2019-nCoV IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma)
Package Insert

REF INCP-402B	English

A rapid test for the qualitative detection of IgG and IgM antibodies to 2019-nCoV in human whole blood, serum or plasma specimens.
For professional in vitro diagnostic use only.

【INTENDED USE】

The 2019-nCoV IgG/IgM Rapid Test Cassette is a lateral flow chromatographic immunoassay for the qualitative detection of IgG and IgM antibodies to 2019-nCoV in human whole blood, serum or plasma specimen.

【SUMMARY】

Early January 2020, a novel coronavirus (2019-nCoV) was identified as the infectious agent causing an outbreak of viral pneumonia in Wuhan, China, where the first cases had their symptom onset in December 2019.[1]

Coronaviruses are enveloped RNA viruses that are distributed broadly among humans, other mammals, and birds and that cause respiratory, enteric, hepatic, and neurologic diseases.[2] Six coronavirus species are known to cause human disease.[3] Four viruses — 229E, OC43, NL63, and HKU1 — are prevalent and typically cause common cold symptoms in immunocompetent individuals.[3] The two other strains — severe acute respiratory syndrome coronavirus (SARS-COV) and Middle East respiratory syndrome coronavirus (MERS-COV) — are zoonotic in origin and have been linked to sometimes fatal illness.[4]

Coronaviruses are zoonotic, meaning they are transmitted between animals and people.

Common signs of infection include respiratory symptoms, fever, cough, shortness of breath and breathing difficulties. In more severe cases, infection can cause pneumonia, severe acute respiratory syndrome, kidney failure and even death.[5]

Standard recommendations to prevent infection spread include regular hand washing, covering mouth and nose when coughing and sneezing, thoroughly cooking meat and eggs. Avoid close contact with anyone showing symptoms of respiratory illness such as coughing and sneezing. [5]

【PRINCIPLE】

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.

【REAGENTS】

The test contains anti-human IgM and anti-human IgG as the capture reagent, 2019-nCoV antigen as the detection reagent. A goat anti-mouse IgG is employed in the control line system.

【PRECAUTIONS】

1. For professional *in vitro* diagnostic use only. Do not use after expiration date.
2. Do not eat, drink or smoke in the area where the specimens or kits are handled.
3. Do not use test if pouch is damaged.
4. Handle all specimens as if they contain infectious agents. Observe established precautions against microbiological hazards throughout all procedures and follow the standard procedures for proper disposal of specimens.
5. Wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are assayed.
6. Please ensure that an appropriate amount of samples are used for testing. Too much or too little sample size may lead to deviation of results.
7. The used test should be discarded according to local regulations.
8. Humidity and temperature can adversely affect results.

【STORAGE AND STABILITY】

Store as packaged in the sealed pouch at room temperature or refrigerated (2-30°C). The test is stable through the expiration date printed on the sealed pouch. The test must remain in the sealed pouch until use. **DO NOT FREEZE.** Do not use beyond the expiration date.

【SPECIMEN COLLECTION AND PREPARATION】

- The 2019-nCoV IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma) can be performed using whole blood (from venipuncture or fingerstick), serum or plasma.
- To collect **Fingerstick Whole Blood Specimens**:
  - Wash the patient's hand with soap and warm water or clean with an alcohol pad. Allow to dry.
  - Massage the hand without touching the puncture site by rubbing down the hand towards the fingertip of the middle or ring finger.
  - Puncture the skin with a lancet. Wipe away the first sign of blood.
  - Gently rub the hand from wrist to palm to finger to form a rounded drop of blood over the puncture site.
  - 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.
- Separate serum or plasma from blood as soon as possible to avoid hemolysis. Use only clear non-hemolyzed specimens.
- Testing should be performed immediately after the specimens have been collected. Do not leave the specimens at room temperature for prolonged periods. Serum and plasma specimens may be stored at 2-8°C for up to 7 days, for long term storage, serum/plasma specimens should be kept below -20°C. 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 etiological agents.
- EDTA K2, Heparin sodium, Citrate sodium and Potassium Oxalate can be used as the anticoagulant for collecting the specimen.

【MATERIALS 】

Materials provided

- Test cassettes
- Package insert
- Buffer

Materials required but not provided

- Specimen collection containers
- Lancets (for fingerstick whole blood only)
- Capillary tubes
- Droppers
- Centrifuge (for plasma only)
- Timer
- Pipette

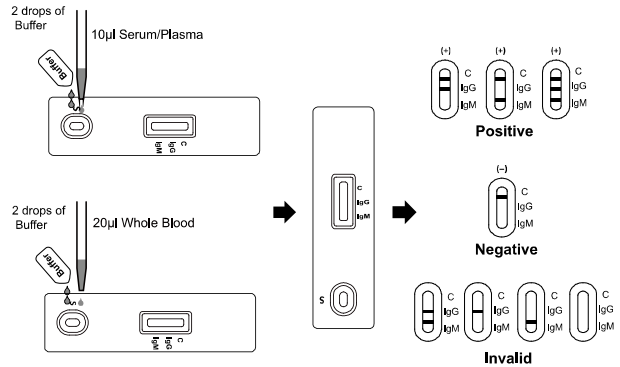
【DIRECTIONS FOR USE】

Allow the test, specimen, buffer and/or controls to reach room temperature (15-30°C) prior to testing.

1. Remove the test cassette from the foil pouch and use it within one hour. Best results will be obtained if the test is performed immediately after opening the foil pouch.
2. Place the cassette on a clean and level surface.
   For **Serum or Plasma** specimen:
   - To transfer **10 μL** of specimen to the specimen well(**S**) with a pipette or a dropper, then **add 2 drops of buffer** (approximately 80 μL), and start the timer

   For **Venipuncture or Fingerstick Whole Blood** specimen:
   - To transfer **20 μL** of specimen to the specimen well(**S**) with a pipette or a dropper, then **add 2 drops of buffer** (approximately 80 μL), and start the timer
3. Wait for the colored line(s) to appear. **Read results at 10 minutes.** Do not interpret the result after 20 minutes.

Note: It is suggested not to use the buffer, beyond 6 months after opening the vial.



【INTERPRETATION OF 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.

【QUALITY CONTROL】

Internal procedural controls are included in the test. A colored line appearing in the control region (C) is an internal procedural control. It confirms sufficient specimen volume and correct procedural technique. Control standards are not supplied with this kit; however, it is recommended that positive and negative controls be tested as a good laboratory practice to confirm the test procedure and to verify proper test performance.

【LIMITATIONS】

1. 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.
2. 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.
3. As with all diagnostic tests, all results must be considered with other clinical information available to the physician.
4. 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.
5. The hematocrit level of the whole blood can affect the test results. Hematocrit level needs to be between 25% and 65% for accurate results.
6. The test will show negative results under the following conditions: The titer of the novel coronavirus antibodies in the sample is lower than the minimum detection limit of the test, or the novel coronavirus antibody has not appeared at the time of sample collection (Asymptomatic stage).

