AgPlus diagnostics

The role of Clinical Tests in the management of the global pandemic induced by SARS-COV-2

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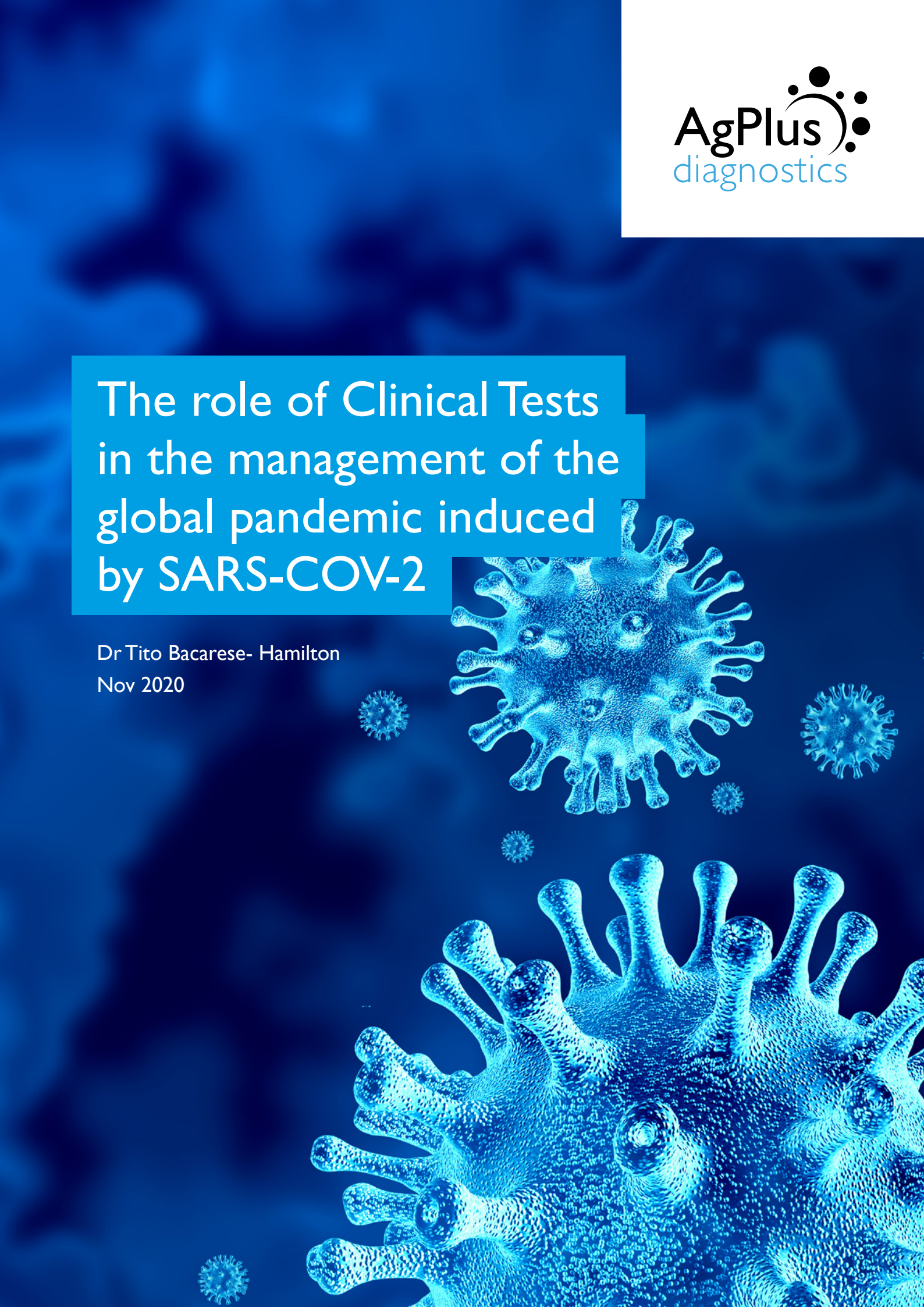

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KEY POINTS

In less than 12 months SARS-CoV-2 has infected > 55 million people and has been implicated in the death of > 1.3million

