Review Article

Classical and Molecular Virology in the Context of SARS-CoV-2

Shailesh D Pawar[1], Babasaheb V Tandale[2], Deeksha S Tare[3], Sachin S Keng[4], Sadhana S Kode[5], Priya Abraham[6]

Diagnostic virology has evolved as a discipline from being confined to hospital laboratories to dedicated state-of-the art research facilities around the world, working on different aspects of diagnosis and research on viral diseases. As the world struggles with the ongoing pandemic of Coronavirus Disease-2019 (COVID-19) caused by the Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2), the need for the development and upgradation of new and existing tools has resurfaced. Latest technological advances in the field of molecular diagnosis have paved the way for providing faster turn-around times for tests, leading to expedited treatment and quarantine decisions. The use of classical and molecular virology tools leads to a better understanding of the virus in the context of the host. The initial diagnosis of the SARS-CoV-2 was carried out by using Next Generation Sequencing (NGS) platforms. Currently, real-time RT-PCR is the gold standard for the laboratory diagnosis of SARS-CoV-2. Serological assays are being used for the detection of antibodies against SARS-CoV-2 for serodiagnosis and to understand the parameters like infection rate and seroprevalence in the community. The present article describes the advancements in the field of viral diagnostics and the role of classical and molecular virology in the context ofthe COVID-19 pandemic response and research.

[*J Indian Med Assoc* 2021; **119(1):** 24-30]

Key words **: Classical virology, Molecular virology, COVID-19, Pandemic, Serology, Epidemiology.**

Viruses make up over two-thirds of all new human pathogens[1]. On an average, more than two new species of human virus are reported every year[2].The current Coronavirus disease (COVID-19) pandemic caused by the Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) is a public health emergency across the world. During a pandemic situation, diagnostics are fundamental as a primary response for treatment and implementation of prevention and control measures.

The pioneering methods in diagnostic virology were the complement fixation test to detect antibodies and virus isolation using tissue culture during 1929 and 1948 respectively. Eventually rapid diagnosis was possible with the advent of techniques using monoclonal antibodies in the 1970s and molecular methods such as the polymerase chain reaction (PCR) in 1985[3]. The DNA sequencing by chain termination method was first described by Sanger *et al* in the year 1977, and has revolutionized the field of biology[4]. The present article discusses briefly the advancements in the field of viral diagnostics and the role of classical and molecular virology in the context of COVID-19 pandemic response and research.

Editor's Comment :

- **Concerted efforts including classical and molecular virology along with epidemiological studiesin pandemic situation would help in impact assessment, containment and mitigation.**

Role of Classical Virology in COVID-19 :

Since a living system is required to culture viruses, host systems such as cell culture, embryonated chicken eggs and mammalian hosts are used for isolation depending on the preference of the viruses.The influenza virus was first isolated using embryonated chicken eggs in the year 1933[5]. The first two human viruses to be isolated using tissue culture were Mumps and Influenza viruses in 1948[6].

The advantages of virus isolation are that it makes virus available for serological and molecular diagnostic assays, vaccine development, antigenic characterization, studies on morphological and structural aspects, pathogenesis studies and antiviral studies. The only disadvantage of virus isolation is that it is time-consuming,thus cannot be used for diagnostic purposes. SARS-CoV-2 is a culturable virus and has been isolated using Vero CCL-81 and Vero E6 cell lines[7]. It has been shown that SARS-CoV-2 uses the SARS-CoV receptor angiotensin-converting enzyme

ICMR-National Institute of Virology, Pune 411021

[1]MSc, PhD, Scientist 'E' and Nodal Officer, Poliovirus Essential Facility and Corresponding Author

[2]MD (PSM), PG Diploma-Bioethics, Scientist 'F' and Group Leader

[3]MSc, ICMR-Senior Research Fellow

[4]MSc, Technical Officer 'B', Poliovirus Essential Facility

[5]MSc, Senior Technical Officer '3', Poliovirus Essential Facility

[6]MD, FRCPath, PhD, Director

Received on : 17/11/2020
Accepted on : 19/11/2020

2 (ACE2) for entry and the cellular serine protease TMPRSS2 for S protein priming. Moreover, SARS-CoV-2 shows increased binding affinity to ACE2 as compared to SARS-CoV, thereby making it more pathogenic[8]. India was the fifth country in the world to isolate the virus.