【PERFORMANCE CHARACTERISTICS】

Sensitivity and Specificity

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.

IgG Result

Method		PCR		Total Results
2019-nCoV IgG/IgM Rapid Test	Results	**Positive**	**Negative**	
	Positive	20	1	21
	Negative	0	49	49
Total Result		20	50	70

Relative Sensitivity: 100% (95%CI*: 86.0%-100%) *Confidence Interval
Relative Specificity: 98.0% (95%CI*: 89.4%-99.9%)
Accuracy: 98.6% (95%CI*: 92.3%-99.96%)

IgM Result

Method		PCR		Total Results
2019-nCoV IgG/IgM Rapid Test	Results	**Positive**	**Negative**	
	Positive	17	2	19
	Negative	3	48	51
Total Result		20	50	70

Relative Sensitivity: 85.0% (95%CI*: 62.1%-96.8%) *Confidence Interval
Relative Specificity: 96.0% (95%CI*: 86.3%-99.5%)
Accuracy: 92.9% (95%CI*: 84.1%-97.6%)

Cross-reactivity

The 2019-nCoV IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma) has been tested for anti-influenza A virus, anti-influenza B virus, anti-RSV, anti-Adenovirus, HBsAg, anti-Syphilis, anti-H. Pylori, anti-HIV and anti-HCV positive specimens. The results showed no cross-reactivity.

Interfering Substances

The following compounds have been tested using the 2019-nCoV IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma) and no interference was observed.

Triglyceride: 50 mg/dL Ascorbic Acid: 20mg/dL Hemoglobin 1000mg/dL
Bilirubin: 60mg/dL Total cholesterol : 6mmol/L

【BIBLIOGRAPHY】

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2. Weiss SR, Leibowitz JL. Coronavirus pathogenesis. Adv Virus Res 2011;81:85-164. PMID:22094080 DOI:10.1016/B978-0-12-385885-6.00009-2
3. Su S, Wong G, Shi W, et al. Epidemiology, genetic recombination, and pathogenesis of coronaviruses. Trends Microbiol 2016;24:490-502. PMID:27012512 DOI:10.1016/j.tim.2016.03.003
4. Cui J, Li F, Shi ZL. Origin and evolution of pathogenic coronaviruses. Nat Rev Microbiol 2019;17:181-192.PMID:30531947 DOI:10.1038/s41579-018-0118-9
5. World Health Organization (WHO). Coronovirus. https://www.who.int/health-topics/coronavirus

Index of Symbols

IVD	For in vitro diagnostic use only	Σ	Tests per kit	EC REP	Authorized Representative
2°C 30°C	Store between 2-30°C		Use by		Do not reuse
	Do not use if package is damaged	LOT	Lot Number	REF	Catalog #
	Manufacturer		Consult Instructions For Use		

Hangzhou AllTest Biotech Co., Ltd.
#550, Yinhai Street
Hangzhou Economic & Technological Development Area
Hangzhou - 310018, P. R. China
www.alltests.com.cn

EC REP
MedNet GmbH
Borkstrasse 10
48163 Muenster
Germany

WARNING STATEMENT

• 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 or other interference factors.
• Not for the screening of donated blood.

Number: 146222400
Effective Date: 2020-03-20

EC Declaration of Conformity

Manufacturer:
Name: HANGZHOU ALLTEST BIOTECH CO., LTD.
Address: #550, Yinhai Street, Hangzhou Economic & Technological Development Area, Hangzhou -310018, P.R. China

European Representative:
Name: MedNet GmbH
Address: Borkstrasse 10, 48163 Muenster, Germany

Product Name: 2019-nCOV (Novel Coronavirus) IgG/IgM Rapid Test
(Whole Blood/Serum/Plasma)
Model: Cassette
Classification: Other Device of IVDD 98/79/EC
Conformity Assessment Route: IVDD 98/79/EC Annex III
EDMA Code: 15 70 90 90 00

We, HANGZHOU ALLTEST BIOTECH CO., LTD., herewith declare that we are exclusively responsible for this declaration of conformity. We herewith declare that the above mentioned products meet the transposition into national law, the provisions of the following EC Council Directives and Standards. All supporting documentations are retained under the premises of the manufacturer.

DIRECTIVES

General applicable directives:

DIRECTIVE 98/79/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 27 October 1998 on in vitro diagnostic medical devices

Standard Applied: EN ISO 13485:2016, EN ISO 14971:2012, EN 13975:2003, EN ISO 18113-1:2011, EN ISO 18113-2:2011, EN 13612:2002/AC:2002, EN ISO 17511:2003, EN ISO 23640:2015, EN 13641:2002, EN ISO 15223-1:2016

Place, Date of Issue: in Hangzhou on 16/02/2020

Signature: Soar Gao
Name: Soar Gao (Position: General Manager)

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INFORME SOBRE ESTRATEGIA DE DIAGNÓSTICO MICROBIOLÓGICO DEL COVID-19 (Actualización)

Madrid 19 de abril de 2020

Estrategia diagnóstica actual. La técnica de PCR

El diagnóstico microbiológico del COVID-19 se ha basado hasta ahora en todo el mundo, en la detección del material genético (**ARN**) **viral del SARS-CoV-2** mediante técnicas de **PCR** (siglas de Reacción en Cadena de la Polimerasa), en muestras respiratorias (**exudado nasofaríngeo** principalmente) de pacientes con síntomas compatibles (*Procedimiento de actuación frente a la enfermedad por SARS-CoV-2. Ministerio de Sanidad*). La PCR es una técnica muy sensible y específica, que se realiza en los laboratorios de microbiología para el diagnóstico de muchas infecciones (*Documento de posicionamiento de la SEIMC sobre el diagnóstico microbiológico de COVID-19*). Por estos motivos, **actualmente la PCR es la técnica diagnóstica de referencia**.

Según los datos recopilados en las últimas semanas, los hospitales y centros sanitarios españoles están realizando al menos **15.000 PCRs al día** desde el comienzo de la pandemia. Según los datos recopilados y notificados al Ministerio de Sanidad por las CCAA, en las semana del 23 al 29 de marzo se hicieron entre **22.000-25.000 PCRs diarias** en los hospitales españoles, cifra que se mantuvo en la semana del 30 de marzo. Las compañías de diagnóstico están distribuyendo semanalmente más de 300.000 reacciones de PCR en los centros sanitarios españoles desde el 20 de marzo de 2020. Tras el aumento de capacidades de los centros sanitarios españoles, las últimas cifras recogidas (semana 13-19 abril) indican que en España se realizan todos los días **entre 30.000 y 50.000 PCRs**.

El número de determinaciones diarias **no implica que se analicen todos esos nuevos casos** sospechosos cada día, ya que también se incluyen PCRs de seguimiento, de alta de enfermos y repeticiones por resultados no concluyentes.