Use of novel gene sequencing and protein expression techniques have rapidly elucidated the genetic structure and the detailed proteomics of the virus

Knowledge of the genetic and protein make-up of the virus has spawned the development of an unprecedented number of both molecular and serological tests to diagnose and monitor the condition throughout the classic disease dynamics of incubation, early, progressive and recovery phases

The main molecular test established for disease diagnosis is based on gene sequencing of the virus using samples obtained by swabbing the upper and/or lower respiratory tract; the key serological test for confirming prior infection and/or monitoring of the condition is based on the measurement of IgG antibodies in patient blood samples

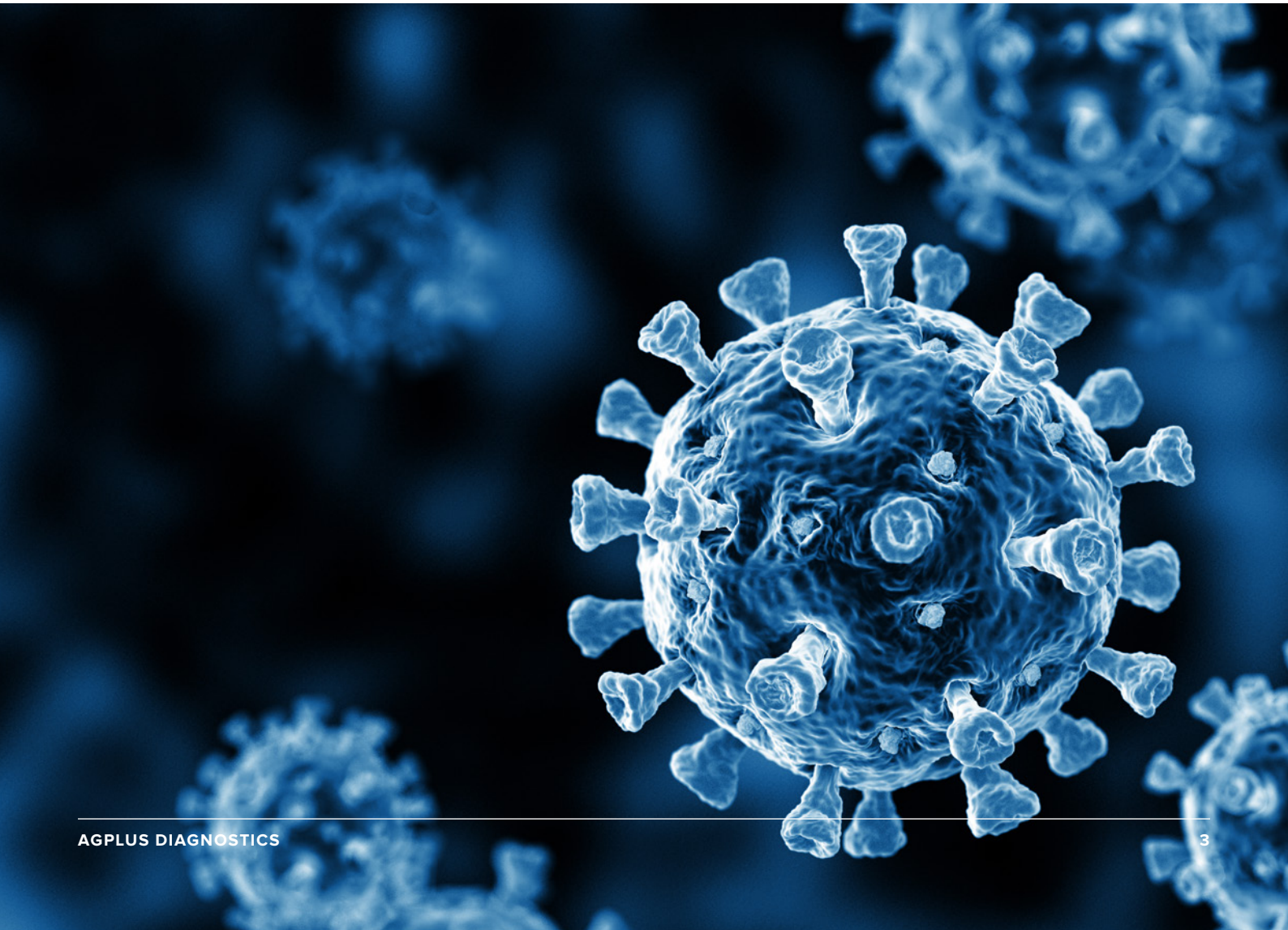
Although much is known of the virus and the condition it induces there remain big gaps in our knowledge relating to infectivity and correlates of protection

