



Nirmidas COVID-19 (SARS-CoV-2) IgM/IgG Antibody Detection Kit (NBPC-0001-xx)

Instructions for Use

xx Tests/Kit (kit sizes will vary)

For prescription use only

For in vitro diagnostic use only

For Emergency Use Authorization only

1. PRODUCT NAME

Nirmidas COVID-19 (SARS-CoV-2) IgM/IgG Antibody Detection Kit

2. INTENDED USE

The Nirmidas COVID-19 (SARS-CoV-2) IgM/IgG Antibody Detection Kit is a lateral flow immunoassay intended for the qualitative detection and differentiation of IgM and IgG antibodies to SARS-CoV-2 in human serum and plasma (dipotassium EDTA and Lithium heparin). The Nirmidas COVID-19 (SARS-CoV-2) IgM/IgG Antibody Detection Kit is intended for use as an aid in identifying individuals with an adaptive immune response to SARS-CoV-2, indicating recent or prior infection. At this time, it is unknown for how long antibodies persist following infection and if the presence of antibodies confers protective immunity. The Nirmidas COVID-19 (SARS-CoV-2) IgM/IgG Antibody Detection Kit should not be used to diagnose acute SARS-CoV-2 infection. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C 263a, that meet requirements to perform moderate or high complexity tests. Results are for the detection of SARS CoV-2 antibodies. IgM and IgG antibodies to SARS-CoV-2 are generally detectable in blood several days after initial infection, although the duration of time antibodies are present post-infection is not well characterized. Individuals may have detectable virus present for several weeks following seroconversion. Laboratories within the United States and its territories are required to report all results to the appropriate public health authorities. The sensitivity of the Nirmidas COVID-19 (SARS-CoV-2) IgM/IgG Antibody Detection Kit early after infection is unknown. Negative results do not preclude acute SARS-CoV-2 infection. If acute infection is suspected, direct

testing for SARS-CoV-2 is necessary.

False positive results for the Nirmidas COVID-19 (SARS-CoV-2) IgM/IgG Antibody Detection Kit may occur due to cross-reactivity from pre-existing antibodies or other possible causes. Due to the risk of false positive results, confirmation of positive results should be considered using second, different IgG or IgM assay.

The Nirmidas COVID-19 (SARS-CoV-2) IgM/IgG Antibody Detection Kit is only for use under the Food and Drug Administration's Emergency Use Authorization.

3. PRINCIPLE OF THE TEST

This test uses the principle of colloidal gold immunochromatography, and capture method to detect COVID-19 (SARS-CoV-2) IgM and IgG antibodies in human serum and plasma. When the sample contains COVID-19 (SARS-CoV-2) IgM / IgG antibody and the concentration is greater than or equal to the minimum detection limit, the antibody binds to the S1 and RBD subunits of spike protein immobilized in test region M / test region G and captured by the secondary antibody to produce a red reaction line. The result is considered positive when a red reaction line appears in either test region. The result is considered negative when no red reaction line is in either test region. The test is valid when the control line (C) produces a red reaction line.

4. REAGENTS AND MATERIALS PROVIDED

- Individually foil-pouched Nirmidas COVID-19 (SARS-CoV-2) IgM/IgG Antibody Test Cards with desiccant. The Test Card consists of a membrane, a sample pad, a colloidal gold pad and absorbent paper.
- 1 vial of Sample Diluent per 1 test

5. MATERIALS REQUIRED BUT NOT PROVIDED

A timer and a permanent marker are needed to process Test Cards. Volumetric pipette (10 and 100 microliters) and tips.

6. STORAGE AND EXPIRATION

The test kit should be stored at 4-25°C in original packaging condition.

Do not freeze.

Properly stored kits are usable until the expiration date indicated on the kit labels. The Test Card should be used (apply sample and Sample Diluent) within 15 minutes after removing from the foil pouch.

7. SAMPLE REQUIREMENTS

- Serum and plasma (K₂EDTA or lithium heparin) samples can be used for testing.
- Serum and plasma samples should be tested as soon as possible or stored at 4-8°C and tested within 3 days.
- Serum and plasma specimens which cannot be tested within 3 days after collection should be stored frozen at -20°C or lower and tested within a month.
- Sample should be warmed to room temperature before testing. Avoid repeated freezing and thawing.
- Avoid grossly hemolytic (bright red), lipemic (milky), or turbid samples (after centrifugation).