Use of Serological Assays :

Serological assays are required for the detection of specific antibodies against a virus. These include the complement fixation, enzyme-linked immuno-sorbent assay (ELISA), immunofluorescence assay (IFA), hemagglutination inhibition and neutralization assays which could be used for different purposes. The advantages of serological assays are that they could be used to estimate immunity levels and spread of the disease by undertaking large-scale serosurveys. Such studies also enable researchers to determine the stages of infection among the infected individuals, such as acute or convalescent phase. Use of serological assays for vaccine-efficacy studies during clinical trials enables the determination of protective immune response against the vaccine. Immuno-fluorescence assays using virus-infected Vero E6 cells spotted on glass slides and ELISAs using extracts or supernatant of infected cells were among the first assays used in serological diagnosis of the Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) of 2002[9]. There have been several advancements in the field of diagnosis since the SARS-CoV epidemic. It has been reported that serology testing could be useful to assist sero-diagnosis of SARS-CoV-2[10]. It has also been speculated that in addition to the use of serological data to identify and contain cases, serological studies can also be used to assess how much community transmission has occurred, to determine the immunity of healthcare workers and also to identify individuals who had mounted a strong immune response as possible donors for plasma therapy[11].

The ELISAs, lateral flow immunoassays (LFIAs), or chemiluminescent immunoassays (CLIAs) are the three serological assays which are being widely used for COVID-19 diagnosis. In a meta-analysis carried out for studies reporting serological assays for COVID-19 diagnosis, the pooled sensitivity of ELISAs measuring IgG or IgM was reported as 84.3%, of CLIAs was 97.8% and of LFIAs was 66.0%, which was the lowest[12].The World Health Organization (WHO) also advices cautious use of such rapid immunodiagnostic tests due to the high possibility of false positive results, due to cross-reactivity with other pathogens.

Use of Rapid diagnostic Tests (RDTs) for detection of antibodies and antigensagainst SARS-CoV-2 :

Lateral-flow immunoassays, in the form of Rapid Detection Tests (RDT)are employed for the detection of several diseases and viruses. They facilitate rapid detection of antigens and antibodies. However, they have the limitation of poor sensitivity and specificity. The pooled sensitivity for LFIAs, the potential point-of-care method, was reported lower than that for ELISA and CLIA[12]. The detection of antigens by using RDTs is generally preferred, as the development of antibodies requires time after infection. There are different types of antibodies based on their function like neutralizing, complement fixing, cross-reacting and hemagglutination inhibition antibodies. RDTs are not able to distinguish the exact properties of the antibody. RDTs can also not determine the antibody titres which may be required to study disease progression. The WHO has encouraged research on the performance and potential diagnostic utility of serological rapid detection tests for COVID-19.

In case of respiratory pathogens, the detection of antigens is easier as the virus is shed in the respiratory fluids. The ICMR has issued an advisory on the on the use of an RDT kit called the 'Standard Q COVID-19 Ag detection kit', for the diagnosis of COVID-19[13]. It was reported that the test has a specificity ranging from 99.3 to 100%, and a sensitivity ranging from 50.6% to 84% in two independent evaluations, depending upon the viral load of the patient. Higher viral load correlated with higher sensitivity. The turn-around time for this test was 15 minutes. Though there is no data on the application of this test on a large sample size in clinical setting.

Role of molecular virology in COVID-19 :

The development of PCR techniques has revolutionized the field of diagnostics and research. Multiplex PCR technology has made the diagnosis of multiple pathogens possible at the same time, from a single sample[14]. Molecular assays for the detection of microorganisms can be designed even when only partial nucleic acid sequence information is available. This is valuable for the identification and diagnosis of new diseases and emerging pathogens since the rapid development of in-house assays becomes possible[15]. The advent of real time PCR has enabled detection and absolute quantification of the amplified products in lesser turn-around times, making real time PCR the Gold-standard for molecular diagnosis for viral diseases like influenza H1N1 and more recently, SARS-CoV-2[16]. Although the reverse transcriptase-PCR (RT-PCR) for the diagnosis of COVID-19 is highly specific, further optimization to mitigate the false negative rate has been recommended on high priority[17]. These assays are also expensive and need to be carried out with utmost precautions, because a lack of PCR discipline causes cross-contamination and can be catastrophic. For COVID-19 testing in India, two more molecular based tests are available apart from RT-PCR, namely, True Nat and CBNAAT (cartridge based nucleic acid amplification test)[18]. The advisory on the testing strategy for COVID-19 has been issued by the ICMR, and has been summarized in Box 1[19].

