COVID-19 IgG Serum BioCard™

Catalogue No: CS033A & CS33B

Instructions for Use

INTENDED PURPOSE AND PRINCIPLE OF TEST

The Bio-Diagnostics (UK) COVID-19 spike specific IgG serum BioCard™ is a unique ultra-rapid qualitative IVD screen and/or confirmatory method for the specific detection of circulating IgG Antibodies to the Spike of the SARS-CoV-2 virus.

Presence of specific IgG is a marker used as a tool to assist in identifying past SARS-CoV-2 infection given the spike is unique to SARS-CoV-2 virus.

A positive result demonstrates the presence of specific spike IgG antibodies to SARS-CoV-2 virus, strongly indicative of past exposure.

Due to a small number of people failing to generate, and/or sustain antibodies, a negative result does not infer with 100% certainty a patient has not had COVID-19, but does provide evidence that they do not have antibodies specific to it. (*1 & 2).

This version of the device is specifically validated for serum from either venous or finger prick obtained bloods, which in validation studies demonstrated a sensitivity of 100% and specificity of 99.4%.

The test is intended to be used on samples no earlier than day 14 after onset of symptoms or PCR positive result. As some patients who later develop antibodies, may not develop them by day 14, retesting later is recommended for efficient use. (*1).

The test is intended to give a positive result only when spike specific IgG antibodies are present in the sample. Failure to detect spike specific IgG antibodies that are not present, is not a failure of this test. (*3)

This test is intended to be used by competent healthcare professionals.

This test is not suitable for diagnosing active infections.

SUMMARY

The Bio-Diagnostics COVID-19 IgG Serum BioCard™ was wholly developed and manufactured within the UK working under ISO13485 accreditation within the associated regulatory requirements. COVID-19 is the infectious disease caused by the SARS-CoV-2 virus, which can cause mild to severe respiratory illness. First identified in China, and has now spread globally, including the UK. The COVID-19 BioCard specifically detects IgG antibodies which bind to the spike protein, considered to be unique to the SARS-CoV-2 virus. Results should not be compared to nonspecific tests which may also detect generic Coronavirus antibodies, including those which may cause some forms of the common cold.

Attaining optimal accuracy for clinical results

This device would ideally be utilised together with clinical history, other clinical data and prior confirmed PCR results where available. Use of a confirmed PCR (to the extent of its true accuracy), when determining results can be applied to the population incidence and the resultant associated PPV/NPV. When calculating the PPV and NPV consider the "population" being studied. If the group is tested without clinical certainty of exposure, then the "prevalence" of that sample group is limited to the presumed incidence of the whole demographic area.

When selecting a confirmatory method, it is necessary that it also utilises the Spike antigen for accurate correlation utilising the same binding domain.

The BioCard should be interpreted by competent healthcare professionals able to visually discern any level of binding at normal reading distance (wearing prescribed lenses when required).

Whilst in validation studies all PCR positive and negatives were correctly identified, it is not improbable that in a global population of 8.8 billion people, a number of erroneous binding antibodies may occur when SARS-CoV-2 antibodies are not present. Similarly, whilst it was not seen in validation trials, it remains possible antibodies could fail to react in the test window and cause a false negative result.

Good clinical practice of running all samples on a second confirmatory method using an Orthogonal testing algorithm is recommended.

PERFORMANCE CHARACTERISTICS

Relative Sensitivity and Specificity
Studies were performed using sera from COVID-19 positive patients and normal blood donors. These samples were tested on the COVID-19 Serum BioCard and the results compared to several alternative methods within context of a clinical history where available.

		Reference Method Result		Sensitivity	Specificity
		+ve	-ve		
BioCard Result	+ve	68	1	100%	99.4%
	-ve	0	178		

Validation Method

In absence of 100% accurate PCR tests, COVID-19 patients can be identified definitively as having suffered from SARS-CoV-2 infection when evidenced from a past ICU admission due to extreme clinical symptoms definitively pertaining to SARS-CoV-2.

In absence a definitive gold standard or other established accurate test methods for specific spike IgG determination, sera from such patients are essential for initial design, proof of concept and validation studies. Commercial sources of such sera are available and helpfully provide independent laboratory analysis of IgG presence, evidenced by a Certificate of Analysis.

However, potentially valid critique has been made in this particular regarding the use of positive control selection for antibody tests for COVID-19 when sera is exclusively selected from previously severely symptomatic patients and drawn >6-8 weeks after onset, to define test accuracy. As this serum is likely to have very high IgG levels and exclude low and borderline samples, sera acquired from commercial serum suppliers and selected to ensure it is predefined to have high IgG levels, is clearly not exclusively representative of wider population samples.

