International Journal of
Virology & Infectious Diseases

Letter to Editor

Clinical Interpretation of Serological Tests in Patients with Covid-19 Mutations; Care is needed -

Adel AA Ismail*

Consultant in Clinical Biochemistry and Chemical Endocrinology; Director of pathology services (RTD) Mid-Yorkshire and Leeds Hospitals Trust, West Yorkshire; UK

***Address for Correspondence:** Adel AA Ismail, Consultant in Clinical Biochemistry and Chemical Endocrinology, Director of pathology services (RTD) Mid-Yorkshire and Leeds Hospitals Trust, West Yorkshire, UK,
E-mail: adelaaismail@aol.com

Submitted: 05 March 2020; **Approved:** 05 March 2021; **Published:** 08 March 2021

Cite this article: AAA Ismail. Clinical Interpretation of Serological Tests in Patients with Covid-19 Mutations; Care is needed. Int J Virol Infect Dis. 2021 March 08;6(1): 024-027.

ISSN: 2766-5070

Analytical inaccuracies of both covid-19 (SARS-CoV-2) antibodies and RT-PCR tests are recognised [1-3]. Recent mutations of the virus have the potential to increase further the inaccuracy of these laboratory tests warranting considerable care to avoid the mis-interpretation of the data derived from these tests.

Covid-19 is a single stranded positive-sense RNA-envelop virus with fewer than 30,000 nucleotides encoding 29 proteins. Of these 29, four are surface structural proteins namely spike (S), nucleocapsid (N), envelop (E) and membrane (M) proteins. The M-protein also contributes to viral infectivity and cell-fusion by the viral spike S-protein [4]. The other 25 are non-structural proteins such as the virus's own protease, polymerase, replicase and helicase enzymes. Covid-19 non-structural proteins also play major roles in maintaining viral integrity, transmission, infectivity, regulation and replication. Mutations are caused by (a) copying errors during viral replication (common) (b) recombination or re-assortment with other viruses which fortuitously happen to be present in the same infected cell and (c) adaptive mutations to neutralising agents [5].

Viral replication cycles are innately error prone. Although these errors are random some would occur in the snippets of structural proteins used in the vaccines such as the spike S-proteins. Recent mutations have enhanced viral transmissibility and probably pathogenicity too, not only in the elderly but also in the young causing serious illness even in those without known comorbidities. Rampant infection rates in some parts of the world are therefore expected to produce more replication cycles producing more mutations. Clinically important mutations so far identified are N501Y, K417N, E484K, D614G and A701B (see footnote for decoding). The E484K and N439K are adaptive "escape" mutations. The E484k replaces 484th aminoacid glutamate (E) by lysine (K), switching a negative charge to a positive one, allowing the virus to evade and escape recognition by the immune system. This mutation appears to have been evolved independently in South Africa, Brazil and the UK (Bristol area). Such escape mutations could be further enhanced when combined with other mutations.It is therefore abundantly clear that future mutation, as yet unidentified are inevitable, unpredictable and could only be identified retrospectively.

Escape mutations could adversely impact the analytical accuracy of serological and possibly some RT-PCR tests [3,5,6] which were formulated and constructed against the original "unmutated" covid-19 strain. In addition, individuals with escape mutations could avoid the immune system and may not be producing antibodies leading to lower or even negative diagnostic results despite infection. Adverse impacts on the accuracy of RT-PCR could occur if the probes/primers used do not properly anneal in RT-PCR tests [5,6]. It is therefore imperative that the reliability of these analytical tests is constantly re-assessed and if necessary updated for use in samples from patients infected with various mutations of the virus.