The advent of vaccine therapies against SARS-CoV-2 will accelerate the war against this pathogen, tests will still be fundamental to control the management of the disease and especially for surveillance and epidemiological purposes

INTRODUCTION

Pathogenic coronaviruses have presented a challenge to public health over the past two decades. These viruses caused the Severe Acute Respiratory Syndrome (SARS) outbreak in 2002-03, followed in 2012-13 by the Middle East Respiratory Syndrome (MERS) (Ref 1). In January 2020 the Chinese CDC announced the outbreak of a novel respiratory syndrome caused by a pathogenic strain of common coronaviruses which was christened SARS-CoV-2. Unlike SARS and MERS, the spread of SARS-CoV-2 has reached pandemic proportions and has been declared a global public health emergency (Ref 2). At the time of writing, approximately 55M cases and 1.3M deaths in > 210 countries have been attributed to this novel pathogen. Unsurprisingly, many aspects of the pathogen and the associated clinical syndrome, COVID-19, have been investigated and a voluminous amount of literature published. There is broad-based recognition that clinical tests are required to address this health crises and many hundreds of these tests have been developed and commercialised for use (Ref 3).

This brief overview summarises the key known knowns (things we know we know) and the known unknowns (things we know we do not know).

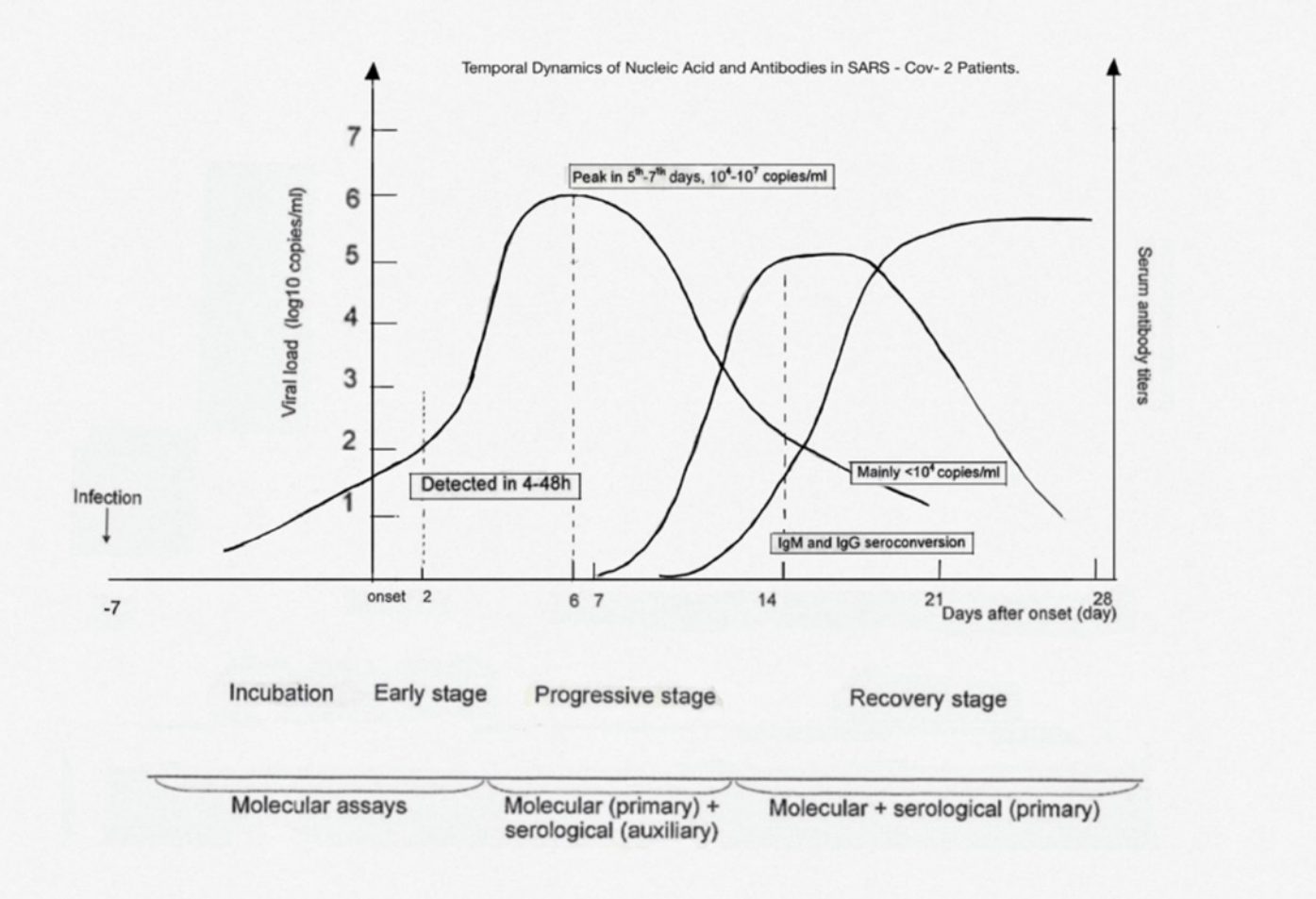


KNOWN KNOWNS

- The genomic structure and phylogenetics of SARS-Cov-2 have been documented as have the proteins expressed by these genes (Ref 4).
- The mechanism via which the virus enters a human cell and the process by which it invades the lung have been elucidated
- The typical transmission characteristics of infection (Ref 5) are an incubation period followed by early, progressive and recovery phases. The temporal dynamics of nucleic acid and antibodies in infected subjects are as follows (diagram abridged from (Ref 4):

IN SUMMARY:

