Coronavirus Disease 2019 (COVID-19) Serologic test Recommendations

Version 1.0

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Executive Summary:

Serologic methods have been developed and will have important public health and clinical uses to monitor and respond to the COVID-19 pandemic [(1)].

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[(1)].

Currently, there is no identified advantage of assays testing for IgG, IgM and IgG, or total antibody [(1)].

It is important to minimize false positive test results by choosing an assay with high specificity (> 99.5% is recommended) and by testing populations and individuals with an elevated likelihood of previous exposure to SARS-CoV-2[(1)].

Strategies to enhance testing performance include orthogonal testing algorithm (i.e., employing two independent tests in sequence when the first test yields a positive result) which can be used when the expected positive predictive value of a single test is low [(1)].

Antibodies most commonly become detectable 1-3 weeks after symptom onset, at which time evidence suggests that infectiousness is likely greatly decreased and that some degree of immunity for future infection has developed[(1)].

Recommendations of this working group for the use of serologic tests include clinical care, occupational health, epidemiological and research applications.

Additional data are needed before establishing final public health recommendations based on serologic test results, including decisions on discontinuing physical distancing and using personal protective equipment[(1)].

Introduction:

Currently, RT-PCR is the gold standard test used for the diagnosis of COVID-19 infection. However, other assays such as antigen and antibody assays (serology assays) can also be used to diagnose COVID19 cases with a fast turn-around-time.

- Serologic antibody assays can help in the diagnosis of late COVID-19 infections when RT-RCR is expected to be negative, seroprevalence and contact tracing and may later on support return to work decisions as more data becomes available.
- Serology assays can also be used to determine eligibility of plasma donation from patients recovered from COVID-19 disease.
- It also has a great value in the epidemiological studies of the SARS-CoV-2 virus pandemic.

COVID-19 Diagnostic Guidelines Panel
The recommendations in the guidelines will be updated as new information on the Diagnosis of COVID-19 becomes available

In an effort to provide guidance on the utilization of serologic tests, a working group representing members from the National Crisis & Emergency Management Authority (NCEMA- UAE) Laboratory Task Force, expert consultants in infectious diseases, microbiology, immunology, clinical pathology, occupational health, Emirates National Accreditation System (ENAS) and academic institutions reviewed the available guidelines and published data to develop this interim guideline on serologic testing for SARS-CoV2 in the UAE. Members of this working group are listed in appendix 1.

This guideline will be updated periodically as more data becomes available and based on changes in National Guidelines and federal decisions.

References reviewed and discussed are all listed under the references section.

Objectives:

The objective of this working group is to develop guidelines and recommendations with regards to serological testing for SARS-CoV-2 antibodies as it pertains to the following three aspects:

1. **Assay characteristics and test performance.**
   This includes but is not limited to assay types, antigenic targets, antibody targets, performance characteristics, FDA – Emergency Use Authorization (EUA), minimal verification and quality assurance requirements and reporting guidelines.

2. **Applications of serologic tests and testing strategies.**
   This includes the following domains, clinical patient care, occupational health, convalescent plasma donors, public health, and research applications.

3. **Limitations of serological tests and inappropriate applications.**

1. Assay characteristics and test performance:

1.1 Assay Types

Below is the list of the variable assay types available:

- Lateral flow, Point of Care Tests (POCT)
- Enzyme-Linked Immunosorbent Assays (ELISA), (Multistep, Qualitative & Semiquantitative)
- Chemiluminescent immunoassays (CLIMA) (Multistep, Qualitative, Semiquantitative, Quantitative)
- Neutralization assays
- 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 the type of neutralization assay used [(1)].

Assay should have sensitivity & specificity > 99.5% and preferably to include orthogonal testing algorithm, to optimize outcomes and be of a maximal clinical utility at a prevalence < 5%.

The use of POCT kits is NOT recommended by the working group as the current kits available to date don't have the desired specificities and sensitivities.

1.2 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 protein and is required for packaging of viral RNA into the viral particle during viral assembly.