The latest technology of Next-Generation Sequencing (NGS) enables analysis of the complete genomes of organisms in a relatively shorter time from small quantities of the sample detecting the most subtle differences in the virus genomes. Sequencing and next generation sequencing can reveal the aspects of the origin, pathogenicity markers, antiviral resistance markers, markers for adaptation in humans, etc. The first detection of SARS-CoV-2 was achieved using NGS, which underscored the importance of such platforms for quick diagnosis of unknown etiology[20].The full genome sequencing of the first two SARS-CoV-2 viruses from India was carried out using the Miniseq platform. They were found to represent separate introductions of SARS-CoV-2. In the study, potential T-cell and B-cell epitopes, as potential vaccine targets, were also identified using bioinformatics tools[21]. Modern technologies like ultrastructural analysis, transcriptomic, proteomic, interactome analyses, single cell RNA seq, RNA interference, CRISPR gene editing etc., have revolutionized the field of biology as a whole. Master Regulator Analysis, which elucidated regulatory networks among the SARS-CoV-2 and host cell proteins revealed the parts of the human interactome which are most affected by infection[22]. The immuno-pathological aspects of the disease, as well as the immunological landscape after recovery has been studied in COVID-19 patients using the single-cell RNA sequencing techniques[23]. A promising dual CRISPR-Cas12 a based method for simple, ultrasensitive and visual detection of SARS-CoV-2 and Human Immunodeficiency Virus (HIV) has been developed enabling rapid detection with improved sensitivity of even a few copies of the virus. The development of other tests, like the Reverse transcription loop-mediated isothermal amplification and Recombinase polymerase amplification is also promising but not without certain disadvantages of its own like challenges in primer design, difficulty in quantification, interference from incorrectly folded primers, etc[24]. Such technologies could be used to explore therapeutics and cutting-edge diagnostic tools against this new virus.

Bioinformatics studies based on genome sequencing provide an insight into the structural and evolutionary aspects of the virus. Molecular clocks, phylogeny and phylogeography studies estimate the spatio-temporal features of evolution of the virus. The structural and functional aspects of the virus need to be correlated with laboratory studies. Software for selection pressure analysis, epitope prediction, molecular simulations, molecular docking, etc. can estimate several features of the organism. Structural studies on SARS-CoV-2 have revealed insights into the receptor binding of the virus and also towards vaccine targets[25]. However, these provide only predictions. The exact pathogenesis of a virus in the host system, or the efficacy of vaccines cannot be determined without carrying out experimental studies. This highlights the importance of correlation of such work with virology experiments in the laboratory.

Role of Molecular Epidemiology and Seroepidemiology in Pandemic Response :

Studies on disease surveillance, seroepidemiology, epidemiology, molecular epidemiology and vaccine evaluation in clinical and field use are important during pandemic response and research. The first COVID-19 case detected through the 'Seattle Flu Study', in a specimen collected on February 24, 2020, was the first documented case of community transmission in the United States at the time. These results initiated assessment of the spread of the virus in the Seattle region, which in turn accelerated public health efforts to mitigate the emerging pandemic. Surveillance studies such as multisite monitoring for influenza surveillance could help in early detection of emerging and re-emerging viruses, unusual epidemiological trends and estimation of the disease burden[26]. Networks of Virus Research and Diagnostic Laboratories across countries are important to understand disease dynamics in the community in this regard[27]. Serosurveillance studies during influenza A (H1N1) 2009 pandemic revealed that the virus infection was wide spread in all sections of the community, highlighting the importance of wide spread surveillance to understand disease progression and herd immunity[28]. Vaccine safety, immunogenicity, efficacy and antibody persistence studies on newly developed vaccines help understand the performance of newer vaccines in the community[29]. Retrospective studies with archived specimens during the influenza H1N1 virus pandemic in India revealed a low level of cross-reactive antibodies to the virus in humans in the pre-pandemic period in Maharashtra, India[30]. Such studies could also be performed for the determination of the seroprevalence status against coronaviruses among the Indian population. Thus, serological assays can answer several questions pertaining to public health as a whole.

