IFCC Live Webinar Series: COVID-19 Guidelines on Molecular, Serological and Biochemical /Hematological Testing

Speaker Panel: Prof Giuseppe Lippi (Italy), Prof Andrea Rita Horvath (Australia), Prof Khosrow Adeli (Canada)

Question	Country	Answer
Dear Presenters, 2:71RT-PCR positive doesn't mean the individual is infected with SARS-CoV-2. After recovery we are encountering some patients to have a positive PCR result. What could be the justification?	ET	This is true. Recurrence of SARS-CoV-2 positivity is a very important issue, which entails the possibility that a patient who tested negative at one or more naso and oro-pharyngeal swabs, may then turn positive again, later. Some studies have been published on this matter, with recurrence rates of SARS-CoV-2 positivity comprised between 7% and 23%. On average, it can hence be estimated that the mean risk of retesting positive for SARS-CoV-2 after a negative test would be around 12%. In most patients this later recurrence of RT-PCR positivity seems to occur with 1 month from the last negative swab, but later positivities are also reported, up to 2 months after a second negative test. SARS-CoV-2 re-positivization is not seemingly due to active reinfection and, therefore, the infectious potential and the transmission risk shall be considered cumulatively very low in these subjects.
What do you think about the role of interferons in the management of COVID-19 disease? Would the analysis of these tests on a routine basis help us?	TR	This will be discussed in the 3rd lecture soon. Briefly, no we do not recommend measuring these tests routinely but for research use only
What interfering substances can give false negative tests?	IN	Antiviral drugs, perhaps hemolysis (due to release of intracellular degradation enzymes).
Are oropharyngeal swab and saliva same?	NP	Detection rate is nearly overlapping (around 70-80%), but saliva can be collected much more easily
If IgM serological test for COVID19 is positive and IgG Negative, and person is without clinical symptoms. In this case is person potential for viral transmission? Do this person needs to be isolated?	XK	Serology tests for SARS-CoV-2 antibodies identify past infection. Current data suggests IgM and IgG may be expressed concomitantly in the serum. There is no evidence that identification of SARS-CoV-2 IgM antibodies suggests an active infection. Serology testing should not be used as a proxy for isolation decisions.
Are the rapid antigen test kits for COVID-19 reliable for diagnosis? Or should results of such kits still be confirmed by rRT-PCR?	PH	A rapid test shall only be used (a) where NAAT is not immediately available (b) for outbreak investigations (e.g., schools, care homes, cruise ships, prisons, etc.) (c) for monitoring trends in disease incidence in communities and widespread community transmission (early detection and isolation of positive cases)

		and (d) for testing of asymptomatic contacts of cases. A positive test results must always be confirmed by RT-PCR
Dear Dr Adeli, Thank you. I have been reading papers stating IgG come earlier than IgM. Why this is happening?	ET	Many conflicting findings have been reported regarding IgM and IgG expression profiles after SARS-CoV-2 exposure. The reasons behind these conflicting findings are likely due to differential assay performance. It is likely that IgG assays have better performance relative to IgM assays. IgG may also have a stronger response, leading to earlier detectability (especially in severe patients)
Kindly explain more about neutralization antibodies.	NP	In a traditional immune response to viral infection, specific antibodies are released that bind to viral antigen and renders the pathogen no longer infectious (neutralization). Current serology assays can tell us whether antibodies are present in the serum, but cannot determine whether these antibodies neutralize SARS-CoV-2. In some cases, antibodies can even contribute to maladaptive immune response. Cell culture neutralization assays are needed to determine antibody efficacy.
Any RT-LAMP for clinical use?	ET	Can be used for rapid diagnosis, but sensitivity is typically lower than RT-PCR.
How we are certain about immune protection for serological tests of positive result?	ET	Only modest correlation has been shown between the index value of a positive serology tests and the neutralizing antibody titre from the same sample, indicating that they are only a modest proxy for neutralization. However, there is very limited data regarding the correlation of positive serology to long term immune protection. More research is needed and current serology test results should not be used to infer immunity.
Can antigen testing ever replace NAAT/PCR in diagnosis COVID 19?	NO	A rapid test shall only be used (a) where NAAT is not immediately available (b) for outbreak investigations (e.g., schools, care homes, cruise ships, prisons, etc.) (c) for monitoring trends in disease incidence in communities and widespread community transmission (early detection and isolation of positive cases) and (d) for testing of asymptomatic contacts of cases. A positive test results must always be confirmed by RT-PCR
To Prof. Lippi. There is a strong pressure by some Italian Regional Health Services to utilise Rapid Antigen test to detect the nucleocapsid protein in nasopharyngeal swab or saliva instead that perform the RTPCR. In particular for screening at school. What is	IT	A rapid test shall only be used (a) where NAAT is not immediately available (b) for outbreak investigations (e.g., schools, care homes, cruise ships, prisons, etc.) (c) for monitoring trends in disease incidence in communities and widespread community transmission (early detection and isolation of positive cases) and (d) for testing of asymptomatic contacts of cases. A positive test results must always be confirmed by RT-PCR. Saliva is still not validated.