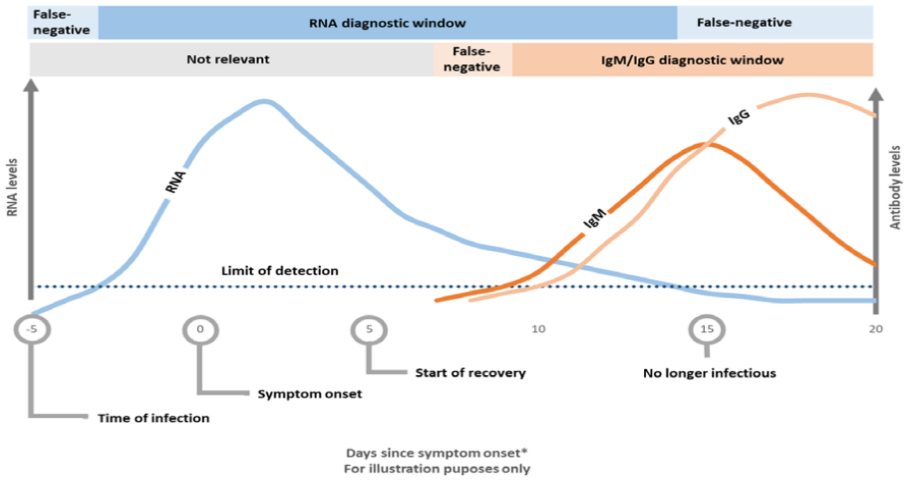
The following are acceptable targets:

- Spike glycoprotein (S) targets
  - S1 + S2
  - S1
  - Receptor binding domain (RBD)
- Nucleocapsid (N) target

1.3 Antibody Types:

Current evidence indicates that SARS-CoV-2 antibodies begin to develop approximately 6 to 10 days after infection with SARS-CoV-2. IgM appears to peak approximately 12 - 15 days after SARS-CoV-2 infection and persists in sufficient quantities for as long as 35 days, after which the quantity declines rapidly. IgG has been observed to peak approximately 17 days after SARS-CoV-2 infection and persist for at least 49 days. Further, IgG has been observed in patients 2 weeks after symptom onset.

Figure ES1: Diagnostic windows for the detection of acute SARS-CoV-2 infection (viral RNA) and the immune response (anti-SARS-CoV-2 IgM and IgG) indicating past exposure to SARS-CoV-2



The above figure is taken from the report of the Health Information & Quality Authority [9], and although it is not exact, it is based on early evidence summaries.

Below are the recommendations regarding antibody type to be selected.

- Total antibodies (IgM & IgG) (Recommended and has slightly better sensitivity than IgG alone)
- Isotype G (Recommended and correlates with previous infection and seroconversion)
- Isotype A (Testing for serum IgA has no clinical utility)
- Isotype M (May play a role, but not superior over total or IgG)

Research has shown varying antibody levels in patients who have recovered from COVID-19, including as many as 5% with no antibodies.

1.4 Current assays approved by FDA:

FDA now requires commercially marketed serologic tests to receive Emergency Use Authorization (EUA).
The working group recommends the use of FDA-EUA kits after verification of its performance characteristics locally as per international standards.

A list of all tests authorized for emergency use under EUA is maintained on an FDA website [2].

Kits with CE Mark (European Conformity) are acceptable as long as there is evidence that the kit / analyzer have been verified through independent conformity assessment body.

Manufacturer should conform to ISO accreditation standards.

1.5 Quality Assurance Requirements:

Following measures are recommended to enhance the confidence in testing;

1. Reagents and consumables
    1.1.1. Each new formulation of examination kits with changes in reagents or procedure, or a new lot or shipment, shall be verified for performance before use in examinations.
    1.1.2. Consumables that can affect the quality of examinations shall be verified for performance before use in examinations.

    Note: Performance can be verified by using any of the mechanism explained in section below.

2. Inter-laboratory comparisons
    2.1. The laboratory shall participate in an inter-laboratory comparison programme(s) (such as an external quality assessment programme or proficiency testing programme) appropriate to the examination and interpretations of examination results.