For studying the seroprevalence against COVID-19, several studies have been carried out across the world. In a study carried out in Nigeria among healthcare workers, 26% IgG positivity was reported against SARS-CoV-2. This indicates a high exposure rate for the hospital staff and patients[31]. A higher rate of seroprevalence of antibodies against the virus has been reported among healthcare workers as compared to the local community[32]. In another study conducted among healthcare workers in Sweden, apart from a high IgG seroprevalence (19.1%) against the virus, a strong association was noted among study participants with patient contact but without known COVID-19 contact[33]. On the contrary, in another study from Belgium, being involved in clinical care, having worked during the lockdown phase, being involved in care for patients with COVID-19, and exposure to COVID-19–positive co-workers were not associated with seroprevalence, but rather, having a household contact with suspected or confirmed COVID-19 was associated[34].

The field of molecular epidemiology, based on genomic information, has contributed immensely in infectious disease research in the modern times in order to study the origins, evolution and host tropism of viral diseases. It has been established that the SARS-CoV-2 had a bat origin. However, since there is no close association of humans with bat colonies, it has been reported that coronaviruses are transmitted to humans by another animal host. The animal hosts of SARS-CoV and MERS-CoV were found to be civet cats and camels respectively, whereas, for SARS-CoV-2, pangolins are being speculated as the intermediate hosts[35]. It has also been reported that the amino acid sequence in the ACE2 receptor responsible for SARS-CoV-2 binding in farm animals and cats closely resembles the human receptor, which explains the possible ease of the cross of species barrier. Thus, molecular epidemiological studies could be useful in elucidating origin of emerging viruses.

COVID-19: Research Avenues :

One of the most pertinent questions in times of a pandemic caused by a new virus is about the pathogenicity of the virus, and the severity of the disease. Animal studies form an integral part of virology research because experiments using laboratory animals help in building analogies of infections in humans. Such studies help determine the pathogenicity, tissue tropism, virus shedding, modes of transmission and disease symptoms. Pathogenesis studies *in vivo* are essential to understand the potential of the etiological agents to cause severe illness. Several animal models are available for studying pathogenesis and also for pre-clinical trials of candidate vaccines. Rhesus macaques and ferrets are infectable with SARS-CoV-2 and evidence of virus replication and virus shedding in their nasal swabs has been reported[37].

Pandemic situations demand identification of suitable antiviral drugs to mitigate the infection. *In vitro* and *in vivo* studies are used to carry out antiviral assays.The animal model is useful for the correlation of molecular markers conferring antiviral resistance with pathogenicity of the virus. Developing assays for the determination of antiviral efficacy using cell culture or animal models provides clues for the discovery and effects of antiviral drugs. Drug repurposing provides a faster alternative to developing new therapeutics during a pandemic situation. *In silico* studies help in screening the various available antiviral molecules for possible interactions with the target proteins associated either with the virus or the host[38]. The embryonated chicken egg model has also been explored for assessing susceptibility to antivirals[39]. RNA interference based technology has been explored for therapeutic applications for SARS-CoV before, and is being tested for SARS-CoV-2 as well[40].

Zoonotic diseases (also known as zoonoses) are caused by pathogens that are transmitted between animals and humans[41]. The phenomenon of 'reverse zoonosis' has also been reported, where inviruses were found to be the second most common pathogen associated with human-to-animal transmission[42]. Presence of antibodies against pandemic influenza H1N1 2009 virus in pigs underscores importance of animal-human interface studies. It has been shown that 61% of all human pathogens are zoonotic, and have represented 75% of all emerging pathogens during the past decade. This elaborates the need for extensive research on the origin and spread of viral diseases. The SARS-CoV outbreak which occurred in the years 2002-2003 highlighted the fact that infections could be rapidly transmitted across the globe due to globalization and rise in international air travel. The SARS-CoV had spread to nearly 40 countries, causing more than 8000 infections and close to 800 deaths within a span of one year[43].

Till date there have been pandemics caused by influenza viruses, HIV and SARS-CoV-2.Influenza viruses have caused four pandemics in 1918, 1957, 1968 and 2009 by influenza H1N1, H2N2, H1N1 and pandemic H1N1 2009 viruses, respectively. Six phases of an influenza pandemic have been described based on the circulation of a zoonotic virus among animals followed by its transmission in humans.These phases are - the prevalence of infection among animals (Phase 1), then infection in humans (Phases 2 and 3) leading to human-to-human transmissions locally, followed by eventual spread of the disease in the community and in other geographic regions (Phases 4, 5 and 6)[44].