Whilst technically correct, by limiting all validation sera to pre-screened, definitively highly positive commercial sera will likely result in a sensitivity significantly higher compared to "real world" samples from mild or asymptomatic patients. Therefore, these have not been exclusively relied upon in the validation of this device. When utilising independently validated sera a broad and complete range of IgG positive antibody levels.

In our validation, bloods were acquired from the domestic population within in the EU, with additional samples from the USA to ensure validation was representative of confirmed incidence in European and Western populations. Consequently, resultant data is designed to be representative of the diverse demographics of patients in those areas as well as being representative of current forms of the virus.

Approximately 1/3 of samples were independently verified positive by a commercial third party by ELISA IgG.

Additionally, 10 PCR certified samples were acquired which were found to include 4 IgG positives, and 6 IgG negatives. Of the samples provided, 1 negative sample* which was taken prior to the parameters of this test, (day 12), and was therefore excluded from our final data.

The remaining samples ("wild") were selected from asymptomatic, mild to moderate patients from the community, aged 3 – 75 and from various ethnic backgrounds, a proportion having been pre-emptively screened via PCR due to being front line workers in healthcare to monitor outbreaks of disease. Clinical histories and epidemiological strategies were employed to determine evidence of past COVID-19 infection.

To establish presence of IgG in all "wild" samples, combination of research and commercial CE marked ELISAs and LF devices were used to screen samples. Multiple lateral flow (LF) tests were then employed to establish evidence when confirming results. Data from LF results proved highly contentious when referencing independent data variance. However, having gained an understanding of each version's limitations and parameters, logical conclusions could be established. Greater significance was afforded to methods utilising solely a Spike protein when determining conformation of IgG antibodies specific to SARS-CoV-2. In house wild sera without external validation unattainable n=10.

The total independently verified and/or externally confirmed positive (including confirmed "wild" sera with IgG n=58.

		PCR Positives	True IgG Positives	BioCard Detected
NIBSC20/130		1	1	1
Commercial SARS-CoV-2 IgG, PCR +ve		20	20	20
Commercial PCR positive (IgG status unverified).		10	4 (5*)	4
"Wild" samples (PCR & Ab independently confirmed).		16	16	16
"Wild" samples (PCR confirmed).		8	8	8
"Wild" samples (Independent Ab confirmed).		0	6	6
"Wild" samples (In house Ab confirmed).		10	10	10
Blind NEQAS (External QA body).		0	3	3
Totals		-	68	68
Total Confirmed Negatives	178			

Summary: 68 true IgG spike Ab positives / 178 True Negatives.

As Bio-Diagnostics is a member of an external quality assurance scheme, copies of reports are available.

A total of 41 samples were previously tested on PCR.

When limiting samples to only commercial sources with independent validation, sensitivity and specificity achieves 100%. These comparative results reflect the methods used more commonly by manufacturers when validating IVDs, without "wild" samples.

Overall, 247 samples were used in validation. When asymptomatic and mildly affected patients from "wild" incidence of the disease are included in validation data the BioCard attained a sensitivity of 100% and specificity of 99.4%.

PRINCIPLE OF THE TEST

The COVID-19 IgG Serum BioCard™ is a rapid immunoassay, which detects the presence of SARS-CoV-2 spike specific IgG to in serum.
When a pre-diluted serum sample is passed through the membrane any anti-SARS-CoV-2 spike IgG present becomes bound to the antigen in the test spot.
Upon addition of the developing reagent, the test spot on the right develops a pink/red colour if the result is positive. The developing reagent also reveals the pink-red control spot on the left, which demonstrates that the reagents are functioning properly. The test device is designed to completely absorb the volume of added reagents.
The presence of two spots in the TEST WINDOW indicates a positive result. A single spot on the left of the TEST WINDOW is a negative result.

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COVID-19 IgG Serum BioCard

KIT CONTENTS
Each COVID-19 IgG Serum BioCard™ kit contains:

- COVID-19 Spike IgG test devices
- Vial(s) of Sample Diluent
- Vial(s) of Developing Reagent
- Instructions for Use

MATERIALS REQUIRED BUT NOT PROVIDED
Pipettes, disposable pipette tips, Eppendorf tubes or equivalent.