2.2. Until a specific inter-laboratory comparison is available, the laboratory may use alternative mechanism e.g.
   2.2.1. certified reference materials;
   2.2.2. retesting of samples previously examined;
   2.2.3. exchange of samples with other laboratories;
   2.2.4. comparison of results for the same sample from new lot with the existing in-use lot

Note1: Currently, only RT-RNA based PTs are available for SARS-COV-2 testing on the worldwide PT database but is continuously being updated. https://www.eptis.org/

Note2: Currently, Reference materials for multiple SARS-CoV-2 serology assay formats are available for both, covering IgG-specific assays and for total antibody tests (e.g. LGC ACCURUN Anti-SARS-COV-2 Reference Materials). Labs may use any suitable reference material intended for this testing.

2. Applications of serologic tests and testing strategies:

Strategies to optimize testing outcomes:

- Choose a test with high specificity (> 99.5%)
- Test individuals with high pre-test probability
- Apply orthogonal testing algorithm (test sample with 2 tests)

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 re-challenged [(1)].

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 or provide some level of protection from re-infection [(1)]. Preliminary research studies have shown that patients who have recovered from COVID-19 have variable levels of neutralizing antibodies including high, low and no titers. (7)

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 [(1)].

Serologic tests can be used for the following applications:

a. Clinical Patient Care

i. Support the diagnosis of COVID-19 illness in late disease presentation with negative PCR (9-14 days)

ii. Support establishing the diagnosis of multisystem hyper-inflammatory syndrome in children or cases presenting late in the course of illness

iii. Selection of Convalescent Plasma (CP) donors for CP therapy*

*For the CP donors quantitative methods are preferred or semi-quantitative methods with the cut-off index. Although some studies have shown a correlation between commercial assays and neutralizing antibody tests, additional data is necessary before commercial tests can be used routinely to determine neutralizing antibody titers. Samples should be stored to be analysed by neutralization assays when available. Neutralizing antibody titre of ≥ 1:160 is recommended by the FDA for selection of such donors. A titer of 1:80 may be considered acceptable if an alternative matched unit is not available [10].

b. Occupational Health

iv. Prevalence in high risk jobs (examples are listed below*) for the following indications / applications:

1. Determine previous infection status
2. Have baseline seroprevalence data
3. Serology can be an additional test with the two negative RT-PCR results used to clear the patient from infection and determine seroconversion status for HCWs
4. Evaluate development of immunity in asymptomatic HCWs with persistent positive RT-PCR for > 2- 3 weeks (IgG specific)
5. To determine who should receive SARS-CoV-2 vaccine when available

*Health care workers (HCWs), Home Health care staff, Paramedics, Areas with high condensations (Labour camps), Ships, Taxi & bus drivers, Security guards, Meat factories, Oil field – offshore workers, etc..

Serologic tests should NOT be used for the following applications:

1. The test should not be used for the diagnosis of acute COVID-19 infections.
2. The test should not be used to make decisions about grouping
3. The test should not be used to make decisions about return to work (RTW)
4. The test should not be used to change PPE guidelines for health care workers
5. The test should not be used to issue immunity passports until the durability and duration of immunity is established

c. Public Health Applications

v. Epidemiologic studies of disease prevalence in the community and to get an accurate estimate of the case fatality rate

vi. Contact tracing

vii. Verification of vaccine response once antibody protection is proven

d. Research Applications:

Research is required and encouraged for the following areas and questions:

viii. Epidemiological studies to study disease prevalence in the population

ix. Timing and kinetics of antibodies production

x. Duration of antibody detection

xi. Effectiveness of antibodies in producing protective immunity

xii. Type of antibody necessary for providing long term protective immunity?

xiii. Significance of serology negative COVID-19 patients; how are they different? Are they truly neagative or should different antibody assays with different antigen targets be used?

xiv. Are people with high levels of neutralizing antibodies less likely to contract the virus again?