La realización de un número tan elevado de determinaciones conlleva la necesidad de **suministrar de manera continuada no sólo los kits de PCR**, sino otros muchos materiales necesarios como **torundas** y medios de transporte para la toma de muestras, soluciones de inactivación, reactivos de **extracción** y diferentes tipos de material **plástico**. Las determinaciones analíticas con PCR tienen que ser realizadas por personal experimentado y suelen necesitar varias horas hasta obtener resultados.

No obstante, se están tomado medidas para poder **aumentar estas capacidades** de análisis de muestras por PCR. Estas medidas son:

1. **Validación clínica de kits de PCR** comerciales desarrollados por empresas **españolas**. Hasta la fecha se han validado kits de cuatro compañías españolas (GENOMICA, VIRCELL, CERTEST y GENETIC PCR SOLUTIONS) y se ha localizado otra empresa española con una técnica ya validada (PROGENIE MOLECULAR). Estas empresas están produciendo 80.000 reacciones de PCR semanales y sus capacidades máximas se calculan en 675.000 reacciones semanales. Además hay kits de otras compañías españolas en fases finales de diseño que serán validados por el ISCIII en próximas semanas.

2. Validación clínica de **reactivos de extracción** de empresas españolas (empresas VIRCELL, CANVAX y MASTER DIAGNOSTICA en proceso de validación en el ISCIII).
3. Desarrollo de procedimientos de PCR que ahorren pasos en el procesamiento de muestras, **evitando el paso de la extracción** de ácidos nucleicos. No se han obtenido resultados satisfactorios con ninguno de los protocolos ensayados ya que se inhibe un porcentaje relevante de PCRs. **Las técnicas sin extracción sólo deben usarse como último recurso en situaciones en las que no haya otra solución**.
4. **Capacitación** de **centros** de **investigación y universidades, y de centros veterinarios o militares,** como laboratorios de apoyo de análisis de muestras clínicas con COVID-19 coordinados por la autoridades sanitarias de cada CCAA. Hasta la fecha **19** centros ya están en activo. Otros centros están en fases avanzadas de capacitación y podrían activarse en próximas fechas.
5. **Aumento de la automatización de las técnicas de PCR.** Se está en contacto con empresas que suministran reactivos en formato automatizado (los llamados robots o plataformas) para que incrementen la producción de reactivos en este formato y, de esta forma, aumentar la realización de tests. Las compañías que tienen esta capacidad son multinacionales (Roche, Abbot, Thermofisher entre otras). Hasta la fecha se ha conseguido que **17 hospitales españoles puedan empezar a trabajar con robots** COBAS 6800 de Roche, aunque la cantidad de suministros que llegan en este formato es aún limitada (3000 PCRs al día). Por otro lado, por iniciativa de varias empresas e investigadores, el ISCIII ha recibido la donación de **4 robots Opentrons**. Estos han sido emplazados en los hospitales de La Paz de Madrid, Clinic de Barcelona y Vall d´Hebrón de Barcelona, y el último en el propio ISCIII. Estos robots se están poniendo en funcionamiento y tienen la capacidad máxima de realizar 2400 PCRs al día cada uno.

Por las razones expuestas en los párrafos precedentes, la estrategia diagnostica principal debe ser **aumentar significativamente las capacidades para realizar más análisis de PCR**, ya que permite detectar y descartar casos con gran fiabilidad. Además continúa siendo el criterio definitivo de curación (PCR negativa) y, por el momento, es la única forma de asegurar que una persona no es contagiosa.

Test rápidos de detección del COVID-19. ¿Qué son?

Hasta la fecha se han comercializado un total de **114 técnicas en la UE** para detectar el COVID-19 que no están basadas en la amplificación de ácidos nucleicos**, 13 antigénicas y 101 de anticuerpos**. La mayor parte utilizan el formato de test rápido de detección basándose en la técnica de **inmunocromatografía** de difusión (lateral-flow) marcada con oro coloidal en pequeños kits para hacer las determinaciones individualmente. También existen dos modalidades de inmunocromatografía, las que **detectan antígenos y las de anticuerpos**, siendo estas últimas las más comunes (90% de las comercializadas). Las pruebas rápidas de detección del COVID-19 permiten obtener resultados en 15 minutos y tienen un formato que el personal sanitario puede realizar con facilidad.

Los **antígenos** son componentes del virus, generalmente proteínas, y suelen detectarse en los primeros días de infección cuando el virus se encuentra en las vías respiratorias altas. Las técnicas de **anticuerpos** detectan la respuesta inmune de los pacientes y aumentan según

avanza la infección, por lo son detectables más tardíamente, cuando la infección está más avanzada.

Todas estas técnicas rápidas para el **diagnóstico del COVID-19** han obtenido las diferentes **certificaciones** necesarias para ser utilizadas como técnicas de diagnóstico clínico, incluyendo el marcado CE, que obliga a realizar estudios de **validación clínica**. En estos estudios de validación se han utilizado como resultados de referencia los obtenidos por PCR. Con estos resultados como comparador, los fabricantes de casi todas las técnicas rápidas declaran en su documentación unos porcentajes de sensibilidad por **encima del 80% y los de especificidad superan el 95%**.

Fiabilidad de las técnicas rápidas de COVID-19

En este momento **no se dispone de experiencia** a nivel internacional que pueda resultar de utilidad para la toma de decisiones con respecto a la utilización de técnicas de diagnóstico rápido. En China y Corea del Sur no se utilizaron masivamente, ya que se estaba desarrollando en los momentos más críticos de la pandemia. En ambos países, la estrategia de diagnóstico microbiológico que se desplegó, se sustentó en **la realización de PCR, la técnica de referencia**. En China en un elevado número de casos moderados y graves, el diagnóstico se sustentó en datos clínicos y radiológicos, considerándose que ante la alta prevalencia no era imprescindible la confirmación microbiológica. En Corea se utilizó un sistema de obtención de muestras rápido ("drive-throug") pero la muestras se analizaron utilizando PCR.

Apenas existen **publicaciones** científicas que avalen su utilización. Los estudios publicadas en revistas médicas reportan datos que indican que alrededor de un 50-60% de los enfermos tienen anticuerpos detectables en la primera semana de infección, ascendiendo a más del 90% tras unos 10 días de infección y a casi el 100% tras dos semanas del contagio. Sobre las técnicas de antígenos hay menos información.

Estudios de fiabilidad de las técnicas rápidas de detección del COVID

Las técnicas rápidas también tienen **limitaciones** por lo que se ha observado cuando se han desarrollado para otras infecciones. Las técnicas de detección de antígenos disponibles para otras infecciones respiratorias virales (gripe, adenovirus, etc.) tienen una **sensibilidad variable** (40-60 % en muchos casos), y suelen ser más útiles en las primeras fases de la infección, cuando pueden detectarse proteínas y otros componentes virales en muestras respiratorias de pacientes con síntomas catarrales. Las de detección de anticuerpos son más útiles en fases más avanzadas de la enfermedad, cuando se generan en el cuerpo humano anticuerpos como respuesta a la infección. Los fabricantes de técnicas de anticuerpos indican que pueden realizarse con sangre, suero o plasma y en algunos casos utilizando la digitopunción.