8. ASSAY PROCEDURE

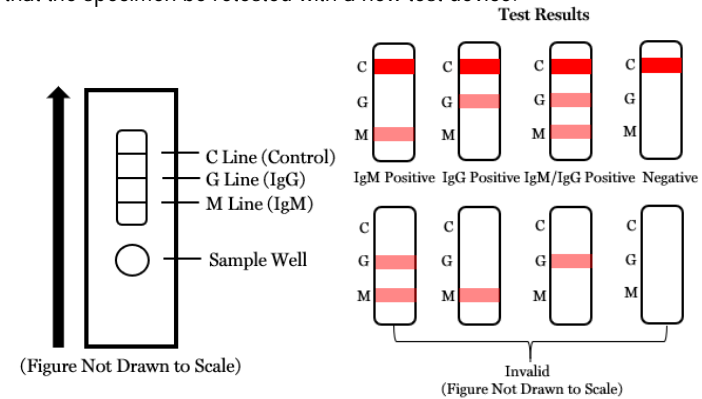
- Bring specimen, Test Card and Sample Diluent to room temperature (15-25°C) before testing.
- Remove the Test Card from the foil pouch and place on a flat surface. Do not use if foil pouch is not intact. Use a permanent marker to mark the Test Card with appropriate details.
- **For processing serum and plasma samples:**
 - (1) Using a volumetric pipette, dispense 10 µL of the sample directly into the Sample Diluent vial. Close vial and mix well.
 - (2) Using a volumetric pipette, add 100 µL of the mixture to the Sample Well of the Test Card.
 - (3) Start a timer. Read the test result **10-15 minutes** after sample/diluent mixture is added. Do not read the result after 15 minutes. Discard the Test Card after result is read to avoid confusion.
- **For processing Positive and Negative Control** – Treat Positive and Negative control as a serum or plasma samples using the above steps.

9. INTERPRETATION OF RESULTS

- **Positive for COVID-19 (SARS-CoV-2) IgM:**
A red line appears in test region M as well as at control line (C).
- **Positive for COVID-19 (SARS-CoV-2) IgG:**
A red line appears in test region G as well as at control line (C).
- **Positive for Both COVID-19 (SARS-CoV-2) IgM and IgG:**
A red line appears in both test region M and G, as well as at control line (C).
- **Negative for COVID-19 (SARS-CoV-2) IgM/IgG:**
A red line appears at control line (C) but no line appears in test region M or test region G.
- Faint pink lines in test regions M or G are considered positive.

- **Invalid Result:**

If no red line appears at control line (C), the test is invalid. It is recommended that the specimen be retested with a new test device.



10. Positive and Negative Controls

Positive Control / Negative Controls kits are sold separately. They are manufactured by Nirmidas, Inc. and can be purchased using Catalog #NBPC-0009. It is recommended that both controls be processed on the Nirmidas COVID-19 (SARS-CoV-2) IgM/IgG Antibody Detection Kit under the following circumstances:

- A new operator uses the test kits for the first time.
- A new shipment of test kits is received.
- Device storage falls out of the 4-25°C range.
- To verify a higher or lower than expected frequency of positive or negative results.
- To investigate the cause of repeated invalid results
- A new test environment is used.
- If the expected control results are not achieved, repeat the control solution with a new test. If still not correct, contact Nirmidas at support@nirmidas.com

11. WARNING AND PRECAUTIONS

- *This test has not been FDA cleared or approved; this test has been authorized by FDA under an EUA for use by laboratories certified under CLIA, that meet requirements to perform moderate or high complexity tests.*
- *This test has been authorized only for the presence of IgM and IgG antibodies against SARS-CoV-2, not for any other viruses or pathogens.*
- *This test is only authorized for the duration of the declaration that*

circumstances exist justifying the authorization of emergency use of in vitro diagnostic tests for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug and Cosmetic Act, 21 U.S.C. § 360bbb3(b)(1), unless the authorization is terminated or revoked sooner.

- It is recommended that all specimens be handled in accordance with Biosafety Level 2 practices as described in the CDC/NIH Publication, Biosafety in Microbiological and Biomedical Laboratories or other equivalent guidelines.
- Always wear gloves when performing this procedure and treat all specimens and used devices as potentially infectious.
- The foil pouch containing the Test Card must be completely sealed. Do not use if foil pouch seal is not intact.
- Do not use the test device beyond the expiration date.
- Do not reuse the test device. It is for single-use.
- Contact the manufacturer if you have any questions during the use of this product.