Key characteristics [7] of antibodies are essentially four; (a) titre; assesses the total quantity of antibodies but not their quality or efficacy as neutralising agents. Nevertheless, the titre alone is commonly used in recent covid-19 studies as the predictor of humoral immunity, the protection's durability, viral clearance, modulation of the infection's severity, the efficacy of vaccines' administration and the therapeutic use of antibodies in convalescent plasmas; (b) specificity; assesses the binding of antibodies to the targeted antigenic determinants (epitopes) of covid-19 and their cross-reactivity with other antigens. Covid-19 proteome shares regions of conservation such as the trimeric S-protein with its highly conserved C-terminal S2-subunit epitopes. This is shared with other endemic CoVs [8] such as the four betacoronoviruses-1and the two more pathogenic namely SARS-CoV-1 and MERS-CoV which have caused earlier pandemics. It appears that antibodies against some of these strong conserved region(s) in this family could, if successful be the holy grail, providing wide divergent functional cross-reactivity and enhances the chance of effective neutralising antibodies against wide range of potential mutations (c) avidity/affinity; reflects the strength of binding between covid-19 domains (epitopes) and the complementary antibodies (paratopes). Avidity/affinity defines the strength and speed of antibody interaction with the virus. Affinity reflects interaction between a single antibody paratope and a single covid-19 epitope. The simplest antigen has multiple epitopes and the binding reaction involves a number of complementary paratopes. An individual epitope has a linear/continuous primary structure of 5-8 aminoacids complexed into secondary and tertiary configurations; and constitutes a surface area between 0.4 and 8 nm^2 (covid-19 is a large virus with a diameter of 120 nm compared with only 10 nm for IgG. Endogenously produced antibodies are invariably polyclonal involving multiple binding sites. Avidity is a measure of the overall strength of binding between all epitopes and the corresponding antibodies' paratopes. Avidity/affinity is measurable and expressed as 10^n where n is an exponent. For example, an affinity/avidity of ~10^7 would be considered poor as a neutralising agent even if the antibody titre is high. On the other hand, an avidity/affinity of 10^9-10^{10} being respectively 100 and 1000 times stronger even at low titres of antibody. Furthermore, the avidity/affinity of antibodies produced against a specific antigen (such as covid-19) is not uniform and varies among individuals. Finally, (d) efficacy; evaluates the performance of antibodies as neutralising agents and may be therefore perceived as essentially the summation of all antibodies' characteristics. The standard procedure is the plaque reduction neutralisation test (PRNT) is utilised for determining efficacy and is considered to be the gold standard. The test though complex and very time consuming (3-7 days) is highly sensitive and specific. After mixing a standardised quantity of viral particles with serially diluted plasma samples, the suspension is then incubated with a confluent semisolid monolayer of susceptible host-target cells covered with agar or carboxymethyl cellulose. The number of plaques (regions of infected cells) is determined microscopically (following the addition of a dye) and their numbers reflect the ability of antibodies in the plasma to neutralise the virus (effective neutralising antibody produces fewer plaques). The efficacy of antibodies produced to any individual vaccine and/or in convalescent plasmas and their ability to neutralise viral mutants is paramount hence the need for fuller evaluation.

A simplified test which takes 2 days is the Microneutralization (MN) assays [9]. The test detects the viral antigens in virus-infected cells in microtiter plates in combination with an immunological and dye end point such as ELIZA. The detection of viral antigens indicates the absence of neutralizing antibodies at that serum dilution. The main advantage of MN assays is its potential for high-throughput automation. But both PRNT and MN tests require viral culture growth performed in a properly equipped BSL-3 laboratory. Further simplifications which removed the need for live viruses and could therefore be performed in BSL-2 laboratories are pseudo or surrogate viruses neutralising tests [10,11] with results available in 1-2 hr. However, the reliability and accuracy of these shortened and modified tests are not independently verified.

Data which rely on antibody titres alone in evaluating a given antibody's efficacy may have been a factor contributing to the conflicting outcomes and conclusions between studies in which convalescent plasmas are administered as neutralising agents [12-16]. Another source of clinical misinterpretation is that recent viral mutations including escape mutations could be producing effective neutralising endogenous antibodies by the host which may not be detected by serological titre methods, ignoring what may be effective neutralising agents. Similarly, interpretation of epidemiological data based on un-validated laboratory tests alone without additional correlates warrant care.

In conclusion,

(1) Effective covid-19 neutralising antibodies should have an affinity/avidity greater than 10^9 combined with wider-cross reactivity involving a well-conserved domains that are structurally shared between SARS-CoV family and potential mutations. Measurements of titre alone are simple and pragmatic; however, their limitations must be taken into account.

(2) Mutations could avoid the immune system. Assessment, revalidation and upgrading of lab tests' accuracy against new mutations would be prudent or even mandatory to diminish clinical and epidemiological mis-interpretation of these tests.

(3) Last but not least, acquiring accurate test results is essential not only for upholding but also consolidating the tremendous successes so far achieved in timely management and eventual suppression of covid-19 and its future mutations.