Por ello, el Instituto de Salud Carlos III y varios hospitales del SNS están realizando **estudios de fiabilidad de las técnicas diagnósticas rápidas de COVID-19** que han llegado a España adquiridas o donadas al Ministerio de Sanidad y las CCAA.

Hasta la fecha se han realizado estudios de fiabilidad con **seis técnicas rápidas** de COVID-19.

Los resultados más significativos se resumen a continuación:

1. Todas las técnicas evaluadas tienen **licencia para diagnóstico clínico**, marcado CE y sensibilidad >83% y especificidad >95%, según estudios presentados por fabricantes
2. Se han evaluado hasta ahora **dos** técnicas de detección de **antígenos** y **4** de **anticuerpos**.
3. Los estudios de fiabilidad se realizan:
    a. Antígenos: en muestras respiratorias de pacientes con síntomas de COVID-19. Se toman dos hisopos y con uno se hace la determinación de antígeno y con el otro la PCR.
    b. Anticuerpos: muestras sanguíneas con PCR COVID-19 positiva y controles negativos con sueros conservados y extraídos antes del 8 de diciembre de 2019.
4. Las determinaciones de anticuerpos pueden realizarse por **venopunción** o por **digitopunción** con resultados de **concordancia es elevada en algunas técnicas**. Aunque hay comunicaciones de algunos hospitales de que la **sensibilidad por digitopunción puede ser inferior** que con muestras de sangre por venopunción o de suero.
5. La **tabla 1** muestra los resultados más destacados de los estudios de fiabilidad:

TABLA 1. Resumen de resultados (S: Sensibilidad, ESP: Especificidad)

TÉCNICA	**FABRICANTE**	**DATOS SEGUN MARCADO CE**	**ESTUDIO DE FIABILIDAD**	**DATOS SOBRE ESTUDIO FIABILIDAD**
2019-nCoV Ag GICA Rapid Test REF: YRLG22202025	SHENZHEN BIOEASY BIOTECHNOLOGY CO LTD	83% S, 100% ESP	S: 25% ESP: 100%	Estudio ISCIII **N=48 pacientes**
2019-nCoV Ag Test Fluorescence IC Assay REF: YRLF04401025	SHENZHEN BIOEASY BIOTECHNOLOGY CO LTD	92% S, 100% ESP	S: 58% ESP: 97%	Estudio Hospital Clínico Madrid, G Marañón y La Paz **N=121 pacientes**
COVID-19 IgG/IgM Rapid Test Cassette REF: GCCOV-402a	ZHEJIANG ORIENT GENE BIOTECH	88% S, 97% ESP (distingue IgM/IgG)	S: 58% ESP: 100% (S=85% en pacientes con >10 días de evolución) (SUERO)	Estudio Hospital de Toledo e ISCIII **N=250 pacientes**
2019-nCoV IgG/IgM Rapid Test Cassette REF: INCP-402	HANGZHOU ALL TEST BIOTECH CO LTD	100% S, 97% ESP (distingue IgM/IgG)	S: 56,5% ESP: 100% (S >75% en pacientes con >7 días de evolución) (SUERO)	Estudio Hospital Clínico de Madrid y Ramón y Cajal **N=119 pacientes**

SARS-CoV-2 Antibody Test REF: W1 95	GUANGZHOU WONDFO BIOTECH CO LTD	100% S, 90% ESP (Ac totales)	S: 66,3% ESP: 100% (S >75-80% en pacientes con >7 días de evolución) (SUERO)	Estudio la Princesa, Ramón y Cajal, Gregorio Marañón, Hospital de Toledo y Hospital Clínico de Madrid **N=386 pacientes**
SGTi flex COVID 19 IgM/IgG REF: COVT02SE	SUGENTECH INC	94% S, 96% ESP (distingue IgM/IgG)	S: 74% ESP: 90% (S=94% en pacientes con >10 días de evolución) (SUERO)	Estudio Hospital de la Princesa y Hospital Clínico de Madrid **N=200 pacientes**

6. Con estos datos el **valor predictivo positivo de todas las técnicas rápidas es >95%**.

Además en la última semana se han empezado a realizar **estudios en pacientes recuperados**, principalmente **personal sanitario** diagnosticado por PCR, que ha superado la enfermedad y se está **reincorporando a su puesto de trabajo**.

En estos estudios se ha realizado toma de muestras por **venopunción y por digitopunción**, **y analizado sangre total y suero**

La **tabla 2** muestra resultados de estudios realizado en sangre total y suero con las técnicas de anticuerpo, y comparando venopunción con digitopunción

TABLA 2. Resumen de resultados en pacientes recuperados y comparaciones venopunción vs digitopunción. (S: Sensibilidad, ESP: Especificidad)

TÉCNICA	**FABRICANTE**	**DATOS SEGUN MARCADO CE**	**ESTUDIO DE FIABILIDAD**	**DATOS SOBRE ESTUDIO FIABILIDAD**
COVID-19 IgG/IgM Rapid Test Cassette REF: GCCOV-402a	ZHEJIANG ORIENT GENE BIOTECH	88% S, 97% ESP (distingue IgM/IgG) Sangre/suero/plasma. El fabricante recomienda digitopunción	S en SUERO: 85,5% ESP en SUERO: 98.1% S en SANGRE: 82,1% ESP en SANGRE: 99% S en DIGITOPUNCIÓN: 82,1% ESP en DIGITOPUNCIÓN: 99%	**150 pacientes** Estudio ISCIII **56 pacientes** Hospital Clínico de Madrid **56 pacientes** Hospital Clínico de Madrid