12. LIMITATIONS

- *The test is only to be used in CLIA certified laboratories and not in point-of-care or at-home testing settings.*
- *Performance has only been established with the specimen types listed in the Intended Use. Other specimen types have not been evaluated and should not be used with this assay.*
- *Results from antibody testing should not be used to diagnose or exclude acute SARS-CoV-2 infection or to inform infection status.*
- *It is not known at this time if the presence of antibodies to SARS-CoV-2 confers immunity to re-infection.*
- *SARS-CoV-2 IgG antibodies may be below detectable levels in patients who have been exhibiting symptoms for less than 15 days. SARS-CoV-2 IgM antibodies may be below detectable levels in patients who have been exhibiting symptoms for less than 8 days.*
- *Testing with a molecular diagnostic should be performed to evaluate for acute SARS-CoV-2 infection in symptomatic individuals.*
- *A negative result for an individual subject indicates absence of detectable anti-SARS-CoV-2 antibodies. Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Antibodies may not be detected in the first several days of infection; the sensitivity of the Nirmidas COVID-19 (SARS-CoV-2) IgM/IgG Antibody Detection Kit early after infection is unknown. False positive results for IgM and IgG antibodies may occur due to cross-reactivity from pre-existing antibodies or other possible causes.*
- *A positive result may not indicate previous SARS-CoV-2 infection. Consider other information including clinical history and local disease prevalence, in*

assessing the need for a second but different serology test to confirm an immune response.

- *A negative result can occur if the quantity of the anti-SARS-CoV-2 antibodies present in the specimen is below the detection limits of the assay, or the antibodies that are detected are not present during the stage of disease in which a sample is collected.*
- *Positive results may be due to past or present infection with non-SARS-CoV-2 coronavirus strains such as HKU1, OC43, NL63, or 229E.*
- *Cross-reactivity may also occur due to past or present infection with Flu A, Flu B, CMV, HSV, EBV, or presence of ANA.*
- *Not for the screening of donated blood.*

13. CONDITIONS OF AUTHORIZATION FOR THE LABORATORY

The Nirmidas COVID-19 (SARS-CoV-2) IgM/IgG Antibody Detection Kit Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Recipients, and authorized labeling are available on the FDA website:

<https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/in-vitro-diagnostics-euas>.

Authorized laboratories using the Nirmidas COVID-19 (SARS-CoV-2) IgM/IgG Antibody Detection Kit ("the product" in the conditions below), must adhere to the Conditions of Authorization indicated in the Letter of Authorization as listed below:

- *Authorized laboratories* using the product will include with test result reports, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.*
- *Authorized laboratories using the product will use the product as outlined in the authorized labeling. Deviations from the authorized procedures, including the authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to use the product are not permitted.*
- *Authorized laboratories that receive the product will notify the relevant public health authorities of their intent to run the product prior to initiating testing.*
- *Authorized laboratories using the product will have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.*
- *Authorized laboratories will collect information on the performance of the product and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and Nirmidas Biotech, Inc (<https://www.nirmidas.com/technical-support> or info@nirmidas.com) any suspected occurrence of false positive or false negative results and significant*

deviations from the established performance characteristics of the product of which they become aware.

- All laboratory personnel using the product must be appropriately trained in immunochromatographic techniques and use appropriate laboratory and personal protective equipment when handling this kit and use the product in accordance with the authorized labeling. All laboratory personnel using the assay must also be trained in and be familiar with the interpretation of results of the product.
- Nirmidas Biotech, Inc, authorized distributors, and authorized laboratories using the product will ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.

*The letter of authorization refers to, "Laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform moderate or high complexity tests" as "authorized laboratories."

14. PERFORMANCE SUMMARY

A retrospective clinical study was performed evaluating 86 stored unique clinical specimens positive for COVID-19 (by PCR), 58 clinical specimens negative for COVID-19 (by PCR), and 424 clinical samples collected pre-outbreak.

Table 1a: Negative agreement for specificity for IgG/IgM. Serology testing performed on samples from COVID-19 PCR negative samples.