3. Limitations of Serologic Tests:

- At present, the immunologic correlates of immunity are not well defined.
  - Level of antibody required for immunity
  - Duration of protection
  - Kinetics of the antibody response
  - Ability to protect from reinfections
  - The correlation between binding antibody titers to neutralization abilities
- Cross reactivity with other coronaviruses may lead to false positive result

Recommendations:

1. Information impacting serologic recommendations is rapidly evolving and this guideline will be updated as more information becomes available.

2. The working group recommends the use of serologic assays that have been approved by FDA or have been granted Emergency Use Authorization (EUA) since their test performance data have been reviewed by FDA. Kits with CE Mark (European Conformity) are acceptable as long as there is evidence that the kit / analyzer have been verified through independent conformity assessment body.

3. Assays with specificity > 99.5% are recommended. The use of POCT kits is NOT recommended by the working group as the current kits available to date don't have the desired specificities and sensitivities.

4. 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) will increase the positive predictive value (PPV).

5. The use of two step test approach or an orthogonal testing algorithm is also recommended to increase the PPV.

6. 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.

7. Until more information is available about the dynamics of IgA detection in serum, testing for IgA antibodies is not recommended.

8. Serologic testing should be ordered as a method to help establish a diagnosis when COVID-19 patients present late in the course of disease or in patients with late complications of COVID-19 illness, such as multisystem inflammatory syndrome in children.

9. Serologic testing should not be used for the diagnosing an acute COVID-19 infection or for the exclusion of infection in patients with compatible COVID-19 clinical presentation.

10. Serologic testing should be considered for high risk occupations to determine previous exposure and prior to vaccination should vaccine become available.

11. Serologic tests should be used for population seroprevalence studies and can also aid in contact tracing.

12. 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.

13. Serologic test results should not be used to make decisions about returning persons to the workplace.

14. Serologic test results should not be used for the diagnosis of acute infections.

15. Serologic testing should not be used to issue immunity passports until the presence, durability, and duration of immunity is established.

16. Research in the areas of SARS-CoV-2 serology is encouraged in this part of the world, particularly as it relates to protective immunity and durability of the antibodies.

17. The working group emphasizes the importance of education of the community regarding the current limitations of serological testing.

18. Test Interpretation:

The following comments or similar disclaimers shall be used to help interpreting results:

18.1 Negative result:

"This sample does not contain detectable SARS-CoV-2 IgG (or IgG/IgM as applicable) antibodies. This negative result does not rule out SARS-CoV-2 infection. Correlation with epidemiologic risk factors and other clinical and laboratory findings is recommended. **Serologic results should not be used as the sole basis to diagnose or exclude recent or past SARS-CoV-2 infection.** This assay was performed using (specify platform & technology used)"

18.2 Positive result:

"Results suggest recent or prior infection with SARS-CoV-2. Correlation with epidemiologic risk factors and other clinical and laboratory findings is recommended. Protective immunity cannot be inferred based on these results. Infrequently**, false positive results may be due to prior infection with other human coronaviruses.** Serologic results should not be used as the sole basis to diagnose or exclude recent or past SARS-CoV-2 infection. This assay was performed using (specify platform & technology used)"

18.3 Results of antibody testing should be interpreted with caution in immunocompromised patients (immunodeficiency, cancer, transplant, use of biologics, etc).

19. The working group recommends the support in establishing antibody neutralization assays in the UAE in designated laboratories by the National Crisis & Emergency Management Authority (NCEMA- UAE).

20. The working group recommends support in coordinated research to support public health policy in the field of SARS-CoV2 serology to be governed and supported by the National Research Committee.

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COVID-19 Diagnostic Guidelines Panel
The recommendations in the guidelines will be updated as new information on the Diagnosis of COVID-19 becomes available

Appendix 1: Members of the Working Group

Name	Designation & Specialty	Affiliation
Dr. Laila Abdel Wareth	Chair of working group. Consultant Clinical Pathologist	National Reference Laboratory- Mubadala
Dr. Eiman AlZaabi	Chair of NCEMA laboratory Subcommittee. Consultant Hematopathologist & Transfusion Medicine	Sheikh Shakhbout Medical City- SEHA
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COVID-19 Diagnostic Guidelines Panel
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