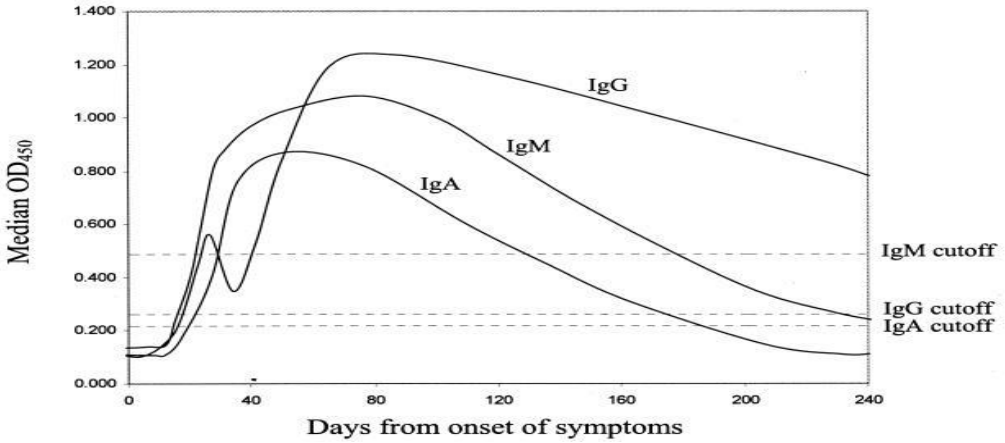
SARS-CoV-2 Antibody Test REF: W1 95	GUANGZHOU WONDFO BIOTECH CO LTD	100% S, 90% ESP (Ac totales) Sangre/suero/plasma.	S en SUERO: 77,8% ESP en SUERO: 95% S en SANGRE: 84,5% ESP en SANGRE: 100% S en DIGITOPUNCIÓN: 61,5% ESP en DIGITOPUNCIÓN: 100%	**45 pacientes** Hospital Gregorio Marañón **97 pacientes** Hospital Clínico de Madrid **52 pacientes** Hospital Gregorio Marañón y Hospital Clínico de Madrid
2019-nCoV IgG/IgM Rapid Test Cassette REF: INCP-402	HANGZHOU ALL TEST BIOTECH CO LTD	100% S, 97% ESP (distingue IgM/IgG) Sangre/suero/plasma. El fabricante recomienda digitopunción	S en SUERO: 86% ESP en SUERO: 85% S en DIGITOPUNCIÓN: 74% ESP en DIGITOPUNCIÓN: 95%	**56 pacientes** Hospital Ramón y Cajal y Gregorio Marañón **47 pacientes** Hospital Gregorio Marañón

Interpretación de estos resultados

1. La sensibilidad de los tests de **antígenos podría ser inferior** a la de anticuerpos, sobre todo en fases avanzadas de la infección, lo que es similar a lo observado en otras infecciones virales, donde los test de antígenos son de utilidad diagnóstica limitada.
2. Las técnicas de detección de **antígeno** deben hacerse en no más de 30 minutos desde que se toma la muestra, por lo que deberían situarse en formato point-of-care (POC) en servicios de urgencia, lo que **resulta complicado en muchos centros sanitarios**.
3. En lo que se refiere a las técnicas de **detección de anticuerpos**, ya sean IgM e IgG por separado o de detección de anticuerpos totales debe indicarse:
    a. Aunque la sensibilidad en nuestro medio es inferior a la reportada previamente, la pruebas rápidas **detectan con rapidez un porcentaje significativo** de casos.
    b. Esta sensibilidad media más baja se debe a que gran parte de los enfermos con un resultado negativo **no habrán producido anticuerpos** con un título suficiente para ser detectado.
    c. En pacientes que llevan **más de 7 días de evolución** de COVID-19, la sensibilidad de la detección de anticuerpos **asciende a más de 75%.**
4. La **detección** de anticuerpos IgM, IgG o anticuerpos totales en paciente con **síntomas compatibles con COVID-19 debe considerarse diagnóstico** de infección, sobre todo en situaciones de alta prevalencia de la infección.
5. En cuanto al significado de **detectar una inmunoglobulina u otra** debe indicarse, que la cinética de producción de anticuerpos frente al COVID-19 no se conoce suficientemente. Los datos recogidos en los estudios de fiabilidad muestran que un 40% de los enfermos sintomáticos tienen IgG detectable, y que hasta un 70% de los pacientes recuperados asintomáticos mantienen IgM detectable. Los estudios realizados con el coronavirus SARS-1 probaron que los periodos de producción de inmunoglobulinas se solapan y se

mantienen en el tiempo (ver figura 1) (Woo et al. Clin Diagn Lab Immunol. 2004; 11: 665–668)

FIGURA 1. Perfil de IgG, IgM, and IgA en pacientes con neumonía por SARS-CoV.



6. Hasta que no se dispongan de test **serológicos cuantitativos** (ELISA, inmuno-ensayos) suficientemente fiables no se podrá analizar la cinética de las diferentes inmunoglobulinas en respuesta a la infección COVID-19. Se han localizado 8 de estas técnicas que se están evaluando en el Centro Nacional de Microbiología. Hay otras muchas en desarrollo, de las que se espera tener un prototipo en pocas semanas.
7. En **pacientes recuperados**, todos los tests rápidos evaluados presentan una sensibilidad en suero y sangre total **alrededor del 80%**. Los datos que hay sobre **la digitopunción indican que su sensibilidad puede ser inferior** en algunos casos y para algunas técnicas.

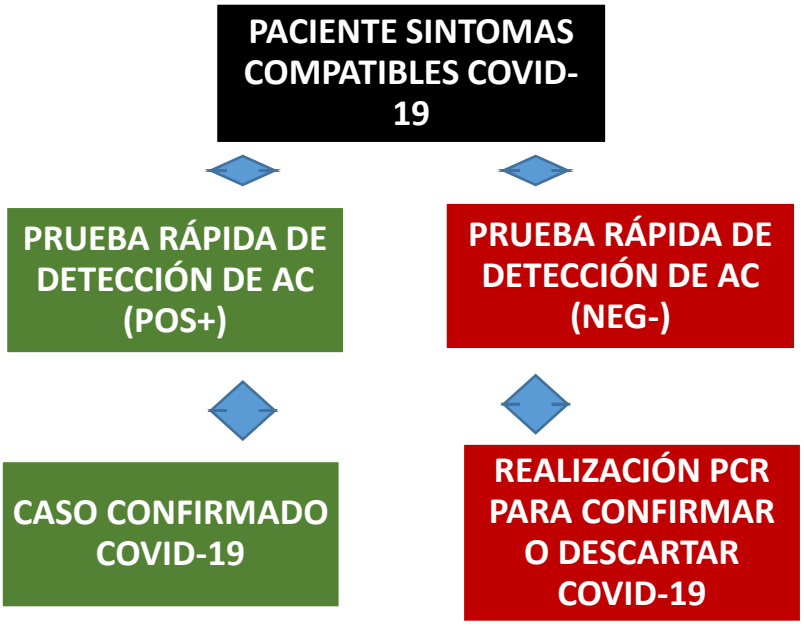
Propuesta de estrategia

Tras lo expuesto en los apartados anteriores se propone lo siguiente:

1. La realización de **PCRs debe considerarse el pilar fundamental** de la estrategia. Deben aumentarse las capacidades:
    a. **Mantener e incrementar** la distribución de reactivos en los **centros sanitarios españoles**, priorizando en estos momentos la obtención de más reactivos de extracción y aumentando progresivamente la distribución de reacciones de PCR.
    b. Se debe aumentar la **automatización en el proceso de realización de PCRs**, obteniendo reactivos en este formato y poniendo en funcionamiento los robots y plataformas disponibles.
    c. Se debe **incrementar** el número de **centros** universitarios, de investigación, veterinarios, militares o de cualquier otra clase, **con capacidad** para realizar análisis de PCR y apoyar al Sistema Nacional de Salud, siempre en coordinación con las **autoridades sanitarias de cada CCAA y supervisados por los servicios de microbiología clínica.** Esta capacitación sólo podrá ser eficaz si se aumenta la consecución total de reactivos de extracción y de PCR, ya que de otro modo, su puesta en marcha podría detraer reactivos de los centros sanitarios.

