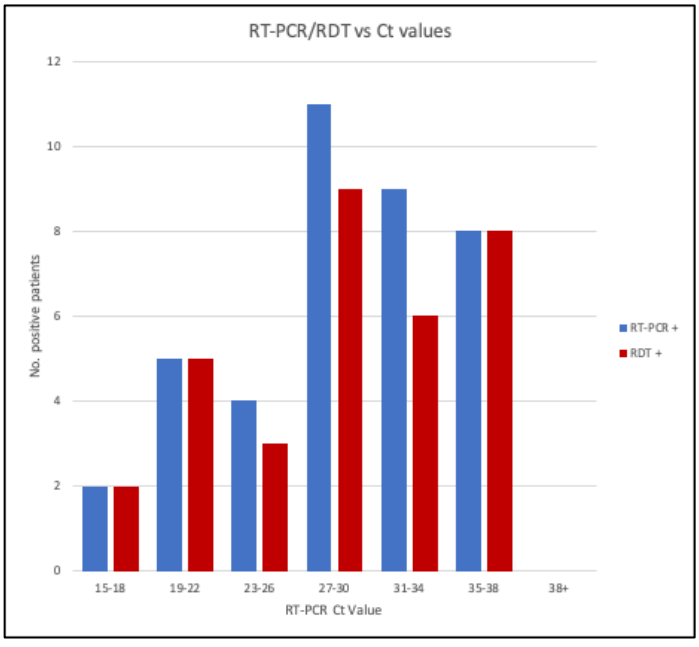
Days since symptom onset	Cumulative RT-PCR Positive (+)	Cumulative Sona COVID19 LFA (+)	PPA (Sensitivity)	95% Confidence Interval	Average Ct count
1 to 2	1	1	100%	68.8 - 100	17.2
3 to 4	3	3	100%	70.6 - 100	27.5
5 to 6	7	7	100%	73.9 - 100	28.9
7 to 8	18	17	94%	76.0 - 100	27.1
9 to 10	30	26	87%	76.4 - 97.6	30.0
11 to 12	31	27	87%	77.2. - 96.8	33.0
13 to 14	35	30	86%	78.7 - 93.3	30.0
15 to 16	37	32	86%	80.3 - 91.7	34.1
17 to 18	39	33	85%	80.2 - 89.8	31.8

Figure 1: Most Sona Missed Results Had CT Counts Above 30



High viral loads are associated with RT-PCR Ct counts of ≤25 with low viral load levels determined as CT counts of >30 with viral load typically equating to relative infectiousness. The Sona LFA identified 9/9 RT-PCR positive patients with low Ct counts (<25) (100% sensitivity) and 18/20 RT-PCR positive patients with medium Ct counts (<25-30) (90% Sensitivity) and 33/39 RT-PCR positive patients with high Ct counts (30-35) (85% Sensitivity). High CT counts are widely thought to reflect instances in which the RT-PCR is picking up remnants of dead virus in patients who are no longer infectious.

Figure 2: Sona Test Had 100% Agreement to RT-PCR for CT Counts Below 25



Conclusions

This paper outlines evidence that Sona's rapid antigen test performs extremely well in its ability to identify patients with the highest risk of transmission.

Access and deployment of rapid tests is key to combating the current pandemic. With only a handful of such tests available, it is imperative that as many developers and manufacturers as possible can bring these to market and make them widely available in a bid to dramatically reduce community transmission rates that are currently rising as many countries enter a second wave.

Rapid antigen detection tests, such as Sona's, can be utilised as screening tools among at-risk populations to allow for infected individuals to isolate at home and therefore cut or reduce transmission chains. These diagnostic tools can be used in screening systems that identify potentially positive workers and customers from long-term care facilities, manufacturers, airlines, schools and other environments in which populations are not able to maintain social distancing in order serve their function.

References:

1.Wu, F., Zhao, S., Yu, B., Chen, Y. M., Wang, W., Song, Z. G., ... & Yuan, M. L. (2020). A new coronavirus associated with human respiratory disease in China. Nature, 579(7798), 265-269.

2.Wu, D., Wu, T., Liu, Q., & Yang, Z. (2020). The SARS-CoV-2 outbreak: what we know. International Journal of Infectious Diseases.

3.World Health Organization Coronavirus disease 2019 (COVID-19) Situation Report – 73; 2 April 2020.

4.Hu, Z., Song, C., Xu, C., Jin, G., Chen, Y., Xu, X., ... & Wang, J. (2020). Clinical characteristics of 24 asymptomatic infections with COVID-19 screened among close contacts in Nanjing, China. Science China Life Sciences, 63(5), 706-711.

5.Nishiura, H., Kobayashi, T., Miyama, T., Suzuki, A., Jung, S. M., Hayashi, K., ... & Linton, N. M. (2020). Estimation of the asymptomatic ratio of novel coronavirus infections (COVID-19). International journal of infectious diseases, 94, 154.

6.Corman, V. M., Landt, O., Kaiser, M., Molenkamp, R., Meijer, A., Chu, D. K., ... & Mulders, D. G. (2020). Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Eurosurveillance, 25(3), 2000045.

7.World Health Organization Coronavirus disease 2019 (COVID-19) technical guidance: Laboratory testing for 2019-nCoV in humans.