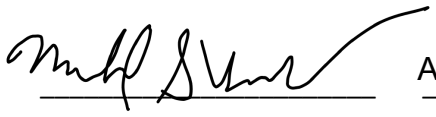
A 20/20 BioResponse initiated investigation

Report: Quality Control/Clinical Study of CoronaCheck™ (distributed by 20/20 BioResponse)

Date: March/April 2020

Study Director: Michael S. Lebowitz, PhD, Chief Scientific Officer



April 8, 2020

Purpose:

20/20 BioResponse is a US distributor of rapid serological tests to detect patient immune responses (IgM/IgG) to SARS-CoV-2, the virus that causes COVID-19 disease. The company has forged a relationship with reputable, CFDA-approved Chinese manufacturer of such tests. The Chinese manufacturer has performed significant verification and validation studies (in China) of their tests, including cross-reactivity studies, analytical and clinical validation. To confirm expected device functionality, 20/20 BioResponse performed further testing in the United States.

Background:

COVID-19 is caused by a novel coronavirus, SARS-CoV-2.

The standard diagnostics tests for SARS-CoV-2 infection are primarily nucleic acid-based testing involving RT-PCR amplification of specific viral amplicons. These tests detect the presence of viral RNA in a patient's respiratory tract obtained by nasopharyngeal swabs. The RT-PCR test is known to be less than perfect; false negatives may result due to improper sampling technique, presence of the virus in the lower but not in the upper respiratory tract, as well as other reasons (Patel R, *et al.* (2020) mBio 11:e00722-20, doi: 10.1128/mBio.00722-20).

Serology tests measure the presence of human antibodies (IgM and/or IgG) in the blood of potentially infected individuals. Information regarding the time required after infection for a detectable immune response to SARS-CoV-2 to develop is limited at this time. While some reports initially suggested that immune responses may be detectable as early as 3-6 days post infection (Li, *et al.* (2020) *J.Med.Virol.* doi: 10.1002/jmv.25727) other more recent reports are driving to a consensus position that much longer times are required for the development of antibody responses; perhaps ~10 days or more after the onset of symptoms (Lee, *et al.* (2020) *J.Microbiol.Immunol.Infect.*, doi: 10.1016/j.jmii.2020.03.003; To, *et al.* (2020) *Lancet*, doi: 10.1016/S1473-3099(20)30196-1; Zhao, *et al.* (2020) *Clin.Infect.Dis.* doi: 10.1093/cid/ciaa344).

CoronaCheck™ distributed by 20/20 BioResponse is a lateral flow immunoassay, detecting IgM and IgG against SARS-CoV-2 S-protein (RBD) and N-protein.

Many experts have stated that serology testing for antibodies that bind to SARS-CoV-2 antigens are likely to be highly valuable in epidemiological efforts to control the spread of the virus, and in understanding personal risk from the virus after recovering from a first infection with the virus.

Goal of the Study:

To confirm the manufacturer's stated performance characteristics (*i.e.*, sensitivity and specificity) of CoronaCheck™ at multiple clinical sites treating COVID-19 patients in the United States.

Study Design:

Six (6) U.S. clinics and hospitals agreed to participate in the study. Sites were located in New Jersey, Colorado, South Carolina, Pennsylvania and Maryland. Each site received tests under an RUO label, and were asked to test at least 5 individuals who had previously tested positive for SARS-CoV-2 by a PCR based test and 5 individuals who had previously tested negative for SARS-CoV-2 by a PCR based test. Sites were suppled a record sheet for report of resultant data, including the following information: Test

date, patient age, gender, symptoms, date of symptom onset, test results including positive or negative for IgG, IgG control line, IgM and IgM control line, PCR samples collection date and PCR test result (positive/negative). Sites were also encouraged to supply completely anonymized data concerning the physician's observations and conclusions regarding the respiratory disease under investigation.

Results:

Results were reported on a total of 65 patients. Two test cassettes failed; control lines did not properly develop, therefore 63 validated samples including 24 confirmed PCR positive patients and 39 confirmed PCR negative patients. Of a total of 24 confirmed PCR positive patients 18 were positive for IgM only (5), IgG only (7) or both IgM and IgG (6). Of 34 confirmed PCR negative patients 32 were negative for both IgM and IgG. Thus, the resulting sensitivity is 18 out of 24 or 75% and the resulting specificity is 37 out of 39 or 95%. However, It should be noted that for the two confirmed PCR negative patients that were positive for both IgM and IgG, the physician reported that these 2 patients presented clinical signs of COVID-19 disease including positive chest x-ray, and that physician had concluded that both patients had COVID-19 despite the negative RT-PCR result. If one accepts that the physician is correct about the diagnosis, the test would then have 77% sensitivity and 100% specificity relative to the whole clinical record (not just PCR). The manufacturer's reported sensitivity is 87% and reported specificity is 100%. Because of the small samples size, these test results reported are statistically consistent with the manufacturer's stated performance characteristics.

Five of the six sites recorded the date of symptoms onset, which allows for some insight into the relationships between time of symptom onset and presence of IgM and/or IgG in patient blood. Table 1 lists the 14 patients arranged in order of test day post symptom onset. 6 out of 14 patients IgM positive from day 8-18; 6 out of 14 patients IgG positive from day 12-24; 3 out of 14 patients negative for both - day 20-24. As expected, IgM tends to be present earlier and recede as the immune response continues, while IgG is present later and remains.

