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CASE SERIES COLUMN

Is a negative COVID-19 RT-PCR enough to rule out COVID-19? –A case series

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Background:

The COVID-19 pandemic caused by the SARS-CoV-2 virus began when the first case was reported in December 2019 in the city of Wuhan, China and has been ongoing since then. There are two different modalities of COVID-19 testing available per the United States Food and Drug Administration (FDA), including Antigen test and Molecular testing.[1] Antigen test options include rapid antigen tests at a testing center (laboratory or Hospital) and rapid at-home tests. Currently there are over 45 antigen tests approved by the FDA for use in health care facilities, including 17 tests that are cleared for use at home. [2]

Per the Cochrane Systematic review in 2022, the average sensitivity of rapid test was higher in symptomatic patients at about 73.0% compared to asymptomatic participants at 54.7% sensitivity. The average sensitivity was higher in the first week after symptom onset at 80.9% than in the second week of symptoms at 53.8%. [3]

Molecular test options include – RT- PCR (Reverse Transcriptase Polymerase Chain Reaction), CB-NAAT (Cartridge based nucleic acid amplification test) and Bio-Fire.

- RT-PCR is the gold standard for diagnosing COVID-19 and is aimed at detecting the RNA of the virus in respiratory samples such as nasopharyngeal swabs or bronchial aspirate[4]. A systematic review done in 2022 showed that 58% of COVID-19 patients may have initial false-negative RT-PCR results.[5] Some studies say that a combination of clinical, molecular, and serological diagnostic tests is highly recommended to achieve adequate sensitivity and specificity.[5]
- Cartridge-based nucleic acid amplification test (CB-NAAT, GeneXpert,) is an automated cartridge-based molecular technique.[6] It employs a two-stage isothermal amplification assay capable of producing a large copy number within 1 hour [6] and requires fewer cycles than RT-PCR, resulting in reduced incubation time and a lower overall frequency of error. It is robust, sensitive, and specific for single stranded RNA (ssRNA) detection [7].
- The Bio-Fire RP2 (Bio-Fire Diagnostics, LLC) was US FDA cleared in May 2017 for the simultaneous qualitative detection and identification of nucleic acids from multiple common viral and bacterial respiratory pathogens in nasopharyngeal swabs[8]. The Bio-Fire Film Array system utilizes an automated sample purification and multiplex-nested PCR and melting analysis approach. In response to the outbreak of COVID-19, which began in December 2019, the existing Bio-Fire RP2 and Bio-Fire Film Array respiratory panel 2 plus (RP2plus) products were modified to add assays for detection of SARS-CoV-2. The Bio-Fire RP2.1 obtained EUA status from FDA on May1, 2020 and subsequently received marketing authorization using De Novo pathway on March 17, 2021. [9]

Several studies[10,11] have shown that COVID-19 RT-PCR exhibit the same analytical performance as the BioFire RP 2.1 assay. Furthermore, the objective of the study to determine if solely using COVID-19 RT-PCR testing is enough to exclude the possibility of COVID-19 during the early and late stage of infection.

Table 1: Comparison between bio-fire and RT-PCR

Assay Feature	**BIOFIRE**	**3B Black Bio RTPCR**
Methodology	Nested Multiplex PCR	RT qPCR (Dual target singleplex)
Lower Limit Of Detection	160 COPIES/ML	6000 COPIES/ML
Gene Targets	M & S	E, N, RdRp
Analysis	High resolution melt curve analysis	qPCR

In India, the Indian Council of Medical Research (ICMR) set standards for COVID-19 molecular test interpretation for all laboratories across India. As per the ICMR's circular, all RT-PCR tests with a cycle threshold (CT) value of less than or equal to 35 are declared as positive and those with a CT value greater than 35 are declared as negative, and all CB-NAAT tests with a CT value less than or equal to 40[12] are declared as positive and greater than 40 are declared as negative.

Discussion and Case Review:

Here we discuss several cases where we had a negative PCR (run at Dr Dang's Lab (DDL)) but were positive on Bio-Fire with the same sample (run at U.S. Embassy Health Unit). Indian public health protocols required all COVID-19 tests to be reported via an ICMR-accredited laboratory, so all positive COVID-19 tests done within the U.S. Embassy Health Unit were sent our for split sample confirmation at DDL.

Cases Review:

CASE 1

A 32-year-old obese Indian female (BMI 34.8 kg/m^2) without any significant pre-existing medical conditions developed symptoms of COVID-19 infection, including sore throat, cough, nasal congestion, headache, and body aches. The day she developed the symptoms, she tested positive via Bio-Fire. However, the same sample tested with RT-PCR at DDL was reported negative. She was advised home isolation. Contact tracing was performed by the U.S. Embassy Health Unit and all the contacts were advised appropriately. In view of contrasting test results, she tested again with Yashoda Hospital lab the next day and was reported as negative again. She was recommended to repeat COVID-19 RT-PCR sample again two days later, again run at DDL, and she tested COVID-19 positive. The patient's symptoms of illness resolved fully, and her isolation period was uneventful.

CASE 2

A 59-year-old obese American female (BMI 39.2 kg/m^2) without any significant pre-existing medical conditions residing in New Delhi, India developed symptoms suspicious of COVID-19 infection, including sore throat, cough, nasal congestion, fever and body aches. She was seen and was tested on Bio-Fire, which was positive. Subsequently the same sample was sent for COVID-19 RT-PCR at DDL, which came back negative. She was placed into home isolation and contact tracing was performed by the U.S. Embassy Health Unit and all the contacts were advised appropriately. The patient's symptoms of illness resolved fully, and her isolation period was uneventful.