your opinion? Thanks		
Thank you very much, nice presentation sir. If I want to test COVID -9 by RT-PCR test of swab sample in outer packaging materials of food products, what are the additional method verification requirements need.	IN	No, can use the same reagents and procedure as for biological testing
Do you have any experience with immunoblot-based anti-SARS-CoV-2 assays? Are those considered too valuable as a means of antibody presence confirmation?	PL	Few studies have been published on immunoblot-based SARS-CoV-2 antibodies. More research is needed to ascertain their value. However, their clinical implementation would likely be logistically and technically challenging.
a question for Rita Horvath. are there data for Calprotectin as an inflammatory biomarker useful in discriminating high-risk patients? thank you	CH	Assuming the question addresses serum calprotectin rather than fecal calprotectin; although the latter was also found to be elevated in COVID-19 cases with diarrhoea, which is not surprising given fecal calprotectin being used as a marker of inflammatory bowel disease. *Serum* Calprotectin has been described as a neutrophil activation marker in the diagnosis of acute respiratory infection and, similarly to procalcitonin, it was found to be elevated more in bacterial than in viral infections. In COVID-19 increased neutrophil count correlates with disease severity, and thus increased Calprotectin release from neutrophils is not surprising and shows the same clinical correlation. Calprotectin levels were also found to be correlated with CRP, ferritin, fibrinogen and D-dimer levels in severe cases. Whilst publications are emerging about monitoring Calprotectin increase and the loss of a non-classical monocyte subset as early markers of an incipient cytokine storm, in resource poor times, measuring this biomarker in addition to the routinely available tests mentioned above does not necessarily help guide patient management and therefore the added clinical utility and prognostic value of serum Calprotectin testing remains to be seen. However, serum Calprotectin, similarly to cytokines, can be used for research studies and may help uncover further pathophysiological aspects of SARS-CoV-2 infection that could aid in the triage and prognosis of patients or in finding new treatment options.
Seroprevalence/Antibody level will decease just after 2/3 months of infection. So, the likely recommendation could be just conducting a timely serosurvey. And this could also be another challenge for LMICs.	ET	Indeed, the timing of antibody response significantly complicates the completion of serosurveys. Most seroprevalence studies are completed in populations with unknown exposure thus timing seroprevalence studies to correspond with SARS-CoV-2 exposure would be very challenging. As research is completed regarding antibody durability in COVID-19 patients, we will update our recommendation regarding the feasibility and scientific value of serosurveys.