For the current pandemic of SARS-CoV-2, four stages have been defined for early stage of the disease in people with travel history (Stage 1), local transmission in clusters (Stage 2), community transmission (Stage 3) and stage of uncontrollable spread (Stage 4)[45]. At every phase of a pandemic, laboratory diagnosis is of utmost importance. Table 1

Table 1 — *Applications of Classical and Modern Virology techniques in COVID-19 pandemic response and research*

Assay Types	Technique	Clinical and Diagnostic use	Public health	Basic Research
Molecular assays	NAAT	Nucleic acid detection-qualitative & quantitative	Rapid diagnosis for case confirmation, prevention and control and surveillance	Virology studies
	Sequencing (Sanger and NGS)	Confirmation of sequences, Virus identification	Molecular epidemiology and transmission tracking studies	Genome sequence, Molecular markers, structural bioinformatics, phylogeny, Metagenomic analysis etc.
	Proteomics/ interactomics	Suceptibility / pathogencicity	Contribution in understanding the disease	Host cell regulatory factors, gene expression, identification of drug targets
	Gene manipulation	Intervention following diagnosis	Development of diagnostic tests such as antigen detection tests using recombinant antigens therapeutics such as monoclonal antibodies	Studying pathogenesis, gene splicing, use of recombinant DNA, monoclonal antibodies
Serological assays	ELISA	Antigen/ antibody detection for serodiagnosis	Herd immunity, serosurveillance, seroprevalence, vaccine trials	Host immune response
	RDT/LFIA	Rapid point-of-care serodiagnosis	Screening of susceptibles, studying population level immunity	Serosurveillance
	RDT	Antigen detection	Rapid diagnosis for case confirmation, prevention and control and surveillance	Virology studies
	CLIA	Total antibody detection	Herd immunity, serosurveillance, seroprevalence	Serosurveillance
	IFA	Virus detection in infected cells	Laboratory diagnosis at referral centers, validation of diagnostic assays	Confirmatory for virus isolation, Host-virus interaction studies
	NT	Useful to determine whether antibodies are protective or not	Herd immunity, serosurveillance, vaccine immunogenicity, antibody persistence, efficacy	Protective immune response, Antigen/ epitope identification, Neutralizing antibodies
Virological assays	Virus isolation in cell culture	Confirming diagnosis	Vaccine development	Infectivity, pathogenicity and virulence studies. Basic material used in all branches of research
	Antiviral assays	No	For prophylaxis and treatment	Drug-repurposing,antiviral susceptibility
	Animal experiments	No	Preclinical vaccine trials	Pathogenesis, virus fitness, tissue tropism, monoclonal antibody generation
	Receptor specificity	No	Zoonosis	Pathogenicity, host range

NAAT Nucleic Acid Amplification Tests; ELISA Enzyme Linked Immunosorbent Assay; RDT Rapid Detection Test; CLIA Chemiluminescent Immunoassay; IFA Immunofluorescence Assay; LFIA Lateral Flow Immunoassay; NT Neutralization Test.

ICMR Testing Strategy for COVID-19 testing in India		
Setting	Recommended tests (in order of priority)	Whom to test
Containment zones : routine surveillance & screening	1. Rapid Antigen Test 2. RT-PCR or TrueNat or CBNAAT	Symptomatic individuals: healthcare & frontline Asymptomatic individuals: contacts and high-risk
Non-containment zones: routine surveillance	1. RT-PCR or TrueNat or CBNAAT 2. Rapid Antigen Test	Symptomatic individuals: with travel history, direct contacts, healthcare & frontline
	1. Rapid Antigen Test 2. RT-PCR or TrueNat or CBNAAT	Asymptomatic high-risk contacts
Hospitals	1. RT-PCR or TrueNat or CBNAAT 2. Rapid Antigen Test	All symptomatic patients, neonates Asymptomatic patients: high-risk, undergoing invasive procedures, pregnant women
Testing on demand	Whichever applicable	As per request
Advisory for testing strategy is available on the ICMR portal https://www.icmr.gov.in/cteststrat.html		