# PCR negative at any time	Nirmidas IgG/IgM Results		
	# of negative results	NPA	95% CI
58	55	94.83%	85.86% - 98.23%

Table 1b: Negative agreement for specificity for IgG/IgM. Serology testing performed on samples from preCOVID-19 banked samples. A few samples were collected before 2014. Most samples (at least 403) were collected between 2017 to 2019.

# preCOVID-19 samples	Nirmidas IgG/IgM Results		
	# of negative results	NPA	95% CI
424	415	97.88%	96.02% - 98.88%

Table 2a: Positive Agreement of IgG using 86 stored clinical samples (23 plasma and 63 serum), according to days post symptoms onset. All samples are SARS-2 PCR positive.

Days post symptom onset	# PCR positive at any time	Nirmidas IgG Results		
		# of positive results	PPA	95% CI
0 - 7	18	5	27.78%	12.50% – 50.87%
8 - 14	34	26	76.47%	60.00% – 87.56%
≥15	34	34	100%	89.85% - 100%

Table 2b: Positive Agreement of IgM using 86 stored clinical samples (23 plasma and 63 serum), according to days post symptoms onset. All samples are SARS-2 PCR positive.

Days post symptom onset	# PCR positive at any time	Nirmidas IgM Results		
		# of positive results	PPA	95% CI
0 - 7	18	5	27.78%	12.50% – 50.87%
8 - 14	34	28	82.35%	66.49% – 91.65%
≥15	34	33	97.06%	85.08% – 99.48%

Independent Clinical Agreement Validation Study

The Nirmidas COVID-19 (SARS-CoV-2) IgM/IgG Antibody Detection Kit was tested on July 9 and September 11, 2020 at the Frederick National Laboratory for Cancer Research (FNLCR) sponsored by the National Cancer Institute (NCI). The test was validated against a panel of previously frozen samples consisting of 58 SARS-CoV-2 antibody-positive serum samples and 97 antibody-negative serum and plasma samples. Each of the 58 antibody-positive samples were confirmed with a nucleic acid amplification test (NAAT) and both IgM and IgG antibodies were confirmed to be present in all 58 samples. The presence of antibodies in the samples was confirmed by several orthogonal methods prior to testing with the Nirmidas COVID-19 (SARS-CoV-2) IgM/IgG Antibody Detection Kit. The presence of IgM and IgG antibodies specifically was confirmed by one or more comparator methods. Antibody-positive samples were selected at different antibody titers. All antibody-negative samples were collected prior to 2020 and include: i) Eighty-seven (87) samples selected without regard to clinical status, "Negatives" and ii) Ten (10) samples selected from banked serum from HIV+ patients, "HIV+".

Testing was performed by one operator using one lot of the Nirmidas COVID-19 (SARS-CoV-2) IgM/IgG Antibody Detection Kit. Confidence intervals for sensitivity and specificity were calculated per a score method described in CLSI EP12-A2 (2008).

For evaluation of cross-reactivity with HIV+, it was evaluated whether an increased false positive rate among antibody-negative samples with HIV was statistically higher than the false positive rate among antibody-negative samples without HIV (for this, a confidence interval for the difference in false positive rates was calculated per a score method described by Altman). Study results and summary statistics are presented in Table 3a and Table 3b, respectively.

Table 3a: Summary results of independent evaluation.

Nirmidas COVID-19 (SARS-CoV-2) IgM/IgG Antibody Detection Kit	Comparator Method			Total
	Positive (IgM/IgG) +	Negative (IgM/IgG)-	Negative, HIV+	
IgM+/IgG+	49			49
IgM+/IgG-	5	1	1	7
IgM-/IgG+	2			2
IgM-/IgG-	2	86	9	97
Total	58	87	10	155

Table 3a: Summary statistics of independent evaluation.

Measure	Estimate	95% CI
IgM Sensitivity	93.1% (54/58)	(83.6%; 97.3%)
IgM Specificity	97.9% (95/97)	(92.8%; 99.4%)
IgG Sensitivity	87.9% (51/58)	(77.1%; 94.0%)
IgG Specificity	100.0% (97/97)	(96.2%; 100%)
Combined PPV for prevalence = 5.0%	71.1%	(39.2% - 90.2%)
Combined NPV for prevalence = 5.0%	99.8%	(99.3% - 99.9%)
Cross-reactivity with HIV+	10.0% (1/10), may be present	

Limitations of the study:

- Samples were not randomly selected, and sensitivity and specificity estimates may not be indicative of the real-world performance of the device.
- These results are based on serum and plasma samples only and may not be

indicative of performance with other sample types, such as whole blood, including finger stick blood.