Your opinion...		
Is the IFCC recommending wide-spread asymptomatic serological screening for Sars-COV-2? If so why, given the expected poor positive predictive value of such testing in this low prevalence population, and the significant implications a positive result has on the individual, their close contacts and the wider community?	UK	No, the IFCC is not recommending wide-spread asymptomatic serological screening. The current indications for which serological testing is recommended include the following: • To serve as adjunct to molecular testing in patients presenting with suggestive clinical features (>14 days post symptom onset), but molecular testing for SARS-CoV-2 is negative, undetermined or unavailable. • To serve as adjunct to molecular testing where persistently positive molecular tests occur in the absence of infectious virus, such as late after resolved infection. • To assist in the diagnostic work-up of Multi-System Inflammatory Syndrome in Children (MIS-C). More research is needed before serosurveys are implemented on a wide scale.
how to monitor ferritin and d-dimer in patients hospitalized in ICU and other wards?	EC	We do not make recommendations on the frequency of monitoring biomarkers in hospital inpatients as each case may present at different time point and with variable co-morbidities which makes it hard to come up with a unified monitoring schedule. Whilst higher values for ferritin and D-dimer have definitely been shown to correlate with worse prognosis, one of the limitations of these studies is that samples were collected at different times during the disease course (i.e. at admission, or during the course of illness). A common sense approach may be to measure these markers at baseline and be guided by the clinical features of the illness and the response of the patient to treatment.
To Rita: How is the involvement of TMA (thrombotic micro- angiopathy) or TTP what are similar to DIC but at the platelet site and less on the coagulation.	HU	This paper, referenced in our guideline, may help answering this question: https://link.springer.com/article/10.1007/s11239-020-02134-3. The authors imply that COVID-19-induced coagulopathy is different to TMA. However, another paper reviewing multiple publications of autopsy reports suggests that the coagulopathy associated with COVID-19 is more similar to complement-mediated TMA. This hypothesis is supported by the fact that in COVID-19 bleeding complications are uncommon, fibrinogen is often high rather than low and platelet count is mildly decreased only, unlike in a consumption coagulopathy such as DIC (https://www.nature.com/articles/s41584-020-0474-5)
Comment to the introduction: This week nano-biophysical results: The unique single-particle approach revealed that the surface layer of spikes on SARS-CoV-2 is highly dynamic, the virion is unusually compliant and resilient, and it displays an unexpected	HU	Thanks, I have no further comments

global thermal stability.Title: Topography, spike dynamics and nanomechanics of individual nativeSARS-CoV-2 virions, by the group of Miklos Kellermayer, Your preprint 10.1101/2020.09.17.302380 has posted on bioRxiv: https://biorxiv.org/cgi/content/short/2020.09.17.302380v1		
I would like to know if there are any Red blood cell and Leukocyte inclusions or changes seen in the peripheral blood of a COVID - 19 patient?	PH	This paper referenced in our guideline looked at blood film morphology in infected patients https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7276239/ Viral inclusions will not be seen by standard microscopy as they are too small
Maybe I have missed, do we have a percentage of the cases for COVID in 0-19 years old?	TR	Depends on the country and the prevalence of disease. Can be monitored at: https://coronavirus.jhu.edu/map.html
Can serology tests be used to detect immunity after vaccination? How sensitive and specific would they have to be?	NO	Currently available serological tests from manufacturers make no claim towards the identification of immunity. The role of serological testing in the detection of immunity post-vaccination is currently unknown and will hopefully develop as vaccinations are released. Quantitative serological testing may be more useful in this regard in the future.
What about Protein C reactive?	CO	If asking about CRP, yes, it is associated with severe disease and poor prognosis. See Refs: https://www.sciencedirect.com/science/article/pii/S0009898120302709 and https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7282743/?
We hear about false positive PCR results - what do you think these are?	UK	Are extremely rare and mostly attributable to cross-contamination during sample collection and/or analysis
For areas with limited use for laboratory tests, what will you be recommending to use?	PH	RT-PCR with 5-sample pooling (see: "Upper respiratory samples pooling for screening SARS-CoV-2 infection: ready for the prime time? Available at: https://www.degruyter.com/view/journals/cclm/ahead-of-print/article-10.1515-cclm-2020-1342/article-10.1515-cclm-2020-1342.xml")
How about bradykinin hypothesis and potential new biomarkers?	BG	There is a theory that the bradykinin pathway is responsible for the pathophysiology of COVID-induced ARDS. See Garvin et al. eLife 2020;9:e59177. DOI: https://doi.org/10.7554/eLife.59177 As for additional biomarkers for the renin-angiotensin-bradykinin system – we would not recommend them