Table 1: Presence of IgM and/or IgG Post Symptom Onset

Site #	Patient #	Days Post Symptoms	IgM	IgG	Both
3	2	8	Pos	Neg	**Pos**
4	3	8	Pos	Neg	**Pos**
5	5	9	Pos	Neg	**Pos**
5	9	11	Pos	Neg	**Pos**
3	6	12	Neg	Pos	**Pos**
5	3	12	Pos	Neg	**Pos**
3	5	14	Neg	Pos	**Pos**
3	9	17	Neg	Pos	**Pos**
6	3	18	Pos	Pos	**Pos**
2	1	20	Neg	Neg	**Neg**
4	1	21	Neg	Pos	**Pos**
4	2	21	Neg	Neg	**Neg**
4	4	24	Neg	Pos	**Pos**
6	8	24	Neg	Neg	**Neg**

Discussion:

20/20 BioResponse continues to conduct on-going testing to best serve the medical community's needs during the COVID-19 public health crisis.

Conclusion:

CoronaCheck™ was found to perform within the manufacturer's stated performance characteristics for sensitivity and specificity.

Appendix 1: Letter supplied of instruction supplied to performance sites

Dear Dr. _____:

As you know, 20/20 BioResponse is a US distributor for several Chinese manufacturers of rapid serological tests to detect patient immune responses (IgM/IgG) to SARS-CoV-2, the virus that causes COVID-19 disease. All of our Chinese manufacturers are reputable companies and have performed significant studies and validation in China of their respective devices, including cross-reactivity studies, analytical and clinical validation as well as quality control (QC) of manufactured lots. As a responsible distributor of such kits, we wish to perform local QC to ensure that these test kits function properly.

Please be aware, that due to a miscommunication from the manufacturer, the tests came packaged in a different lot size than originally suggested. As such, we are providing you with a carton that contains 20 tests. Because this kit is for QC purposes, it is labelled as Research Use Only (RUO), but it is of course the same kit that we will be distributing once it has passed QC. We would ask that you specifically test at least 5 individuals who have tested positive for SARS-CoV-2 by a PCR based test and 5 individuals who have tested negative for SARS-CoV-2 by a PCR based test. The remainder of the tests may be used as you see fit; we are just asking that we receive the data from all 20 tests run and as such we are supplying a record sheet for you to return to us with the resultant data. We are seeking a rapid turn-around time of ~48 hours from receipt of the kits as your data will be incorporated into our Quality Assurance program in conjunction with IVD kits. All information sent to us should be deidentified. We do not need any patient identifying information.

PLEASE READ: IMPORTANT INFORMATION

We are supplying these tests as an aid in the management of COVID-19 disease; these tests do not provide a definitive diagnosis of current infection with SARS-CoV-2. It is important to understand the limitations of serology tests in regard to COVID-19 infection.

- Definitive diagnosis of current infection with SARS-CoV-2 requires a test that directly measures the presence of the virus; specifically, nucleic acid-based testing or direct measurement of viral antigens.
- Serology tests measure an individual's immune response to SARS-CoV-2 infection; specifically, they measure the presence of either or both IgM and/or IgG antibodies to SARS-CoV-2 antigens.
- Information regarding the time post-infection for a detectable immune response to SARS-CoV-2 to develop is limited at this time. While some reports initially suggested that immune responses may be detectable as early as 3-6 days post infection (Li, *et al.* (2020) *J.Med.Virol.* doi: 10.1002/jmv.25727) we are now aware of other more recent reports that have identified much longer times for the development of antibody responses; specifically, **~10+ days post onset of symptoms** (Lee, *et al.* (2020) *J.Microbiol.Immunol.Infect.*, doi: 10.1016/j.jmii.2020.03.003; To, *et al.* (2020) *Lancet*, doi: 10.1016/S1473-3099(20)30196-1; Zhao, *et al.* (2020) *Clin.Infect.Dis.* doi: 10.1093/cid/ciaa344).

The FDA requires we label these tests with the following language:

- This test has not been reviewed by the FDA.
- Negative results do not rule out SARS-CoV-2 infection, particularly in those who have been in contact with the virus. Follow-up testing with a molecular diagnostic should be considered to rule out infection in these individuals.

- Results from antibody testing should not be used as the sole basis to diagnose or exclude SARS-CoV-2 infection or to inform infection status.
- Positive results may be due to past or present infection with non-SARS-CoV-2 coronavirus strains, such as coronavirus HKU1, NL63, OC43, or 229E.
- Positive results are NOT an indication of immunity to SARS-CoV-2.
- Not for the screening of donated blood.

We will be in need of ongoing QC of incoming lots in the future. If you would be interested in serving in this capacity on a continuing basis, please let us know. Furthermore, if it might be possible for you to supply us with a tube of de-identified blood, serum or plasma from confirmed, symptomatic COVID-19 patients for future testing, please let us know and we will supply you with the necessary documents to consent the patients.

Thank you for your efforts and support. We are eager to be a responsible part of the effort to mitigate the current personal and public health impacts of the COVID-19 pandemic.

Thank you and best wishes for your health,

MIchael S. Lebowitz, PhD
CSO

Appendix 2: Data record sheet

CoronaCheck Test Results Log

Hospital Name: _____ Department: _____

Prepared by: _____ Approved by:_____

	TEST ID	TEST DATE	AGE	M/F	PRESENTING SYMPTOMS / Date of Onset	IgG +/-	Cont +/-	IgM +/-	Cont +/-	PCR sample collection Date	PCR Confirmed Result (Pos/Neg)
1											
2											
3											
4											
5											
6											
7											
8											
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Please provide any notes or comments in the space below:

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