2. Las técnicas **rápidas de detección de anticuerpos**, aquellas con sensibilidad alrededor del 60% o superior, podrían emplearse como **prueba de cribado rápido** en pacientes **sintomáticos** en áreas/centros/situaciones de alta prevalencia de COVID-19:
    a. Un resultado **positivo** de anticuerpos totales, IgM o IgG **confirmaría** la infección.
    b. Los resultados **negativos** habría que confirmarlos realizando **PCR** en muestras respiratorias del paciente.
    c. Esta estrategia reduciría el número total de PCR diarias a realizar en los hospitales en las situaciones de alta prevalencia.
    d. Las técnicas de detección de anticuerpos pueden realizarse en los laboratorios de microbiología, tras recoger la muestra en unidades de extracción de sangre, sin alterar el funcionamiento hospitalario habitual. Se pueden utilizar muestras de sangre, suero o plasma. Varios fabricantes recomiendan hacer la prueba inmediatamente después de la extracción de la muestra.
    e. En la Figura 2 se muestra una propuesta de algoritmo diagnóstico en los pacientes sintomáticos compatibles con COVID-19.

FIGURA 2. Propuesta de algoritmo diagnóstico en PACIENTES SINTOMÁTICOS



3. Utilizando estas técnicas con una sensibilidad media del 60%, **el porcentaje de PCRs que podrían no hacerse tras obtener un resultado positivo con la técnica rápida sería significativo** y dependería de la frecuencia de COVID-19 en la población:
    a. Los hospitales de las CCAA con mayor número de casos están comunicando resultados positivos de PCR entre 50-80% de los casos sospechosos de COVID-19 analizados diariamente.
    b. Con una sensibilidad de la técnica rápida de detección de anticuerpos alrededor del 60% **se ahorrarían un 36-48% de PCRs cada día**, en estos escenarios de alta prevalencia.

c. En pacientes con enfermedad **más evolucionada**, donde la sensibilidad de la detección de anticuerpos **supera el 75%,** se podría reducir un 60% la necesidad de PCR.
d. En poblaciones en las que la frecuencia de la enfermedad es inferior, como **CCAA con menor prevalencia**, grupos de personas aisladas en **residencias de ancianos**, **personal** de servicios **esenciales** y **sanitarios**, la utilidad diagnóstica de la detección de anticuerpos sería inferior y la PCR seguiría jugando un papel fundamental.
e. Además las técnicas rápidas de detección de anticuerpos podrían tener un uso adicional que es ayudar **a confirmar casos compatibles con COVID-19 con resultados de PCR repetidamente negativos**.

4. Las técnicas de detección de **anticuerpos tienen además** otro papel, que es poder detectar anticuerpos en población que ya ha superado la enfermedad. Para estos usos se recomienda utilizar **técnicas que distingan entre IgM e IgG**, ya que aportarían más información sobre el perfil de anticuerpos en la población general. Estos usos son:
    a. Detectar personas supuestamente **inmunes** que podrían reiniciar sus labores profesionales.
    b. Pacientes **recuperados** a los que se les puede extraer **plasma** para tratamiento de nuevos casos.
    c. Estudios de **seroprevalencia**.
    d. Estudios de respuesta **vacunal**, cuando existan estas vacunas.
    e. Apoyo en **ensayos clínicos** de **profilaxis** que necesiten conocer la presencia de anticuerpos y no introducir sesgos en la evaluación de la pauta profiláctica. En este caso también podría utilizarse la detección de anticuerpos totales.
5. Por último, las técnicas de detección de **antígeno** podrían emplearse en aquellos casos que superen el 60% de sensibilidad y sólo en aquellos centros que tengan la posibilidad de crear POCs sin alterar el funcionamiento del hospital.

INFORME EMITIDO POR

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Centers for Disease Control and Prevention

Coronavirus Disease 2019 (COVID-19)

Interim Guidelines for COVID-19 Antibody Testing

Interim Guidelines for COVID-19 Antibody Testing in Clinical and Public Health Settings

Data that will inform serologic testing guidance is rapidly evolving. Recommendations on the use of serologic tests to determine protective immunity and infectiousness among persons recently infected with SAR-CoV-2 will be updated as new information becomes available.

Summary

Serologic methods have been developed and will have important public health and clinical uses to monitor and respond to the COVID-19 pandemic.

- Serologic assays for SARS-CoV-2 now have Emergency Use Authorization (EUA) by the U.S. Food and Drug Administration (FDA), which has independently reviewed their performance.
- Currently, there is no identified advantage of assays whether they test for IgG, IgM and IgG, or total antibody.
- It is important to minimize false positive test results by choosing an assay with high specificity and by testing populations and individuals with an elevated likelihood of previous exposure to SARS-CoV-2. Alternatively, an orthogonal testing algorithm (i.e., employing two independent tests in sequence when the first test yields a positive result) can be used when the expected positive predictive value of a single test is low.
- Antibodies most commonly become detectable 1-3 weeks after symptom onset, at which time evidence suggests that infectiousness likely is greatly decreased and that some degree of immunity from future infection has developed. However, additional data are needed before modifying public health recommendations based on serologic test results, including decisions on discontinuing physical distancing and using personal protective equipment.

Background

Serologic assays for SARS-CoV-2, now broadly available, can play an important role in understanding the virus's epidemiology in the general population and identifying groups at higher risk for infection. Unlike viral direct detection methods such as nucleic acid amplification or antigen detection tests that can detect acutely infected persons, antibody tests help determine whether the individual being tested was ever infected—even if that person never showed symptoms. Serologic tests detect waning or past SARS-CoV-2 virus infection indirectly, by measuring the host humoral immune response to the virus. Therefore, serology assays do not typically replace direct detection methods as the primary tool for diagnosing an active SARS-CoV-2 infection, but they do have several important applications in monitoring and responding to the COVID-19 pandemic.

Although serologic tests should not be used at this time to determine if an individual is immune, these tests can help determine the proportion of a population previously infected with SARS-CoV-2 and provide information about populations that may be immune and potentially protected. Thus, demographic and geographic patterns of serologic test results can help determine which communities may have experienced a higher infection rate and therefore may have higher rates of herd immunity. In some instances, serologic test results may assist with identifying persons potentially infected with SARS-CoV-2 and determining who may qualify to donate blood that can be used to manufacture convalescent plasma as a possible treatment for those who are seriously ill from COVID-19.

Development of Antibodies and Immunity

Nearly all immune competent individuals will develop an immune response following SARS-CoV-2 infection. Like infections with other pathogens, SARS-CoV-2 infection elicits development of IgM and IgG antibodies, which are the most useful for assessing antibody response because little is known about IgA response in the blood.

Antibodies in some persons can be detected within the first week of illness onset. SARS-CoV-2 infections are somewhat unusual because IgM and IgG antibodies arise nearly simultaneously in serum within 2 to 3 weeks after illness onset. Thus, detection of IgM without IgG is uncommon. How long IgM and IgG antibodies remain detectable following infection is not known.