		at this point out of a research setting and until further evidence is gathered about the clinical utility of these tests
Question for Dr. Rita: What about the severity prediction of MxA biomarker?	RO	Sorry, I am not familiar with this test but checking some publications, the Myxovirus resistance protein A (MxA) is a marker of interferon-induced antiviral host response. Together with CRP it has been used in a lateral flow POCT device which claims to be able to distinguish viral from bacterial respiratory infection. However, just like CRP it is non-specific to SARS-CoV-2 and its diagnostic accuracy was tested in a high COVID-19 prevalence scenario at the peak of local infections in the UK. At this stage, the added value to CRP testing is questionable but using the POCT device may add to the costs of care.
Dear Expert Panel, in your opinion would a baseline/admission determination for 25-hydroxyvitamin D levels be advocated for COVID-19 suspect individuals? Thank you in advance, Harjit Pal Bhattoa	HU	We do not see much value in measuring vitamin D on admission of COVID-19 suspected cases. Association of lower vitamin D levels with susceptibility to infection with SARS-CoV-2 has been widely debated. A statement by NICE (UK) suggests that there is no evidence for giving vitamin D supplements to treat or prevent COVID-19 https://www.nice.org.uk/advice/es28/chapter/Advisory-statement-on-likely-place-in-therapy An international consensus statement also advises against the use of excessive doses of Vitamin D for prevention of COVID-19. However they advise that those who are confined indoors due to long term lock-downs or in isolation and the elderly, especially in the winter season, follow their Government's general vitamin D intake recommendations and a healthy diet. https://nutrition.bmj.com/content/bmjnph/early/2020/05/15/bmjnph-2020-000089.full.pdf
What is the behavior of C-reactive protein in COVID-19?	CO	CRP is associated with severe disease and poor prognosis. See Refs: https://www.sciencedirect.com/science/article/pii/S0009898120302709 and https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7282743/
Given that saliva has a high viral titre why don't we use this in testing rather than nasopharyngeal swaps...	UK	I agree, but this has not been yet validated and thereby recommended by the WHO and/or CDC.
have there been documented cases about patients that have been re-infected?	PH	Yes. Detailed information can be found in the article "SARS-CoV-2 recurrent RNA positivity after recovering from coronavirus disease 2019 (COVID-19): a meta-analysis", available here: https://www.mattioli1885journals.com/index.php/actabiomedica/article/view/10303
Do you think there are tests sensitive enough	NO	The long-term application of serological testing is currently unknown. Most commercially available assays

to detect antibodies also after the levels decreased again after several month?		demonstrate excellent sensitivity in patients with severe COVID-19 up to 60 days post symptom onset. Our knowledge of sensitivity after that time point is limited, but an active area of research.
Any Role of serology testing in Convalescent plasma therapy? Also, role of serology testing in diagnosing Pediatric Inflammatory Multisystem syndrome diagnosis?	IN	Yes, serological testing has a demonstrated role in the clinical work up for MIS-C (it is included in the WHO case definition). Identification of good convalescent plasma donors would likely require a quantitative assay that can detect a high titre as well as one that correlates with neutralization capacity. Currently available commercial assays do not have these properties.
Compare to Ig G and Ig M which can be used as predictor for patients representing covid-19 symptoms	IN	The relation of IgM and IgG antibodies and their correlation to clinical symptoms is not well established. Detecting IgM vs IgG may be a reflection of assay performance rather than clinical significance. More research is needed in this area.
For Khosrow, the second reason to test for antibodies was to assess if the PCR was a false positive. This must only apply to very few patients and why should a PCR be assessed in an asymptomatic patient in a clinical scenario.	UK	Thank you for your point, we are in agreement. This is a special case scenario that is being applied in multiple labs worldwide. PCR testing is currently being used for screening purposes in asymptomatic individuals such as teachers returning to school, hospital workers, travelers among others. The combination of PCR and serology tests may beneficially impact case conclusion when multiple positive PCR results are negative.
how long (hours?) does it take from getting infected until a molecular test can detect the infection	AT	This has not been clearly established, though it seems reasonable to conclude that RT-PCR positivity will develop between 2-5 days after being infected.
how long does it take to be able to test antigen test after being infected by covid?	MM	This has not been clearly established, though it seems reasonable to conclude that antigen positivity on nasopharyngeal swabs will develop between 3-5 days after being infected.
Can you comment on research only parameters in hemogram like augmented variance of monocyte population as prognostic factor for COVID-19 severity?	EE	Monocyte distribution width (MDW) has been found to be useful in early detection of sepsis. Higher MDW, although not specific to SARS-CoV-2 infection, was observed in hospitalised COVID-19 cases. https://www.sciencedirect.com/science/article/pii/S0009898120302667 MDW is a routinely reported parameter in automated haematology testing and its monitoring during treatment may be a helpful adjunct and a prognostic tool at no additional cost.
Good morning, congratulations, a question: in Latin America can a PCR diagnosis be made with a gene for example N gene?	PE	Yes, technically. But most International Organizations and Associations on COVID-19, including the IFCC, are strongly recommending to use two different SARS-CoV-2 gene targets, one of which could be the N gene.
All serological assays detect binding antibodies, as you said neutralizing	IT	As quantitative assays are developed, we can hypothesize that their role will be multi-fold including the detection of severity of immune response, identification of potential convalescent plasma donors as well as