CASE 3

A 46-year-old American male (BMI 25.2 kg/m^2) without other significant pre-existing medical conditions residing in New Delhi, India developed symptoms suspicious for COVID-19 infection, including sore throat, nasal congestion, hoarseness, headache and fever and presented to us on the first day of symptoms. COVID-19 Bio-Fire was positive, however the same sample sent for COVID-19 RT-PCR with DDL was negative. The patient was advised isolation and contact tracing was performed, identifying sixteen close contacts: the patient's wife, daughter (12 years old), and thirteen coworkers. All of them were contacted and given the appropriate advice, and all were subsequently negative for COVID-19 on RT-PCR testing. The patient's infection was attributed to the significant community transmission occurring at the time and no clear source could be identified. The patient's symptoms of illness resolved fully, and his isolation period was uneventful.

CASE 4

A 4-year-old American female (BMI 15.6 kg/m^2) without other known medical history residing in New Delhi, India developed high grade fever. Her parents immediately brought this to the attention of the medical unit. After examination, there was no focus of fever. Her parents were advised to give symptomatic treatment. The next day, she developed mild congestion and as a part of work up, COVID-19 Bio-Fire was done, which came back positive. The same sample was sent for COVID-19 RT-PCR test at DDL was negative. She was placed into home isolation and contact tracing was performed, identifying seven close contacts: the patient's mother, father, nanny, housekeeper, driver, and two children from a neighboring family. All were placed in quarantine, and her parents and nanny also tested positive. Other contacts were all negative for COVID-19 on follow up PCR testing. No clear source of the patient's infection, beyond significant community transmission occurring at the time, was able to be identified. The patient's and her parents' symptoms of illness resolved fully, and their isolation period was uneventful.

CASE 5

A 45-year-old obese American male (BMI 43.8 kg/m^2) with a past medical history significant for orthotopic liver transplant on chronic immunosuppressive therapy with tacrolimus and mycophenolate was identified as an asymptomatic close contact of his toddler daughter, who tested positive for COVID-19 via Bio-Fire after presenting with fever. Given his high-risk status he was also tested via Bio-Fire and found to be COVID-19 positive, but the split sample sent to DDL was negative. He was placed into home isolation and contact tracing was performed, identifying three close contacts: the patient's wife and two household staff. All were placed in quarantine. His wife tested positive several days later after developing symptoms, and her isolation period was uneventful. The household staff were subsequently negative for COVID-19 on follow-up PCR testing. On day 8 of illness the patient's condition worsened and he required oxygen therapy and monoclonal antibody and was medically evacuated to the United States given his high-risk status and unavailability of hospital beds locally. Following transfer to the U.S. he was admitted to hospital, received remdesivir and supportive care, and was discharged after two days. No clear source of the patient's infection, beyond significant community transmission occurring at the time, was able to be identified.

Table 2: Demographic distribution of COVID-19 cases (n=5)

S No	Age (in years)	Gender	BMI (kg/m^2)	Bio-fire	PCR
1	32	Female	34.8	Positive	Negative
2	59	Female	39.2	Positive	Negative
3	46	Male	25.2	Positive	Negative
4	4	Female	15.6	Positive	Negative
5	45	Male	43.8	Positive	Negative

Another important thing to consider is that we had a total of thirty-two tests done from November 2020 to August 2022 run at the US Embassy Health Unit Bio-Fire and PCR at DDL from the same sample. While most of the tests had the same results, five of them were positive for COVID-19 at the US Embassy Health Unit Bio-Fire and negative on PCR at DDL. There was a discrepancy in the results with about 15.62%, but we find that there is no statistically significant difference between the results amongst the two labs running the same sample on two different test variants. (p=0.0528, Fisher's exact test). Now this could possibly change if there are more samples compared between the two different modalities of testing for COVID-19 (Bio-Fire at US Embassy Lab and RT-PCR at DDL).

Table 3: COVID-19 test results at the two labs. (n=32)

	COVID-19 test result	
	Positive	Negative
US Embassy Lab	32	0
DDL	27	5

Conclusion:

The pandemic status of COVID-19 is over, but COVID-19 still exists and there are different modalities available for testing, but Bio-Fire seems to be the most sensitive in picking up the SARS-CoV-2 virus. In this case series, it detected SARS-nCOV-2 even with a low viral load, which happens in the early phase of the infection and sometimes at the tail end of the infection. In such situations, the RT-PCR and CBNAAT can be negative. As seen in these cases, a negative RT-PCR or a negative CBNAAT can sometimes miss COVID-19. When there is a high index of clinical suspicion, clinical correlation, and other advanced testing like Bio-Fire should be used when available. However, the high relative cost and limited availability of Bio-Fire can be a barrier to its use.[13]

Conflicts of interest: None

Disclaimer: The U.S. Embassy New Delhi/ U.S. Department of State/ U.S. Government in no way formally endorses Dr Dang's Lab. They are one of several affiliated private laboratories which the U.S. Embassy Health Unit, New Delhi, India utilizes for medical investigations for the U.S. diplomatic community when needed.

The views expressed in this article are authors' own and not necessarily those of the U.S. Government or US Department of State or Bureau of Medical Services.

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