# Clinical validation of a Simple and Fast Immunochromatographic assay for qualitative determination of specific ImmunoGLObulin IgG/IgM Antibodies to 2019- nCoV in whole bLood, serum or plasma specimen.

## Code: I GLOBAL

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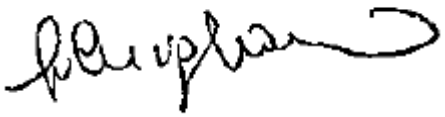
With free support of MEDnoTE that provided kits and High Research for its consultant and organization of the Performance Study Plan.

**PERFORMANCE STUDY PLAN SIGN-OFF PAGE**

I have read and understood protocol and I agree to conduct the study as detailed herein and in compliance with ICH-GCP and applicable ethical requirements

Prof Giuseppe CURIGLIANO MD PhD

Coordinating Investigator (printed name)



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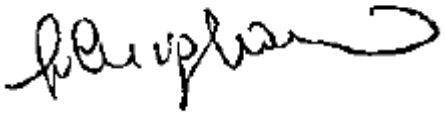
Giuseppe CURIGLIANO

Investigator (printed name)



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Study Type: Observational

Estimated Enrollment: 1000 participants (200 per site)

Observational Model: Cohort

Time Perspective: Prospective

Timelines:

Actual Study Start Date: April 15, 2020

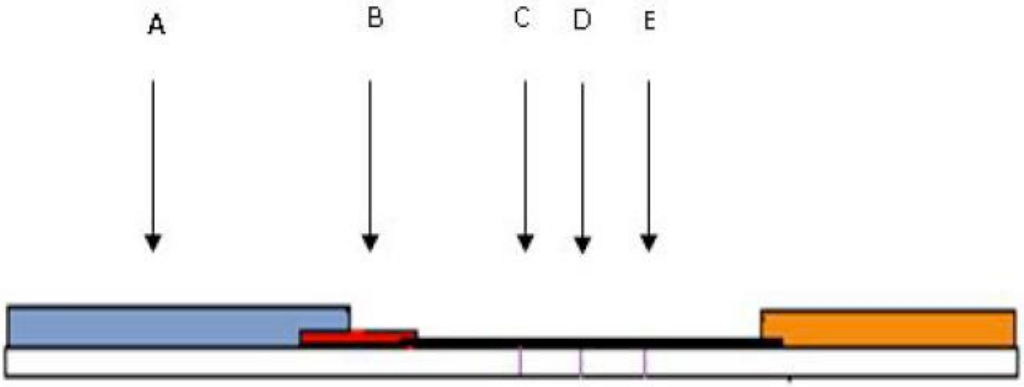
Estimated Primary Completion Date: July 31, 2020

Estimated Study Completion Date: December 31, 2020

## Introduction

The 2019-nCoV IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma) is a qualitative membrane based immunoassay for the detection of IgG and IgM antibodies to 2019-nCoV in whole blood, serum or plasma specimen. This test consists of two components, an IgG component and an IgM component. In the IgG component, anti-human IgG is coated in IgG test line region. During testing, the specimen reacts with 2019-nCoV antigen-coated particles in the test cassette. The mixture then migrates upward on the membrane chromatographically by capillary action and reacts with the anti-human IgG in IgG test line region, if the specimen contains IgG antibodies to 2019-nCoV. A colored line will appear in IgG test line region as a result of this. Similarly, anti-human IgM is coated in IgM test line region and if specimen contains IgM antibodies to 2019-nCoV, the conjugate-specimen complex reacts with anti-human IgM. A colored line appears in IgM test line region as a result. Therefore, if the specimen contains 2019-nCoV IgG antibodies, a colored line will appear in IgG test line region. If the specimen contains 2019-nCoV IgM antibodies, a colored line will appear in IgM test line region. If the specimen does not contain 2019-nCoV antibodies, no colored line will appear in either of the test line regions, indicating a negative result. To serve as a procedural control, a colored line will always appear in the control line region, indicating that the proper volume of specimen has been added and membrane wicking has occurred.

Figure 1: Test principle




As shown in illustration in Fig. 1, the specimen (A) migrates via capillary action along the membrane to react with the gold conjugate (B). 2019-nCoV IgG or/and IgM present in the specimen binds to the conjugate, forming a colored Novel coronavirus antibody-antigen complex. The mouse anti-human IgG and mouse anti-human IgM immobilized in the test zone of the membrane captures the test region (C) and test region (D). The formation of a visible colored line in the test region indicates a positive result (C) or (D). The absence of a colored line in the test zones suggests a negative result. In the control zone of the membrane, immobilized reagents capture colored conjugate regardless of test specimen composition. The resulting visible colored band (E) confirms control line.

Incubation Time: RESULTS AT 10 MINUTES

Storage:

Store the test at 2-30°C. Do not freeze.