summarizes the various tool that could be or are used for the diagnosis and research of SARS-CoV-2. As the pandemic progresses, diagnosing each and every case without any established predisposition is difficult and unnecessary, since it puts unnecessary pressure on the resources leading to shortage. Eventually, the cases are diagnosed based on clinical symptoms and presumptive treatment and preventive actions are implemented accordingly. However, it will depend on the criticality of diagnosis for treatment and prevention measures. The scientific community across the world is carrying out rigorous research aimed at understanding the virus better, and also towards the development of potential cures and vaccines, which bear utmost importance in this public health emergency (Table 1).

From the Indian perspective, the need has been highlighted to revisit responses to a raging pandemic[46]. The production of local technology-driven solutions including point-of-care diagnostics and research relevant to stages of pandemic should be supported. The scientific community across the world has been working on the development of different technologies like whole virus vaccines, recombinant protein subunit vaccines, and nucleic acid vaccines against SARS-CoV-2[46]. These efforts combined with the pursuit of antiviral drugs provide hope for the control of this pandemic.

The field of virology has evolved tremendously in the 21st Century. Several key developments in the field of biology, like the genesis of molecular virology, gene manipulation, gene transfer, whole genome sequencing, growth of immunology, cell biology and biochemistry have been associated with the discipline of modern virology[47]. While unravelling the mechanisms of replication and pathogenesis of novel viruses, new and interesting aspects of cell biology have also emerged in the recent past. Therefore, the emphasis on classical virology approaches along with the molecular virology in the current pandemic would help in containment, mitigation and understanding of the impact of the pandemic on human health.

Conflicts of interest : None

Author Contributions Statement : Conceptualization of the article: SDP, BVT. Wrote the manuscript: SDP, BVT, DST, SSK, SSK. Reviewed and edited the manuscript: SDP, BVT, DST, SSK, SSK, PA.

Acknowledgements

We would like to thank Dr Jayati Mullick for support and Dr. Sarah Cherian for critical review of this article. The Indian Council of Medical Research is also acknowledged.

References

1 Woolhouse M, Gaunt E — Ecological origins of novel human pathogens. *Crit Rev Microbiol* 2007; **33:** 231-42. https://doi.org/10.1080/10408410701647560
2 Woolhouse ME, Howey R, Gaunt E, *et al* — Temporal trends in the discovery of human viruses. *Proc R Soc B* 2007; **275:** 2111-5. https://doi.org/10.1093/biohorizons/hzw010
3 Storch GA — Diagnostic Virology. In: Knipe DM, Howley PM (eds) Fields Virology, 5th Edition. Lippincot Williams & Wilkins 1996; pp 566–604.
4 Sanger FSN, Coulson A — DNA sequencing with chain-terminating inhibitors. *Proc Natl Acad Sci, USA* 2007; **74:** 5463-7. https://doi.org/10.1097/00006250-199004001-00013
5 Smith W, Andrewes CH, Laidlaw PP — A Virus Obtained from Influenza Patients. *Lancet* 1933; 66-8.
6 Weller TH, Enders JF — Production of Henlagglutinin by Mumps and Influenza A Viruses in Suspended Cell Tissue Cultures. *Proc Soc Exp Biol Med* 1948; **69:** 124-8.
7 Harcourt J, Tamin A, Lu X, *et al* — Severe Acute Respiratory Syndrome Coronavirus 2 from Patient with 2019 Novel Coronavirus Disease, United States. *Emerg Infect Dis* 2020; **26:** https://doi.org/10.3201/eid2606.200516
8 Hoffmann M, Kleine-Weber H, Schroeder S, *et al* — SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. Cell 2020; 181: 271-80. e8. https://doi.org/https://doi.org/10.1016/j.cell.2020.02.052
9 Infantino M, Damiani A, Gobbi FL, *et al* — Serological assays

for SARS-CoV-2 infectious disease: Benefits, limitations and perspectives. *Isr Med Assoc J* 2020; **22:** 203-10.

10 Xu Y, Xiao M, Liu X, *et al* — Significance of Serology Testing to Assist Timely Diagnosis of SARS-CoV-2 infections: Implication from a Family Cluster. *Emerg Microbes Infect* 2020; **0:** 1-12. https://doi.org/10.1080/22221751.2020.1752610.