KEY FOR SYMBOLS
Used in this instruction leaflet and on accompanying product packaging:

	Manufacturer	Σ	Contains sufficient for <n> tests
	Temperature limitation	IVD	In vitro diagnostic medical device

WARNINGS AND PRECAUTIONS

1. This test is designed for *in vitro* diagnostic use only.
2. Do not pipette any material by mouth. Do not smoke, eat, or drink where specimens or kit reagents are handled.
3. Use suitable protective clothing and gloves when handling the test components or the patient samples, and while performing the assay.
4. The liquid reagents in the kit contain <0.1% (w/w) sodium azide. Although sodium azide is considered non-hazardous at this low concentration, the reagents should be treated as toxic. They should not be ingested or allowed to come into contact with either the skin or the mucous membranes. Sodium azide may also cause the formation of potentially explosive lead or copper azides in sinks, therefore any excess should be disposed of with large quantities of water.
5. Each test is designed for single use only. After completion of the test, all components should be disposed of as biohazardous waste.
6. Do not use the test components beyond the expiry date that appears on the label.

STORAGE AND STABILITY
Store the COVID-19 IgG Serum BioCard™ at a temperature from +2°C to +8°C.
Do not freeze the test kit.
The stability of the COVID-19 IgG Serum BioCard™ is as indicated by the expiry date on the packaging under the above storage conditions.

SPECIMEN COLLECTION AND STORAGE

1. The COVID-19 IgG Serum BioCard™ test is to be performed on fresh serum only.
2. Serum specimens can be stored at +2°C to +8°C for up to 7 days.
3. All samples should be thoroughly mixed before testing.

TEST PROCEDURE
The COVID-19 IgG Serum BioCard™ procedure must be followed closely.
The test must be performed at room temperature (+18°C to +25°C). Diluted samples should run through the test window within 75 seconds.

Once all components and samples reach room temperature:

1. Remove a test device from its foil pouch by tearing along the notch and place on a flat surface.
2. Label the device with the patient's name and/or identification number, and peel back the lower half of protective label to reveal the test window. The antigen and control will be visible as two pale blue spots.
3. Pipette 75µl of sample diluent and 50µl of serum into an Eppendorf, (or similar), and mix well.
4. Pipette the entire serum/diluent mixture onto the test window and allow the mixture to drain completely into the test device. (do not allow test window to dry out).
5. Gently mix the developing reagent in the plastic tube (labelled Developing Reagent) by inverting several times. Remove the lid and pipette 300µl of the developing reagent onto the TEST WINDOW.
6. Allow the developing reagent to absorb into the test device completely. (approx. 1-2 minutes).
7. Interpret the result of the test as described below.

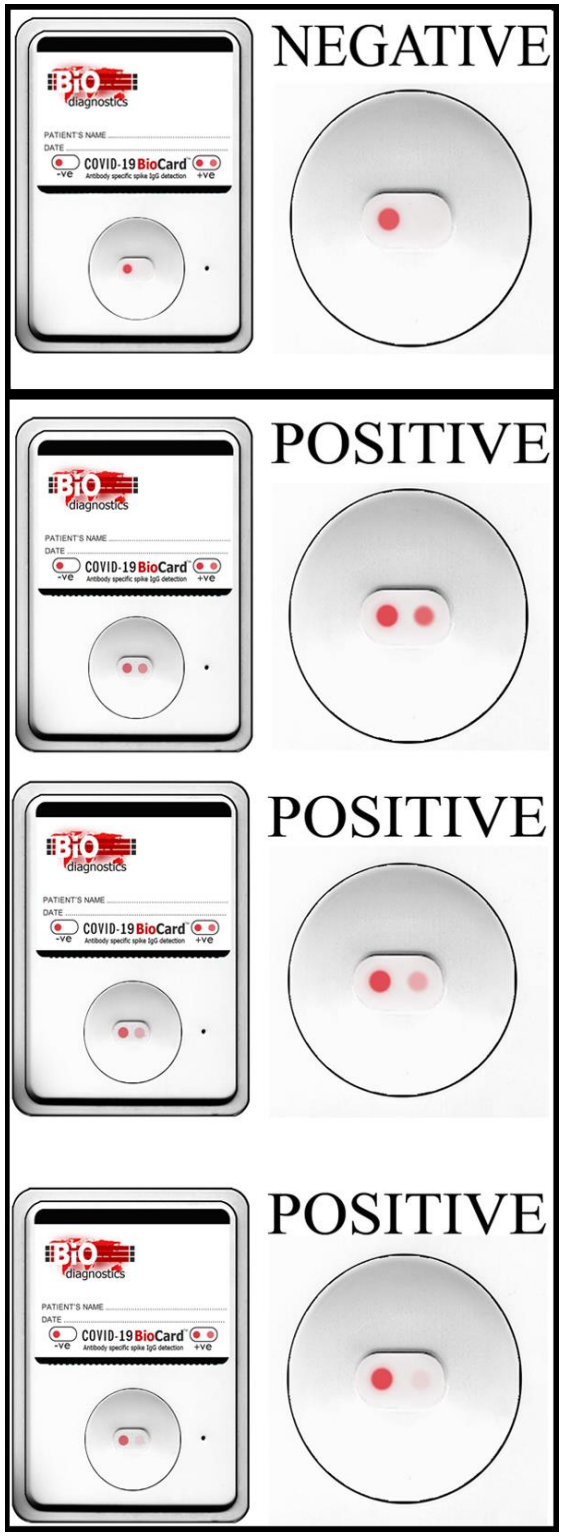
INTERPRETATION OF RESULTS
Results can be interpreted as soon as the developing reagent has drained into the test device. Read the results within 10 minutes of performing the test. On completion of the test, and recording of results, dispose of the test device and all empty component vials as biohazardous material.