***Active ingredient list:***

• Mouse anti-human IgG (capture reagent)

• Mouse anti-human IgM (capture reagent)

• Mouse IgG

• Goat anti-mouse IgG

• 2019-nCov antigen (detection reagent)

***Inactive ingredients list:***

• Adhesive plastic backing

• Buffer

• Label pad

• Absorbent pad

• Sample pad

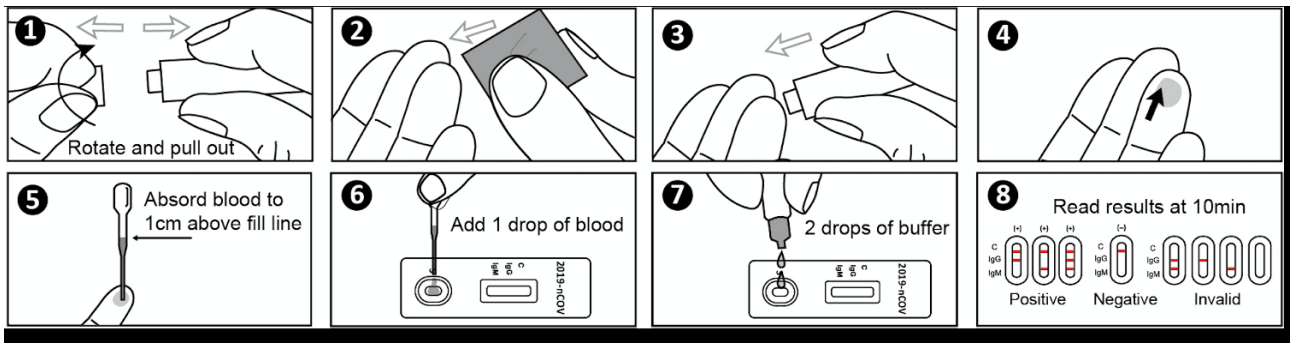
• Nitrocellulose membrane

• Desiccant (in pouch)

• Pouch

• Sample dropper

1.2/Assay procedure:




1) Wash the patient's hand with soap and warm water or clean with an alcohol swab. Allow to dry. Fig 1 and Fig 2.
2) Massage the hand without touching the puncture site by rubbing down the hand towards the fingertip of the middle or ring finger
3) Puncture the finger with a sterile lancet. Wipe away the first sign of blood. Fig 3.
4) Gently rub the hand from wrist to palm to finger to form a rounded drop of blood over the puncture site. Fig 4.
5) Add the Fingerstick Whole Blood specimen to the test by using a capillary tube: Touch the end of the capillary tube to the blood until filled to approximately 20µL. Avoid air bubbles. Fig 5.

6) Place the bulb onto the top end of the capillary tube, then squeeze the bulb to dispense the wholeblood to the specimen well of the test cassette. Fig 6.
7) Dispense two drops of diluent to the specimen well of the test cassette. Fig 7.
8) Read test results after 10 minutes. Fig 8.

**Precautions:**

Bring tests, specimens, buffer, and/or controls to room temperature (15-30°C) before use.

Bring the pouch to room temperature before opening. Remove the test cassette from the sealed pouch and use it within one hour.

Place the test cassette on a clean and level surface.

Testing should be performed immediately after specimen collection. Do not leave the specimens at room temperature for prolonged periods. Whole blood collected by venipuncture should be stored at 2-8°C if the test is to be run within 2 days of collection. Do not freeze whole blood specimens. Whole blood collected by fingerstick should be tested immediately.

Bring specimens to room temperature prior to testing. Frozen specimens must be completely thawed and mixed well prior to testing. Specimens should not be frozen and thawed repeatedly. If specimens are to be shipped, they should be packed in compliance with local regulations covering the transportation of etiologic agents.

EDTA K2, Heparin sodium, Citrate sodium and Oxalate potassium can be used as anticoagulants.

**Interpretation of the results:**

***IgG POSITIVE***:* Two colored lines appear. One colored line should always appear in the control line region (C) and another line should be in the IgG line region.

***IgM POSITIVE***:* Two colored lines appear. One colored line should always appear in the control line region (C) and another line should be in the IgM line region.

***IgG and IgM POSITIVE***:* Three colored lines appear. One colored line should always appear in the control line region (C) and two test lines should be in the IgG line region and IgM line region.

*NOTE: The intensity of the color in the test line regions may vary depending on the concentration of 2019-nCoV antibodies present in the specimen. Therefore, any shade of color in the test line region should be considered positive.

***NEGATIVE***: One colored line appears in the control line region (C). No line appears in the IgG region and IgM region.

***INVALID***: Control line fails to appear. Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control line failure. Review the procedure and repeat the test with a new test. If the problem persists, discontinue using the test kit immediately and contact your local distributor.