Footnote: Every new mutation is named by a letter, followed by a number and another letter. Each of the 20 aminoacids is abbreviated by aletter some of which are straight forward but others not e.g. **A** (alanine), **E**(glutamic acid), **G**(glycine), **S**(serine), **V**(valine), **B**(aspartic acid or asparagine), **D**(aspartic acid), **N**(asparagine), **Y**(tyrosine), **K**(lysine) et cetera. The number between the two letters in this biological shorthand indicates its location in the spike protein; the following letter denotes the substituted aminoacid of the original one cited before the number]

ACKNOWLEDGEMENT

I am grateful to Professor Sandra J Legan who read and made a number of very helpful comments.

REFERENCES

1. Ismail AA. Serological tests for COVID-19 antibodies: Limitations must be recognized. Ann Clin Biochem. 2020 Jul;57(4):274-276. doi: 10.1177/0004563220927053. Epub 2020 May 14. PMID: 32343598.

2. Armstrong S. Covid-19: Government buried negative data on its favoured antibody test. BMJ. 2020 Nov 12;371:m4353. doi: 10.1136/bmj.m4353. PMID: 33184049.

3. Liu G, Rusling JF. COVID-19 Antibody Tests and Their Limitations. ACS Sens. 2021 Feb 5:acssensors.0c02621. doi: 10.1021/acssensors.0c02621. Epub ahead of print. PMID: 33544999; PMCID: PMC7885805.

4. Hu Y, Wen J, Tang L, Zhang H, Zhang X, Li Y, Wang J, Han Y, Li G, Shi J, Tian X, Jiang F, Zhao X, Wang J, Liu S, Zeng C, Wang J, Yang H. The M protein of SARS-CoV: basic structural and immunological properties. Genomics Proteomics Bioinformatics. 2003 May;1(2):118-30. doi: 10.1016/s1672-0229(03)01016-7. PMID: 15626342; PMCID: PMC5172243.

5. Wang R, Hozumi Y, Yin C, Wei GW. Mutations on COVID-19 diagnostic targets. Genomics. 2020 Nov;112(6):5204-5213. doi: 10.1016/j.ygeno.2020.09.028. Epub 2020 Sep 20. PMID: 32966857; PMCID: PMC7502284.

6. Ismail AA. SARS-CoV-2 (Covid-19): A short update on molecular biochemistry, pathology, diagnosis and therapeutic strategies. Ann Clin Biochem. 2021 Feb 10:4563221992390. doi: 10.1177/0004563221992390. Epub ahead of print. PMID: 33478237.

7. Bordeaux J, Welsh A, Agarwal S, Killiam E, Baquero M, Hanna J, Anagnostou V, Rimm D. Antibody validation. Biotechniques. 2010 Mar;48(3):197-209. doi: 10.2144/000113382. Erratum in: Biotechniques. 2010 May;48(5):351. PMID: 20359301; PMCID: PMC3891910.

8. Ladner JT, Henson SN, Boyle AS, Engelbrektson AL, Fink ZW, Rahee F, D'ambrozio J, Schaecher KE, Stone M, Dong W, Dadwal S, Yu J, Caligiuri MA, Cieplak P, Bjørås M, Fenstad MH, Nordbø SA, Kainov DE, Muranaka N, Chee MS, Shiryaev SA, Altin JA. Epitope-resolved profiling of the SARS-CoV-2 antibody response identifies cross-reactivity with endemic human coronaviruses. Cell Rep Med. 2021 Jan 19;2(1):100189. doi: 10.1016/j.xcrm.2020.100189. PMID: 33495758; PMCID: PMC7816965.

9. Liu L, Wen K, Li J, Hu D, Huang Y, Qiu L, Cai J, Che X. Comparison of plaque- and enzyme-linked immunospot-based assays to measure the neutralizing activities of monoclonal antibodies specific to domain III of dengue virus envelope protein. Clin Vaccine Immunol. 2012 Jan;19(1):73-8. doi: 10.1128/CVI.05388-11. Epub 2011 Nov 23. PMID: 22116689; PMCID: PMC3255958.

10. Nie J, Li Q, Wu J, Zhao C, Hao H, Liu H, Zhang L, Nie L, Qin H, Wang M, Lu Q, Li X, Sun Q, Liu J, Fan C, Huang W, Xu M, Wang Y. Establishment and validation of a pseudovirus neutralization assay for SARS-CoV-2. Emerg Microbes Infect. 2020 Dec;9(1):680-686. doi: 10.1080/22221751.2020.1743767. PMID: 32207377; PMCID: PMC7144318.