- The number of samples in the panel is a minimally viable sample size that still provides reasonable estimates and confidence intervals for test performance, and the samples used may not be representative of the antibody profile observed in patient populations.

Class Specificity:

Using 5 samples (testing with 2 replicas each), DTT removal experiments were conducted to remove IgM from samples positive with both IgG and IgM. After DTT treatment, the IgM switched from positive to negative, and IgG results remained positive. This was 100% in agreement with expected outcomes.

Matrix equivalencies

Studies were performed with venous serum, and venous plasma (lithium heparin and dipotassium EDTA) using contrived negative, low positive, and positive samples. Five complete sets of matrices were used in the study, and each assay was performed twice. The agreement was 100% between all matrices compared to serum (and across all matrices).

CONTACT INFORMATION

For technical and product related questions, please contact
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 Tel: +1 (669) 207-9813
 Email: info@nirmidas.com

**Nirmidas COVID-19 (SARS-CoV-2) IgM IgG Positive Control and Negative Controls Kit
(NBPC-0009)**

Instruction for Use

For use under an Emergency Use Authorization Only.

This test has not been FDA cleared or approved; this test has been authorized by FDA under an EUA for use by laboratories certified under CLIA, that meet requirements to perform moderate or high complexity tests.

This test has been authorized only for the presence of IgM and IgG antibodies against SARS-CoV-2, not for any other viruses or pathogens. This test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostic tests for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.

1. Intended Use

The Nirmidas SARS-CoV-2 IgM IgG Positive Control and Negative Control Kit is intended for use as an assayed quality control to monitor the performance of the Nirmidas COVID-19 IgM IgG Antibody Detection Kit (controls sold separately). The performance characteristics of the SARS-CoV-2 IgM IgG Positive Control and Negative Control Kit have not been established for any other assays or instrument platforms.

2. Materials Provided

<p align="center">Positive Control Solution (20 x 50 µL)</p>	<p align="center">Twenty (20), each sufficient for 4 tests. Human serum/plasma reactive for SARS-CoV-2 IgM and IgG antibodies with sodium azide preservative. Single Use Only. Ready to use.</p>
<p align="center">Negative Control Solution (20 x 50 µL)</p>	<p align="center">Twenty (20) vials, each sufficient for 4 tests. Human serum/plasma non-reactive for SARS-CoV-2 IgM and IgG antibodies with sodium azide preservative. Single Use Only. Ready to use.</p>

3. Storage Conditions

The SARS-CoV-2 IgM IgG Positive Control and Negative Control Kit is shipped at 4°C. Upon receipt, immediately store the kit components at -20°C or lower until the expiration date. Once each vial is thawed, use within 24 hours; store at 4°C when not in use.

4. Procedure

- The positive and negative controls are to be treated as a serum/plasma sample and tested following the Instruction for Use provided for the COVID-19 (SARS-CoV-2) IgM IgG Antibody Detection Kit.
- Good laboratory practices include the use of external controls on a regular basis; State and local regulations should be followed.
- Each vial is for single use only, but may be used for up to four (4) times once thawed within

a 24-hour shift.

5. **Warnings and Precautions**

- Controls are not specific to lots of the Nirmidas COVID-19 (SARS-CoV-2) IgM IgG Antibody Detection Assay Kit and may be safely used with multiple Assay Kit lots.
- Do not use kit components beyond the expiration date given on the label.
- All specimens of human origin should be considered potentially infectious and handled with care.
- Observe the normal precautions required for handling all laboratory reagents.
- Waste must be handled with care and disposed of in compliance with laboratory guidelines and the statutory provisions enforced in each country.

Safety Precautions

- Do not eat, drink, smoke or apply cosmetics in the assay laboratory. Do not pipette by mouth.
- Avoid direct contact with potentially infected material by wearing laboratory clothing, protective goggles, and disposable gloves. Wash hands thoroughly at the end of each assay.
- Avoid splashing or forming an aerosol. All drops of biological reagent must be removed with appropriate disinfectants and the means used must be treated as infected waste.