antibodies need to be looked at by viral neutralization assays. Which will be the role of true quantitative binding antibody assays if those will show a very high correlation with the neutralizing activity?		response to vaccination. Current assays have only a modest correlation to neutralization. If quantitative assays were developed with high correlation to neutralization activity, they would be highly useful clinically in the applications mentioned above. However, no such assay currently exist.
Is GenXpert for SARS-CoV-2 equally superior to rRT-PCR?	PH	Depends on the purpose for which the test is use, if for screening (yes) or diagnosis (yes/no).
Could C-reactive protein be considered an early marker of COVID-19 infection?	CO	CRP is a non-specific marker of inflammation and it lags behind some other inflammatory markers
Does Iron play any role in COVID	IN	Not to my knowledge. Ferritin is elevated but this is not related to iron metabolism
In our institution, we inactivate serum samples for clia testing, considering that the viral load in blood is only 1 percent, does this process really helpful in mitigating infection control?	PH	Yes
Is there any data to suggest how long antibodies are detectable for post covid-19 infection?	UK	This is an active area of research. Conflicting results have been reported and may be limited by variable assay performance. Further research is needed in this area.
what is the relevance of Bradykinin Storm in lung damage along with hyaluronic acid elevation and its role in not responding to the oxygen therapy.	TR	The question is probably based on this paper that describes in detail the theoretical model for a RAS-mediated bradykinin storm. Garvin et al. eLife 2020;9:e59177. DOI: https://doi.org/10.7554/eLife.59177 These concepts fit in well with the symptoms and some biomarker changes observed in COVID-19 and could well expand our understanding of the pathophysiology of this condition.
thank you for all nice presentations, would you please inform me about percentage of patients which will be remain serologically negative after2 weeks of exposure	IR	The percentage of patients that remain serologically negative will depend on the clinical nature of exposure (e.g. severe disease vs mild disease), risk factors (e.g. autoimmune disease) as well as assay sensitivity. Negative serology has been reported in asymptomatic patients although the exact percentage cannot be concluded based on current evidence.
On the pre-analytical testing in the first presentation on Molecular testing, on the interference substances, anti-retroviral	MW	All types that can interfere with in vivo COVID-19 replication.

therapy was in mentioned as an interfering substance, my Question, is it all type of anti-retroviral that would affect the test? Or just those specifically used against Cov-19?		
While reporting for molecular testing, how threshold change for particular gene in PCR affect the reporting of sars-cov-2 result?	NP	This issue is critical. This is why the IFCC strongly recommends to follow manufacturers' recommendations about test reporting relative to Ct and/or viral load.
Rita, could you talk a little about the difference in the units of measurement of the dimer, DDU or FEU in the articles and the care taken in adopting values ??in the daily clinic?	BR	This is an important point that we highlight in our recommendations. There is inconsistency in D-dimer reporting formats (either as D-dimer units or fibrinogen equivalent units or variations in units of measure; e.g. ng/mL or µg/L). It is important that when reviewing D-dimer literature for COVID-19, one carefully checks the D-dimer units used and how they compare to those in the individual's laboratory. D-dimer assays are very poorly harmonised which poses a real challenge for translating research findings into clinical practice. For more information see https://www.degruyter.com/view/journals/cclm/58/8/article-p1191.xml
I would like to thank all the presenters for the excellent presentations. I have a question for Prof Lippie. In molecular diagnostics recently the Indian government has approved FELUDA test FNCAS9 Editor-Limited Uniform Detection Assay, what is your opinion on it? My second question is for Prof Adeli. A lot of re-infections are occurring so is it that the antibodies are not persisting for a long duration and that we may not attain herd immunity?	IN	I have no experience about that technology and thereby I cannot comment.
Is a positive IGM a marker of active infection and possible transmission of infection towards others? what is the value of IGM?	AL	Currently there is minimal evidence to suggest identification of IgM indicates active infection. Some studies report concomitant IgM and IgG expression. All serological assays should be used for the identification of past infection at this time.
Dear Prof. Khosrow Adeli, thank you very much for your wonderful presentation, at the	IT	Thank you Sonia for your question. We anticipate antigen testing will be a more rapid identification method for the screening of both asymptomatic and symptomatic individuals. Their use in the identification of