11 Winter AK, Hegde ST — The important role of serology for COVID-19 control. *Lancet Infect Dis* 2020; S1473-3099:30273–4. https://doi.org/10.1016/S1473-3099(20)30322-4.

12 Lisboa Bastos M, Tavaziva G, Abidi SK, *et al* — Diagnostic accuracy of serological tests for covid-19: systematic review and meta-analysis. *BMJ*; **370:** m2516. https://doi.org/10.1136/bmj.m2516.

13 Indian Council of Medical Research (2020) — Advisory on Use of Rapid Antigen Detection Test for COVID-19. https://www.icmr.gov.in/pdf/covid/strategy/Advisory_for_rapid_antigen_test14062020.pdf. Accessed 13 Jul 2020.

14 Storch GA — Diagnostic Virology. In: Reller LB, Weinstein MP (eds) Clinical Infectious Diseases, Special section: Medical microbiology 2000; pp 739-51.

15 Read SJ, Burnett D, Fink CG — Molecular techniques for clinical diagnostic virology. *J Clin Pathol* 2000; **53:** 502-6. https://doi.org/10.5505/TurkHijyen.2015.99705

16 Kiselev D, Matsvay A, Abramov I, *et al* — Current Trends in Diagnostics of Viral Infections of Unknown Etiology 2020; **12:** 211. https://doi.org/10.1111/j.1365-2559.2008.02964.x. Involvement

17 Touma M — COVID-19: molecular diagnostics overview. *J Mol Med* 2020; **98:** 947-54. https://doi.org/10.1007/s00109-020-01931-w

18 Indian Council of Medical Research Strategy for COVID-19 testing in India. https://www.icmr.gov.in/pdf/covid/strategy/Testing_Strategy_v6_04092020.pdf

19 Indian Council of Medical Research (2020) — Information on testing strategies. https://www.icmr.gov.in/cteststrat.html

20 Tan W, Zhao X, Ma X, *et al* — Notes from the Field: A Novel Coronavirus Genome Identified in a Cluster of Pneumonia Cases — Wuhan , China 2019 “ 2020. *China CDC Wkly* 2020; **2:** 61-2.

21 Yadav PD, Potdar VA, Choudhary ML, *et al* — Full-genome sequences of the first two SARS-CoV-2 viruses from India. *Indian Jounral Med Res* 2012; **151:** 200-9. https://doi.org/10.4103/ijmr.IJMR.

22 Guzzi PH, Mercatelli D, Ceraolo C, Giorgi FM — Master Regulator Analysis of the SARS-CoV-2/Human Interactome. *J Clin Med* 2020; **9:** 982. https://doi.org/10.3390/jcm9040982

23 Liao M, Liu Y, Yuan J, *et al* — Single-cell landscape of bronchoalveolar immune cells in patients with COVID-19. Nat Med 2019; https://doi.org/10.1038/s41591-020-0901-9.

24 Molecular-based Tests for COVID-19. https://www.centerforhealthsecurity.org/resources/COVID-19/molecular-based-tests/. Accessed 13 Jul 2020.

25 Robson B — Computers and viral diseases. Preliminary bioinformatics studies on the design of a synthetic vaccine and a preventative peptidomimetic antagonist against the SARS-CoV-2 (2019-nCoV, COVID-19) coronavirus. *Comput Biol Med* 2020; **119:** 103670. https://doi.org/10.1016/j.compbiomed.2020.103670.

26 Chadha MS, Broor S, Gunasekaran P, et al — Multisite virological influenza surveillance in India: 2004-2008. *Influenza Other Respi Viruses* 2012; **6:** 196-203. https://doi.org/10.1111/j.1750-2659.2011.00293.x

27 Gupta N, Potdar V, Praharaj I, *et al* — Laboratory preparedness for SARS-CoV-2 testing in India: Harnessing a network of Virus Research & Diagnostic Laboratories. *Indian Jounral Med Res* 2020; **594:** https://doi.org/10.4103/ijmr.IJMR.

28 Tandale B V, Pawar SD, Gurav YK, *et al* — Seroepidemiology of pandemic influenza A (H1N1) 2009 virus infections in Pune, India. *BMC Infect Dis* 2010; **10:** 255. https://doi.org/10.29333/ejgm/82752.