In addition, development of neutralizing antibodies can also be assessed. Neutralizing antibodies inhibit viral replication in vitro, and as with many infectious diseases, their presence correlates with immunity to future infection, at least temporarily.

Recurrence of COVID-19 illness appears to be very uncommon, suggesting that the presence of antibodies could confer at least short-term immunity to infection with SARS-CoV-2. Consistent with this observation, experimental primary infection in primates and subsequent development of antibodies resulted in protection from reinfection after the primates were rechallenged. Additionally, antibody development in humans correlates with a marked decrease in viral load in the respiratory tract. Taken together, these observations suggest that the presence of antibodies may decrease a person's infectiousness and offer some level of protection from reinfection. However, definitive data are lacking, and it remains uncertain whether individuals with antibodies (neutralizing or total) are protected against reinfection with SARS-CoV-2, and if so, what concentration of antibodies is needed to confer protection.

Current Status of Antibody Testing in the United States

Antigenic targets

The two major antigenic targets of SARS-CoV-2 virus against which antibodies are detected are **spike glycoprotein (S)** and **nucleocapsid phosphoprotein (N)**. While S protein is essential for virus entry and is present on the viral surface, N protein is the most abundantly expressed immunodominant protein that interacts with RNA. Multiple forms of S protein — full-length (S1+S2) or partial (S1 domain or receptor binding domain [RBD]) — are used as antigens. The protein target determines cross-reactivity and specificity because N is more conserved across coronaviruses than S, and within S, RBD is more conserved than S1 or full-length S.

Types of Antibody Testing

Different types of assays can be used to determine different aspects of immune response and functionality of antibodies. The tests can be broadly classified to detect either binding or neutralizing antibodies.

- **Binding antibody detection**: These tests use purified proteins of SARS-CoV-2, not live virus, and can be performed in lower biosafety level laboratories (e.g., BSL-2). With specific reagents, individual antibody types, like IgG, IgM, and IgA, can be determined. In general, IgM is one of the first types of antibodies produced after infection and is most useful for determining recent infection, while IgG generally develops after IgM and may remain detectable for months or years. IgA is important for mucosal immunity and can be detected in mucous secretions like saliva in addition to blood, though its significance in this disease is still to be determined. Depending on the complexity of assays, these tests can be performed rapidly (less than 30 minutes) in a field setting or in a few hours in a laboratory.

  Tests that detect binding antibodies fall into two broad categories.

  - **Point-of-care (POC) tests** generally are lateral flow devices that detect IgG or IgG and IgM, or total antibody in serum, plasma, whole blood, and/or saliva. An advantage of some point-of-care tests using whole blood is that they can be performed on blood samples obtained by fingerstick rather than venipuncture.
  - **Laboratory tests** use ELISA (Enzyme-Linked Immunosorbent Assay) or CIA (chemiluminescent immunoassay) methods for antibody detection, which for some assays may require trained laboratorians and specialized instruments. Based on the reagents, IgG, IgM, and IgA can be detected separately or combined as total antibody.
- **Neutralizing antibody detection**: FDA has not yet authorized the use of neutralization tests for SARS-CoV-2. Neutralization tests determine the functional ability of antibodies to prevent infection of virus in vitro. The test involves incubating serum or plasma with live virus followed by infection and incubation of cells. Testing will require either BSL-3 or BSL-2 laboratories, depending on what form of the SARS-CoV-2 virus is used.

  Two types of neutralization tests are conducted.

  - **Virus neutralization tests (VNT)**, such as the plaque-reduction neutralization test (PRNT) and microneutralization, use a SARS-CoV-2 virus from a clinical isolate or recombinant SARS-CoV-2 expressing reporter proteins. This testing

requires BSL-3 laboratories and may take up to 5 days to complete.

- **Pseudovirus neutralization tests (pVNT)** use recombinant pseudoviruses (like vesicular stomatitis virus, VSV) that incorporate the S protein of SARS-CoV-2. This testing can be performed in BSL-2 laboratories depending on the VSV strain used.

FDA-authorized serologic tests

FDA now requires commercially marketed serologic tests to receive Emergency Use Authorization (EUA) . Tests that are not commercially marketed do not require FDA authorization but developers may voluntarily request authorization. Multiple agencies — including FDA, the National Cancer Institute/National Institutes of Health (NCI/NIH), CDC, and the Biomedical Advanced Research and Development Authority (BARDA) — are collaborating with members of academia and the medical community to evaluate several serology tests using a well-characterized set of clinical samples (serum or plasma) collected before and during the current COVID-19 outbreak. A list of all tests authorized for emergency use under EUA is maintained on an FDA website . All currently authorized tests are qualitative (providing a result that is positive, negative, or indeterminate) rather than quantitative (providing a quantitative assessment of antibody levels).

Both laboratory and rapid serologic assays have received EUA. Serologic testing technologies include single-use, low-throughput lateral flow tests where the presence of antibody is demonstrated by a color change on a paper strip and laboratory-based immunoassays that allow for processing of many samples at the same time.

The EUA letter of authorization includes the settings in which the test is authorized, based on FDA's determination of appropriate settings for use during the public health emergency.

Optimizing Testing Outcomes

Test performance

The utility of tests depends on the sensitivity and specificity of the assays; these performance characteristics are determined by using a defined set of negative and positive samples. In addition, the predictive values of a test should be considered because these values affect the overall outcome of testing. **Positive predictive value** is the probability that individuals with positive test results are truly antibody positive. **Negative predictive value** is the probability that individuals with negative test results are truly antibody negative. Positive and negative predictive values are determined by the percentage of truly antibody positive individuals in the tested population (prevalence, pre-test probability) and the sensitivity and specificity of the test. For example:

- In a high-prevalence setting, the positive predictive value increases — meaning it is more likely that persons who test positive are truly antibody positive – than if the test is performed in a population with low-prevalence. When a test is used in a population where prevalence is low, the positive predictive value drops because there are more false-positive results, since the pre-test probability is low.
- Likewise, negative predictive value is also affected by prevalence. In a high-prevalence setting, the negative predictive value declines whereas in a low-prevalence setting, it increases.

In most of the country, including areas that have been heavily impacted, the prevalence of SARS-CoV-2 antibody is expected to be low, ranging from <5% to 25%, so that testing at this point might result in relatively more false positive results and fewer false-negative results.

In some settings, such as COVID-19 outbreaks in food processing plants and congregate living facilities, the prevalence of infection in the population may be significantly higher. In such settings, serologic testing at appropriate intervals following outbreaks might result in relatively fewer false positive results and more false-negative results.

Testing strategies

In the current pandemic, maximizing specificity and thus positive predictive value in a serologic algorithm is preferred in most instances, since the overall prevalence of antibodies in most populations is likely low. For example, in a population where the prevalence is 5%, a test with 90% sensitivity and 95% specificity will yield a positive predictive value of 49%. In other words,

less than half of those testing positive will truly have antibodies. Alternatively, the same test in a population with an antibody prevalence exceeding 52% will yield a positive predictive greater than 95%, meaning that less than one in 20 people testing positive will have a false positive test result.