1. Nucleic Acid (NA) – after an incubation period of approx. 7 days, viral NA is detected in infected subjects within 4 to 48 hours, levels peak on 5 to 7 days with viral loads of 10^4 to 10^7 copies/ml. These loads decline to < 10^4 copies/ml in the recovery phase, the total viral shedding time is @ 20 days
2. Antibodies – IgM are detectable 5 to 12 days after disease onset, IgG seroconversion occurs > 14 days after onset and they increase rapidly during the recovery phase. IgM typically subside after 10 to 30 days but IgG persist much longer



KNOWN UNKNOWNS

- How Nucleic Acid levels relate to clinical symptoms, infectivity and disease course
- Correlates of Immunity are a major area of investigation as several Vaccine programmes approach the end of Phase 3 of their development. Some of the key questions to be answered:
  - (i) Are measured antibodies able to neutralise the virus and the consequences of re-infection
  - (ii) The antibody titre required for protection
  - (iii) Role of cell-mediated (T-cell) immunity in conferring protection
  - (iv) Duration of immunity – do IgG's confer immune memory i.e. how long do they persist for and do they remain protective?
  - (v) Cross-reaction of measured antibodies to other common coronaviruses; can these cross-reacting antibodies confer immunity?
  - (vi) Identification of epitopes in the viral proteins which are most important to confer immunity
  - (vii) Will mutational changes in the virus require constant updating of clinical tests and of potential vaccine therapies
  - (viii) At a population level, what proportion must be immune to SARS-CoV-2 to generate herd immunity?
- Is Antibody Dependent Enhancement (ADE), as occurs with Dengue, a potential problem (secondary infection induces very severe disease)?
- Can predictive tests be developed which will identify infected patients who will develop very severe symptoms and/or death.

TYPES OF TEST

Clinical tests are based on both NA and antigenic/antibody responses to the virus. At each phase of the disease, NAs, IgM's and IgG's are found in variable concentrations enabling testing strategies combining molecular (i.e. nucleic acid) and serological (i.e. antibody) tests to reveal infection status and disease phase.