INTERNAL QUALITY CONTROL
If the control spot on the left side of the test window fails to react, the test is invalid. If this occurs, repeat the test with new components.
A intense overall pink colour to the whole test window is indicative of a poor-quality sample, for which the test should be considered is invalid.

NEGATIVE RESULT
Only one pink/red control spot appears on the left side of the test window. A negative result indicates no detectable level of anti-SARS-CoV-2 IgG. Negative results do not rule out the diagnosis of past SARS-CoV-2 infection as the specimen may have be drawn before appearance of detectable IgG antibodies.

POSITIVE RESULT
Two pink/red spots appear in the test window.

Any spot which appears on the right side of the test window and is discernible from normal reading distance, regardless of intensity, is a positive result. A positive result means the patient sample contains detectable levels of IgG antibodies to SARS-CoV-2.



TEST LIMITATIONS

1. The COVID-19 IgG Serum BioCard™ is a qualitative assay for the detection of spike specific IgG antibodies to SARS-CoV-2 in serum and is not indicative of the level of antibody titre.
2. The components of the kit have been standardised as a unit so components from different lot numbers are not interchangeable.
3. Strict adherence to the protocol is vital. Any changes made to the procedure are at the discretion of the user.
4. A positive COVID-19 Serum BioCard™ result indicates the presence of spike specific IgG antibodies to SARS-CoV-2. In an infection, there may be no immunological

response to the SARS-CoV-2 antigen for several weeks, therefore a negative COVID-19 BioCard™ result at any time does not preclude the possibility of infection especially if the infection is recent.
5. If any sample flows too slowly during application into the device, (>75 seconds), the test should be repeated.
6. This test is not suitable for diagnosing active infections.

POTENTIAL CAUSES OF ERROR

An erroneous result may be obtained with the COVID-19 IgG BioCard™ if the following occur:
1. Reagents, test device and/or serum have not reached room temperature.
2. Repeated freeze-thaw of serum samples.
3. Use of an inaccurate pipette.
4. Inadequate pipetting of the serum and reagents.
5. Inadequate mixing of the serum/diluent mixture.
6. Failure to add developing reagent.
7. Failure to interpret results within 10 minutes of addition of developing reagent.
8. Failure to take into consideration the warning given in the "Interpretation of Results" section.
9. Diluting samples with anything other than the reagents provided with the device.
10. Components of different batch numbers were mixed.
11. Whole blood should not be applied to the test window directly.
12. Samples should ideally be attained no earlier than day 14 following first onset of symptoms, or positive PCR result.
13. Highly haemolytic and highly lipemic samples should not be used.

(1) Patients develop specific antibodies to the spike of the SARS-CoV-2 varies between 14 days and 23+ days and the amount of antibody will rise and fall over time. Retesting should therefore be carried out regularly to determine when any presumed immunity from antibodies has waned, especially for high risk persons. It was found that > 99% of patients with PCR confirmed COVID-19 had antibodies. Furthermore, 70.4% had high antibody levels, 22.2% had moderate antibody levels and 7.3% had low antibody levels.

(2) Due to potentially significant inaccuracy levels of PCR testing for COVID-19, a proportion of patients without antibodies will be identified due to having previously attained a false result on PCR or equivalent, and so the true percentage of people who do not develop antibodies is likely to alter in line with accuracy of initial testing for the disease, as is "reinfection" which may in reality be a primary infection following a prior false positive SARS-CoV-2 *test result.*

(3) Whether or not a particular patient has, or does not have, specific COVID-19 IgG antibodies within a certain time frame is more relevant to epidemiology studies when establishing a distribution curve of if, and when, patients develop specific COVID-19 antibodies.

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