end of your speech you have mentioned Ag Rapid Assay. In which diagnostic algorithm do you see this kind of tests? For Screening of Symptomatic? How is your perception? Thank you so much for your feedback Warm Regards Sonia		active infection could complement or replace molecular assays. Their role in this pandemic will need to be determined as antigen rapid assays are developed and released.
There are different patterns of seroconversion, so why not look at using an assay that would detect all Ig's [GAM]	US	Many manufacturers have developed assays which detect total antibody (e.g. Roche, Siemens, Ortho). These assays have shown better sensitivity in some studies. However, identification of particular immunoglobulins may assist clinically as we continue to improve our understanding of SARS-CoV-2 immune response.
How do you see the evolvement of Antigen testing instead of PCR testing?	BE	A rapid test shall only be used (a) where NAAT is not immediately available; (b) for outbreak investigations (e.g., schools, care homes, cruise ships, prisons, etc.); (c) for monitoring trends in disease incidence in communities and widespread community transmission (early detection and isolation of positive cases) and (d) for testing of asymptomatic contacts of cases. A positive test results must always be confirmed by RT-PCR.
observations showed that after several months the level of Ab decreased?.so how can a vaccine be effective	IR	The effectiveness of prospective vaccinations cannot be concluded at this time. However, it is important to note that while the antibodies may not be detectable using currently available assays, this does not directly correlate to a non-immune status.
Hello , I'm an Algerian medical technologist, I'm working with a rotor gen Q machine using Biogerm reagents (N and ORF1ab targets) the manufacturer cutoff is 38CT but when i discuss with doctors these patients were not symptomatic should I decrease the cutoff to 35CT thank you	DZ	No, you must rely on manufacturers' declaration, since these have been validated, unless you cannot perform a local re-validation in your facility.
Is it necessary to have full PPE when processing blood samples of covid patient?	SG	No, you must use standard PPE equipment as for all bloodborne pathogens.
In Biochemical/Hematological testing presentation it is stated that CRP provides the related information, therefore PCT is not really recommended. Since PCT increases before CRP, don't you think it is important to	TR	CRP is more consistently elevated than PCT in COVID-19. The role of PCT is identifying patients where there may be concurrent bacterial infection. This is particularly common in children. These patients will likely need both PCT and CRP measurement

catch co-bacterial information early in COVID-19 patients?		
What level of ferritin is more predictive for worse prognosis? How early after the symptom occurs can we make serological test?	MK	In one study, the weighted mean difference between patients with severe and non-severe disease for ferritin was 398ug/L https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7233226 However, it is very hard to translate individual values from published literature, particularly for an assay like ferritin which is not well standardised I would say Serology tests have highest diagnostic accuracy after a minimum of 14 days and for some serology tests 21 days after symptoms
Can you consider an anti RBD ELISA test to assess the neutralizing function of a plasma donor?	RO	No, currently only neutralization assays can be used to determine the neutralization function of a plasma donor. Specific antigenic targets for serological assays have not been shown to confer greater correlation to neutralization status.
@Khosrow Adeli if different PCR test give different result then how can we confirm the true positivity or negativity?	NP	Excellent point. There is potential for different PCR tests to return different results and thus are an imperfect reference standard. However, repeat testing in 48-hour windows has been suggested to decrease the possibility of false negative PCR results. This can also be combined with the clinical picture (e.g. symptoms, CT scan) to have better confidence in RT-PCT results
ACE2 is highly expressed in Intestine and kidneys compared to least expression in the lungs. Yet SarsCov2 primarily infects lungs... WHY?	IN	Because SARS-CoV-2 is a respiratory virus and travels with air and droplets not with food.
I would like to know if the antigen test will be added in the guideline?	CN	Yes, in the next version, as soon as more information will become available.
How can lab interpret discordant result for S protein and N protein based serological assay?	AE	Findings should be reported as inconclusive. Further testing may be necessary, including testing for cross reacting antibodies etc.
I want to add that FELUDA also claims to have 96% sensitivity and specificity	IN	Thanks for this comment.
Any comments from the panel on the role of Clinical Laboratory Experts in advising National government Advisory Committees	AU	The clinical laboratory needs to play a role in public health decisions, guiding policy and practice during this pandemic. We should try to use our expertise to advise non-laboratory professionals in exactly what can be concluded from currently available tests and how this should translate to clinical care and public health