**Limitations:**

The 2019-nCoV IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma) is for in vitro diagnostic use only. This test should be used for detection of IgG and IgM antibody to 2019-nCoV in whole blood, serum or plasma specimens. Neither the quantitative value nor the rate of increase in the concentration of IgG or IgM antibodies to 2019-nCoV can be determined by this qualitative test.

The 2019-nCoV IgG/IgM Rapid Test Cassette (Whole blood/Serum/Plasma) will only indicate the presence of IgG and IgM antibodies to 2019-nCoV in the specimen and should not be used as the sole criteria for the diagnosis of 2019-nCoV infections.

As with all diagnostic tests, all results must be considered with other clinical information available to the physician.

If the test result is negative and clinical symptoms persist, additional follow-up testing using other clinical methods is suggested. A negative result at any time does not preclude the possibility of 2019- nCoV infection.

The hematocrit level of the whole blood can affect the test results. Hematocrit level needs to be between 25% and 65% for accurate results.

## Performance study

***Accuracy, specificity, sensitivity and correlation***

The 2019-nCoV IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma) was compared with a leading commercial PCR; the results show that 2019-nCoV IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma) has a high sensitivity and specificity. Results for IgG and IgM are shown in Table 1 and 2 respectively.

*Table 1: Performance of IgG specimen*

| Method | | PCR | | Total result |
|---|---|---|---|---|
| Rapid test cassette | Results | Positive | Negative | |
| | Positive | 20 | 1 | 21 |
| | Negative | 0 | 49 | 49 |
| Total result | | 20 | 50 | 70 |

Specificity = 98.0% (95%CI: 89.4%~99.9%);

Sensitivity = 100% (95%CI: 86.0%~100%);

Accuracy = 98.6% (95%CI: 92.3%~99.96%).

*Table 2: Performance of IgM specimen*

| Method | | PCR | | Total result |
|---|---|---|---|---|
| Rapid test cassette | Results | Positive | Negative | |
| | Positive | 17 | 2 | 19 |
| | Negative | 3 | 48 | 51 |
| Total result | | 20 | 50 | 70 |

Specificity = 96.0% (95%CI: 86.3%~99.5%);

Sensitivity = 85.0% (95%CI: 62.1%~96.8%);

Accuracy = 92.9% (95%CI: 84.1%~97.6%).

The 2019-nCov IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma) products have been compared with a commercial PCR using clinical specimen. The results show that the relative sensitivity of the 2019-nCov IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma) is 98.6% and the relative specificity is 92.7%.

Refer to data on investigator brochure for other technical data related to interference and cross-reactivity.

**Rational of the study**

In late December 2019, several local health facilities reported clusters of patients with pneumonia of unknown cause that were epidemiologically linked to a seafood and wet animal wholesale market in Wuhan, Hubei Province, China. It is now confirmed that the etiology of this outbreak is a novel coronavirus, namely, SARS-CoV-2. Of critical importance is rapid and simple diagnostic method to be used in clinical settings to timely inform and refine strategies that can prevent, control, and stop the spread of SARS-CoV-2. The 2019-nCoV IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma) is a qualitative membrane based immunoassay for the detection of IgG and IgM antibodies to SARS-CoV-2in whole blood, serum or plasma specimen. This test consists of two components, an IgG component and an IgM component. In the IgG component, anti-human IgG is coated in IgG test line region. During testing, the specimen reacts with 2019-nCoV antigen-coated particles in the test cassette. The mixture then migrates upward on the membrane chromatographically by capillary action and reacts with the anti-human IgG in IgG test line region, if the specimen contains IgG antibodies to SARS-CoV-2.

**Aim of the study**

To validate in a real life study a self diagnostic assay for SARS-CoV-2with the advantages of high speed, simple operation and low cost, and overcomes the shortcomings of the existing molecular detection methods. Clinical specimens from patients who were suspected of being infected with 2019-nCoV will be used to evaluate the performance of the assay. In parallel, we will also use molecular assays for the detection of the presence of the viral RNA from nasopharyngeal swabs since PCR is currently the gold standard.

Primary endpoints

1) To estimate the rate of SARS-CoV-2 positive cancer patients (undergoing chemotherapy, immunotherapy, biological therapy, radiotherapy or experimental treatments or major surgery) and health professionals in a comprehensive cancer center or in a cancer setting. Patients positive are serially tested with SARS-CoV-2 IgM / IgG Rapid Test to evaluate the immune response in IgG negative patients and the reliability of the test in those patients who develop clinical signs of SARS-CoV-2 during the trial.

2) To validate the accuracy of self diagnostic assay by confirmation of IgM and/or IgG positive test with the current gold standard RT-PCR for positive nucleic acid detection of new coronavirus from nasopharyngeal swabs.