Three strategies can be used to improve positive predictive value:

- Choosing a test with a very high specificity, perhaps 99.5% or greater, will yield a high positive predictive value in populations tested with prevalence ≥5%.
- Another strategy is to focus testing on persons with a high pre-test probability of having SARS-CoV-2 antibodies, such as persons with a history of COVID-19-like illness.
- A third approach is to employ an orthogonal testing algorithm in which persons who initially test positive are tested with a second test. Effective orthogonal algorithms are generally based on testing a patient sample with two tests, each with unique design characteristics (e.g., antigens or formats).

Algorithms can be designed to maximize overall specificity while retaining maximum sensitivity. For example, in the example above with a population prevalence of 5%, a positive predictive value of 95% can be achieved if samples initially positive are tested with a second different orthogonal assay that also has 90% sensitivity and 95% specificity. The performance of orthogonal testing algorithms has not been systematically evaluated but can be estimated using an on-line calculator from the FDA. See Table 1 for the potential improvement benefits of the orthogonal testing algorithm.

Limitations of Serologic Tests

At present, the immunologic correlates of immunity from SARS-CoV-2 infection are not well defined. Representatives from BARDA, CDC, FDA, NIH, the Office of the Assistant Secretary for Health (OASH), Department of Defense (DoD), and White House Office of Science and Technology Policy (OSTP) are working with members of academia and the medical community to determine whether positive serologic tests are indicative of protective immunity against SARS-CoV-2. This work includes assessing the level of antibodies required for protection from reinfection, the duration of that protection, and the factors associated with development of a protective antibody response. The kinetics of antibody response, longevity of antibodies, the ability of antibodies to protect from repeat infection, the protective titer of neutralizing antibody, and the correlation of binding antibody titers to neutralization ability are yet to be determined. Although animal challenge studies demonstrate protection in the short run, demonstration of long-term protection in humans will require future study. Hence, pending additional data, the presence of antibodies cannot be equated with an individual's immunity from SARS-CoV-2 infection.

Some tests may exhibit cross-reactivity with other coronaviruses, such as those that cause the common cold. This could result in false-positive test results. Some persons may not develop detectable antibodies after coronavirus infection. In others, it is possible that antibody levels could wane over time to undetectable levels. IgM and IgG antibodies are not present early in infection. Thus, serologic test results do not indicate with certainty the presence or absence of current or previous infection with SARS-CoV-2.

Recommendations for Use of Serologic Tests

Information that might impact serologic recommendations is rapidly evolving, particularly evidence of whether positive serologic tests indicate protective immunity or decreased transmissibility among those recently ill. These recommendations will be updated as new information becomes available.

Choice of test and testing strategy

- Serologic assays that have Emergency Use Authorization (EUA) are preferred for public health or clinical use since their test performance data have been reviewed by FDA.
- Serologic test results should be interpreted in the context of the expected predictive values, positive and negative.
- Positive predictive value should be optimized, particularly if results are returned to individuals, in the following ways:
  - Assure a high positive predictive value (e.g., 95%) by choosing tests with sufficiently high specificity and testing persons or populations with a high pre-test probability of having antibodies (e.g., persons with a history of symptoms compatible with COVID-19 or who are exposed to areas or institutions experiencing outbreaks), OR
  - If a high positive predictive value cannot be assured with a single test, use an orthogonal testing algorithm. See Table 1 for examples of using one or two tests in populations with various prevalences of SARS-CoV-2 antibodies.

- Currently, there is no substantive performance advantage of assays whether they test for IgG, IgM and IgG, or total antibody. Thus, immunoglobulin class should not determine the assay chosen in most circumstances. The detection of IgM antibodies may indicate a more recent infection, but the dynamics of the IgM antibody response are not well defined at present. Over time, it may be important to characterize and evaluate the performance of assays in samples that are IgM negative and IgG positive to ensure that assays remain fit for purpose in population studies as the pandemic progresses and more individuals are expected to have lower IgM levels.
- Serologic testing should not be used to determine immune status in individuals until the presence, durability, and duration of immunity is established.
- Serologic testing can be offered as a method to support diagnosis of acute COVID-19 illness for persons who present late.* For persons who present 9-14 days after illness onset, serologic testing can be offered in addition to recommended direct detection methods such as polymerase chain reaction. This will maximize sensitivity as the sensitivity of nucleic acid detection is decreasing and serologic testing is increasing during this time period.
- Serologic testing should be offered as a method to help establish a diagnosis when patients present with late complications of COVID-19 illness, such as multisystem inflammatory syndrome in children.

Recommendations for persons who test positive for anti-SARS-CoV-2 antibodies

- Although the presence of anti-SARS-CoV-2 antibodies when detected using a testing algorithm with high positive predictive value for the context of use likely indicates at least some degree of immunity, until the durability and duration of immunity is established, it cannot be assumed that individuals with truly positive antibody test results are protected from future infection.
- Asymptomatic persons who test positive by serologic testing and who are without recent history of a COVID-19 compatible illness have a low likelihood of active infection and should follow general recommendations to prevent infection with SARS-CoV-2 and otherwise continue with normal activities, including work.
- Persons who have had a COVID-19-compatible or confirmed illness should follow previous guidance regarding resumption of normal activities, including work.
- There should be no change in clinical practice or use of personal protective equipment (PPE) by health care workers and first responders who test positive for SARS-CoV-2 antibody.

Additional considerations on the use of serologic tests

- Serologic test results should not be used to make decisions about grouping persons residing in or being admitted to congregate settings, such as schools, dormitories, or correctional facilities.
- Serologic test results should not be used to make decisions about returning persons to the workplace.
- Until more information is available about the dynamics of IgA detection in serum, testing for IgA antibodies is not recommended.

* Detection of specific antibody in serum, plasma, or whole blood that indicates new or recent infection provides presumptive laboratory evidence of COVID-19 illness according to the Council of State and Territorial Epidemiologists (CSTE) interim case definition for COVID-19 .

Additional Resources

American Medical Association. Serological Testing for SARS-CoV-2 Antibodies.

Infectious Diseases Society of America. IDSA COVID19 Antibody Testing Primer.

Association of Public Health Laboratories and Council of State and Territorial Epidemiologists. Public Health Considerations: Serologic Testing for COVID-19. Version 1-May 7, 2020.

Table 1: Predictive value positive using one test or two orthogonal tests, by the background prevalence in the population tested.

Prevalence	PPV for one test (SE=90%, SP=95%)	PPV for two orthogonal tests (SE=90%, SP=95%)
2%	26.9%	86.9%
5%	48.6%	94.5%
10%	66.7%	97.3%
30%	88.5%	99.3%

PPV = positive predictive value

SE = sensitivity

SP = specificity

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