in guidance on COVID testing		strategies.
Question for prof Lippi: MDW (Monocyte Distribution Width) in COVID patients	RS	The Monocyte Distribution Width is a diagnostic parameter automatically reported with the Complete Blood Cell Count differential using a specific brand of hematological analyzers, which has earlier been found useful for early detection of sepsis in adult patients. The diagnostic performance of this parameter for identifying patients with SARS-CoV-2 infection was evaluated, displaying an accuracy as high as 91%, with 98% sensitivity and 65% specificity. Moreover, COVID-19 patients in the intensive care unit were also found to have higher values than those with mild symptoms.
I have read an article regarding Jacobson's SarsCov as a bradykinin storm, what are your thoughts about this and are there any lab approaches that could assess bradykinin in SarsCov?	PH	See earlier comments on the same above
Have you noticed anormal în reales în immunoglobulins, especially immunoglobulin A? Thank you!	RO	Most currently available assays do not target IgA and thus our knowledge of this immunoglobulin is limited at this time. Currently available serological tests for the identification of SARS-CoV-2 testing have excellent specificity and thus abnormal increases in SARS-CoV-2 antibodies have not been routinely observed. Although positivity as a result of crossreactivity has been reported for a few assays.
Is D-Dimer test required as a first line test in patients (especially those not to be hospitalized)? Thank You	TR	I am not sure that D-dimer testing would be useful in an asymptomatic infected patient, unless you wish to have a baseline result in case patient's condition deteriorates. D-dimer again is not specific for COVID-19 but it is a good test to monitor progression and assess the severity of disease in hospitalised patients. Patients with a very high D-dimer should be monitored closely for clinical deterioration.
Comment: Differences and Similarities between Disseminated Intravascular Coagulation and Thrombotic Microangiopathy Hideo Wada1*, Takeshi Matsumoto2, Kei Suzuki3, Hiroshi Imai3, Naoyuki Katayama4, Toshiaki Iba5 and Masanori Matsumoto61Department of Molecular and Laboratory Medicine, Mie University Graduate School of Medicine, Tsu, Mie 514-8507, Japan 2Division of	HU	Thanks for this paper and earlier comments that highlight differences between DIC and TMA. More investigations will be needed to fully understand the exact nature of COVID-19 induced coagulopathy. https://link.springer.com/article/10.1186/s12959-018-0168-2 Complement induced TMA is one other mechanism recently reported in the COVID-19 coagulopathy literature: https://www.nature.com/articles/s41584-020-0474-5

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what is your opinion about antigen test? can they replace PCR test in same context?	ES	A rapid test shall only be used (a) where NAAT is not immediately available (b) for outbreak investigations (e.g., schools, care homes, cruise ships, prisons, etc.) (c) for monitoring trends in disease incidence in communities and widespread community transmission (early detection and isolation of positive cases) and (d) for testing of asymptomatic contacts of cases. A positive test results must always be confirmed by RT-PCR
Have you noticed abnormal increases in immunoglobulins, especially immunoglobulin A? Thank you!	RO	Most currently available assays do not target IgA and thus our knowledge of this immunoglobulin is limited at this time. Currently available serological tests for the identification of SARS-CoV-2 testing have excellent specificity and thus abnormal increases in SARS-CoV-2 antibodies have not been routinely observed. Although positivity as a result of crossreactivity has been reported for a few assays.
I would like to ask about what the parameters you recommend to use in a small hospital who don't have hospitalized COVID-19 patients? I mean, which biochemical or hematological parameters you recommend to use for first time for patients that you will derivate to another hospital huger than yours.	ES	This is a good question. We would suggest that at a minimum small hospitals should offer FBC, coagulation screen, troponin, and CRP. Likely EUC and LFT and LDH could be offered also. This appears to cover most of the important COVID-related complications