Infectious disease testing is based on two principal types of test:

(i) Molecular Tests that determine the presence/amount of pathogen in the body, these tests are used primarily for diagnosis and/or to confirm clinical treatment and surveillance activities. These tests can also detect fragments of the pathogen as well as the entire organism so a positive result can be obtained even though the pathogen is no longer able to replicate or cause disease

(ii) Antibody tests (serological tests) determine the body's immune response to infection, and can provide information on current and prior infections. They can detect prior infection after a PCR negative result.

Currently, molecular tests (based on PCR or alternatives like LAMP) are the de facto diagnostic for detecting the virus in the incubation and early phases of disease. However, since they cannot provide information on an individual previously infected but clear of infection, serological tests provide critical data during the progressive and recovery phases with respect to disease severity and immune status of individuals.

PERFORMANCE CHARACTERISTICS

Performance of tests are defined by analytical and clinical parameters:

Analytical parameters include limit of detection, reproducibility, linearity and cross-reactive properties

Clinical parameters are known as Sensitivity (ability for test to deliver a positive result in the presence of disease) and Specificity (ability of the test to deliver a negative result in the absence of disease). Parameters to quantify the probability of generating a positive result (Positive Predictive Value, PPV) and a negative result (Negative predictive Value, NPV) are also commonly quoted. They are extremely sensitive to the Prevalence of disease in a given population such that in scenarios of poor prevalence (< 10%) tests, regardless of their sensitivity/specificity, are at best 50% accurate (Ref 6). This is because of False Positives (FP) and False Negatives (NP) rates.

Examples of False Positives - In molecular tests, any NA contamination will produce a positive result as will fragments of inactive virus; cross-reaction of antibodies to common coronaviruses are a common cause of FP in serology assays

Examples of False Negatives – In molecular tests inadequate or incorrect sample collection (swabbing) are the principal cause; for both molecular and serological tests, poor limits of detection will cause FNs.

The consequences of test errors, both FPs and FNs, are not equivalent eg in a serology test a FN may prevent an individual from returning to work but a FP may initiate an epidemic chain.

CHOICE OF SAMPLE

For molecular tests the choice of sample matrix is critically important as viral load varies depending on the origin of specimens. Viral RNA can be collected/extracted from swabs (nasopharygeal, oropharyngeal), bronchoalveolar fluid lavage (BAFL), saliva and blood. Viral load is highest in respiratory tract samples (BALF> sputum > nasal swab > pharyngeal swab) (Ref 7). Unsurprisingly swabs are in extremely high demand and necessitate the use of Transport Medium to transfer the sample to the test facility. Because of these bottlenecks, saliva samples are under intense evaluation as an alternative to swabs as are blood-based antigen assays using standard immunochemistry.

GOING FORWARD

Tests have been established for diagnosis, identification of convalesced patients to donate antibody therapy and for population surveillance. Going forward, control of the pandemic requires tests for three key populations:

- Those infected
- Those infectious
- Those susceptible to severe disease.

Quantitative, accurate and sensitive Molecular and Serological assays offer a method of identifying infected individuals. For diagnosis, it is acknowledged that molecular assays should be used. However as nucleic acid and antibody concentrations fluctuate throughout the various phases of infection, both molecular and serological assays can play important roles.

More data is required to establish what contributes to infectivity, both in terms of viral load and other factors such as time of exposure, environmental conditions (temperature, humidity) etc. Similarly the key correlates of immunity need to be established in terms of amount and type of antibody required and the duration of immune response.

Also data is required as to factors/symptoms that are predictive and/or prognostic of disease outcomes. Gene-based differences in host responses may be a very important consideration.

Finally there are important considerations as to the samples used for testing and the test availability and time-to-result. Saliva and blood-based tests may become the mainstay as opposed to swabbing which is both uncomfortable and prone to error. The current pandemic has also accelerated the transition of lab-based tests to decentralised centres with near-patient test capabilities.

Research and development activities continue apace to answer many of these questions. The advent of a series of vaccines against SARS-CoV-2 will answer some of these questions but may well give rise to new ones requiring the use of both molecular and serological tests. It is likely new tests based on different sample media e.g. saliva, blood and using different techniques e.g. determination of viral antigen in blood will emerge in the coming months.

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