3) To perform on plasma samples multiplex immunoassay protein quantitation related to markers of inflammation and immunity (IL-6, TNF, INFy, IL1, IL-2). 2.5 ml of plasma will be needed.

Secondary endpoint

A sample of 20 patients in each Centre will be selected by the physician to be monitor using a new cutting-edge medical device working the telemedicine approach. A telehealth virtual office visit can be performed from the patient's home or workplace, decreasing time spent traveling to visit site, time spent in waiting room, and cost to patient. Studying telehealth may improve quality of life in patients during the therapy visit, especially in this SARS-CoV-2 pandemia.

**Study Population**

Patients or health professionals already tested positive for the viral RNA from nasopharyngeal swabs by RT-PCR. Patients or health professional who are suspected of being infected with SARS-CoV-2 in the hospital. Patients who are considered at high risk for infection and eligible for active therapy and major surgery.

**Criteria**

***Inclusion Criteria:***

**1. Suspected cases**

Meet the following 2 at the same time:

a) Epidemiological history: There was a history of contact with confirmed cases before the onset of illness; or subjects with at least *one symptom* in the last week before accrual in the trial. Subjects that have been in contact with people positive for SARS-CoV-2 in the previous 14 days.

b) Clinical manifestations are defined as :

Fever >37.5°; dry cough, muscle aches and/or fatigue, anosmia, subjects with respiratory distress (Respiratory Rate >25/min or O2 Saturation <92%)

or imaging characteristics of pneumonia;

or the total number of white blood cells is normal or decreased with the lymphocyte count decreased in the early stage of onset or there is an abnormal C-Reactive protein. Other symptoms that clinical investigator will relate to SARS-CoV-2 infection.

Subject or cancer patients who have been quarantined for suspect symptoms and have access to hospital to continue therapy or to receive major surgery

**2. Confirmed cases**

Patients or subjects with positive RT-PCR for SARS-CoV-2. On the basis of meeting the criteria for suspected cases, sputum, throat swabs, lower respiratory tract secretions, and other specimens were tested by real-time RT-PCR for positive nucleic acid detection of new coronavirus; or viral gene sequencing was highly homologous with known new coronaviruses. Patients positive are serially tested with SARS-CoV-2 IgM / IgG Rapid Test to evaluate the immune response in IgG negative patients and the reliability of the test in those patients who develop clinical signs of SARS-CoV-2 during the trial.

3. Patients who are considered at high risk for infection and eligible for active therapy and major surgery

- Frailty (age and multiple comorbidities) planned to receive a standard systemic anticancer treatment comprising chemotherapy and/or immunotherapy and/or radiation therapy or to receive an experimental treatment
- Major surgery or surgery after neoadjuvant chemotherapy and or chemo/radiotherapy

***Exclusion Criteria:***

1. Ascertained influenza virus, parainfluenza virus, adenovirus, respiratory syncytial virus, rhinovirus, human metapneumovirus, SARS coronavirus, and other known other viral pneumonia;

2. Ascertained mycoplasma pneumoniae, chlamydia pneumonia, and bacterial pneumonia; non-infectious diseases such as vasculitis, dermatomyositis, and organizing pneumonia.

**Clinical considerations**

All patients with epidemiologic history and/or clinical manifestations suspected for COVID will be proposed, according to the clinical judgement, for RT-PCR test for positive nucleic acid detection, irrespective of serological evidence.

All patients tested as IgM positive will have a confirmatory real-time RT-PCR test for positive nucleic acid detection of new coronavirus on sputum, throat swabs, lower respiratory tract secretions, and other specimens.

The 2019-nCov IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma) will be repeated in patients IgM positive for confirming the subsequent IgG positivity.

The tests will be performed locally in each hospital laboratory.

The ASST of Cremona laboratory could perform:

- Dr Sophie Testa, UO Laboratorio Analisi – ASST di Cremona: SARS-CoV-2 IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma) also for other sites. 1 ml of plasma will be needed;
- Dr Michele Francaviglia- SMeL849 – ASST di Cremona: *multiplex* immunoassay protein quantitation related to markers of inflammation and immunity (IL-6,TNF, INFy, IL1, IL-2, ect). 2.5 ml of plasma will be needed.

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The lab responsible person in Milano IEO is:

Dr.ssa Rita Passerini
Divisione di Medicina di Laboratorio
Istituto Europeo di Oncologia

**Statistical Plan**

All data recorded will be summarized in tables, graphs and listings. In general, categorical data will be presented using counts and percentages, whilst continuous variables will be presented using the mean, standard deviation, median, minimum, maximum and number of patients.

In tabulations, denominators for calculation of percentages will be taken as the number of non-missing responses.

Primary end-point, the accuracy of tests, will be sensitivity and specificity and they will be calculated for all tests and compared with the appropriate statistical test.

**References**

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