29 Tandale BV, Pawar SD, Mishra AC — Immunogenicity and safety of pandemic H1N1 2009 vaccine among adults in field use, India. *Vaccine* 2012; **30:** 2043-44. https://doi.org/10.1016/j.vaccine.2011.10.008.

30 Kode SS, Pawar SD, Tandale BV, *et al* — Low level of cross-reactive antibodies to pandemic influenza (H1N1) 2009 virus in humans in pre-pandemic period in Maharashtra, India. *Indian J Virol* 2012; **23:** 36-8. https://doi.org/10.1007/s13337-011-0055-y.

31 Asuquo MI, Effa E, Otu A, *et al* — Prevalence of IgG and IgM antibodies to SARS-CoV-2 among clinic staff and patients. *Med Rxiv* 2020; 2020.07.02.20145441. https://doi.org/10.1101/2020.07.02.20145441.

32 Schmidt SB, Grüter L, Boltzmann M, Rollnik JD — Prevalence of serum IgG antibodies against SARS-CoV-2 among clinic staff. *PLoS One* 2020; **15:** e0235417.

33 Rudberg A-S, Havervall S, Manberg A, *et al* — SARS-CoV-2 exposure, symptoms and seroprevalence in health care workers. *med Rxiv* 2020; 2020.06.22.20137646. https://doi.org/10.1101/2020.06.22.20137646

34 Steensels D, Oris E, Coninx L, *et al* — Hospital-Wide SARS-CoV-2 Antibody Screening in 3056 Staff in a Tertiary Center in Belgium. *JAMA* 2020; https://doi.org/10.1001/jama.2020.11160

35 Jin Y, Yang H, Ji W, *et al* — Virology, epidemiology, pathogenesis, and control of covid-19. *Viruses* 2020; **12:** 1-17. https://doi.org/10.3390/v12040372

36 Sun J, He W-T, Wang L, *et al* — COVID-19: Epidemiology, Evolution, and Cross-Disciplinary Perspectives. *Trends Mol Med* 2020; **26:** 483-95. https://doi.org/https://doi.org/10.1016/j.molmed.2020.02.008

37 WHO R&D Blueprint, COVID-19 Animal Models. https://www.who.int/blueprint/priority-diseases/key-action/WHO-ad-hoc-Animal-Model-Working-Group_Summary.pdf?ua=1. Accessed 2 May 2020

38 Cherian SS, Agrawal M, Basu A, *et al* — Perspectives for repurposing drugs for the coronavirus disease 2019. *Indian Jounral Med Res* 2020; 160-71. https://doi.org/10.4103/ijmr.IJMR

39 Tare DS, Pawar SD — Use of embryonated chicken egg as a model to study the susceptibility of avian influenza H9N2 viruses to oseltamivir carboxylate. *J Virol Methods* 2015; **224:** https://doi.org/10.1016/j.jviromet.2015.08.009.

40 Wu C, Chan Y — Antiviral applications of RNAi for coronavirus. 2006; 89-97.

41 Zoonotic Diseases | One Health | CDC. https://www.cdc.gov/onehealth/basics/zoonotic-diseases.html. Accessed 2 May 2020

42 Messenger AM, Barnes AN, Gray GC — Reverse zoonotic disease transmission (Zooanthroponosis): A systematic review of seldom-documented human biological threats to animals. *PLoS One* 2014; **9:** 1-9. https://doi.org/10.1371/journal.pone.0089055

43 Marston HD, Folkers GK, Morens DM, Fauci AS — Emerging viral diseases: Confronting threats with new technologies. *Sci Transl Med* 2014; **6:** 1-7. https://doi.org/10.1126/scitranslmed.3009872

44 WHO pandemic phase descriptions and main actions. https://www.who.int/influenza/resources/documents/pandemic_phase_descriptions_and_actions.pdf. Accessed 2 May 2020

45 (2020) Coronavirus: The four stages of a global pandemic | The Economic Times. Econ. times

46 Chen WH, Strych U, Hotez PJ, Bottazzi ME — The SARS-CoV-2 Vaccine Pipeline: an Overview. *Curr Trop Med Reports* 2020; 1-4. https://doi.org/10.1007/s40475-020-00201-6

47 DiMaio D — Is Virology Dead? 2014; MBio 5:. https://doi.org/10.1128/mBio.01003-14.