11. Tan CW, Chia WN, Qin X, Liu P, Chen MI, Tiu C, Hu Z, Chen VC, Young BE, Sia WR, Tan YJ, Foo R, Yi Y, Lye DC, Anderson DE, Wang LF. A SARS-CoV-2 surrogate virus neutralization test based on antibody-mediated blockage of ACE2-spike protein-protein interaction. Nat Biotechnol. 2020 Sep;38(9):1073-1078. doi: 10.1038/s41587-020-0631-z. Epub 2020 Jul 23. PMID: 32704169.

12. Pathak EB. Convalescent plasma is ineffective for covid-19. BMJ. 2020 Oct 22;371:m4072. doi: 10.1136/bmj.m4072. PMID: 33093025.

13. Libster R, Pérez Marc G, Wappner D, Coviello S, Bianchi A, Braem V, Esteban I, Caballero MT, Wood C, Berrueta M, Rondan A, Lescano G, Cruz P, Ritou Y, Fernández Viña V, Álvarez Paggi D, Esperante S, Ferreti A, Ofman G, Ciganda Á, Rodriguez R, Lantos J, Valentini R, Itcovici N, Hintze A, Oyarvide ML, Etchegaray C, Neira A, Name I, Alfonso J, López Castelo R, Caruso G, Rapelius S, Alvez F, Etchenique F, Dimase F, Alvarez D, Aranda SS, Sánchez Yanotti C, De Luca J, Jares Baglivo S, Laudanno S, Nowogrodzki F, Larrea R, Silveyra M, Leberzstein G, Debonis A, Molinos J, González M, Perez E, Kreplak N, Pastor Argüello S, Gibbons L, Althabe F, Bergel E, Polack FP; Fundación INFANT–COVID-19 Group. Early High-Titer Plasma Therapy to Prevent Severe Covid-19 in Older Adults. N Engl J Med. 2021 Feb 18;384(7):610-618. doi: 10.1056/NEJMoa2033700. Epub 2021 Jan 6. PMID: 33406353; PMCID: PMC7793608.

14. Joyner MJ, Carter RE, Senefeld JW, Klassen SA, Mills JR, Johnson PW, Theel ES, Wiggins CC, Bruno KA, Klompas AM, Lesser ER, Kunze KL, Sexton MA, Diaz Soto JC, Baker SE, Shepherd JRA, van Helmond N, Verdun NC, Marks P, van Buskirk CM, Winters JL, Stubbs JR, Rea RF, Hodge DO, Herasevich V, Whelan ER, Clayburn AJ, Larson KF, Ripoll JG, Andersen KJ, Buras MR, Vogt MNP, Dennis JJ, Regimbal RJ, Bauer PR, Blair JE, Paneth NS, Fairweather D, Wright RS, Casadevall A. Convalescent Plasma Antibody Levels and the Risk of Death from Covid-19. N Engl J Med. 2021 Jan 13:NEJMoa2031893. doi: 10.1056/NEJMoa2031893. Epub ahead of print. PMID: 33523609; PMCID: PMC7821984.

15. Nie J, Li Q, Wu J, Zhao C, Hao H, Liu H, Zhang L, Nie L, Qin H, Wang M, Lu Q, Li X, Sun Q, Liu J, Fan C, Huang W, Xu M, Wang Y. Establishment and validation of a pseudovirus neutralization assay for SARS-CoV-2. Emerg Microbes

Infect. 2020 Dec;9(1):680-686. doi: 10.1080/22221751.2020.1743767. PMID: 32207377; PMCID: PMC7144318.

16. Tan CW, Chia WN, Qin X, Liu P, Chen MI, Tiu C, Hu Z, Chen VC, Young BE, Sia WR, Tan YJ, Foo R, Yi Y, Lye DC, Anderson DE, Wang LF. A SARS-CoV-2 surrogate virus neutralization test based on antibody-mediated blockage of ACE2-spike protein-protein interaction. Nat Biotechnol. 2020 Sep;38(9):1073-1078. doi: 10.1038/s41587-020-0631-z. Epub 2020 Jul 23. PMID: 32704169.