JCTLM
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Database Newsletter

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JCTLM special issue: Member and Stakeholder Activities on COVID-19

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Introduction

Ian Young, Robert Wielgosz and Elvar Theodorsson

The JCTLM recently invited its members and stakeholders to contribute material to the present newsletter focused on "reliability of measurements in COVID-19 diagnostics", to facilitate knowledge transfer on ongoing activities in our community. The primary purpose is to inform readers on new developments that focus on the "reliability of measurements in COVID-19 diagnostics", including reference materials, reference measurement methods, interlaboratory comparisons and relevant research, publications and seminars. What is provided in this newsletter is not an exhaustive list of activities, but only a summary of descriptions submitted, that are within the scope described above. It should also be noted that the vast majority of reference materials and methods mentioned have not been through the JCTLM review process to verify compliance with documented requirements for higher order metrological references, and it is hoped that in due time they will be nominated for review. Reference materials, methods and services that have been reviewed to meet requirements are listed in the JCTLM Database at https://www.bipm.org/jctlm/

CCQM Webinar on 'Ensuring the reliability of measurements in response to the Covid-19 pandemic'

The first in a series of CCQM webinars on activities ensuring the reliability of measurements was held on 7 July 2020. Presentations included were:

- Review of diagnostic modalities involved in the response to the COVID-19 pandemic, Prof. Jacob Moran-Gilad
- International proficiency testing scheme results with molecular diagnostics for SARS-CoV-2, Prof. Dr. Heinz Zeichhardt
- SARS-CoV-2 Antibody testing and what we can learn from interlaboratory studies, Prof. Michael Neumaier
- The Development and Assessment of COVID-19 Serological Platforms, Dr Michael A. Drebot

Abstracts of presentations can be found at https://www.bipm.org/cc/CCQM/Allowed/26/CCQM20-11_COVID-19_webinar_7_July_2020.pdf

A recording of the webinar can be viewed at https://youtube/0Jh65cEPIrI

CCQM P199b SARS-CoV-2 RNA copy number quantification

The ability to accurately measure nucleic acids is crucial for COVID-19 molecular testing which detects the coronavirus' (SARS-CoV-2) genetic material (RNA) using techniques such as reverse transcriptase quantitative PCR (RT-qPCR).

The CCQM Working Group on Nucleic Acid Analysis (CCQM-NAWG) has launched a fast-tracked inter-laboratory study for SARS-CoV-2 RNA genome measurement, coordinated by the National Measurement Laboratory at LGC (UK), NIM (China), NIBSC (UK) and NIST (USA).

The study (CCQM-P199.b), will focus on measuring key genes that are targeted by diagnostic tests for SARS-CoV-2, and uses materials developed in China and the UK.

More than a dozen National Metrology Institutes from around the world are participating in the study along with guest laboratories who are experts in virus detection and quantification including the Center for Biologics Evaluation and Research (U.S. FDA) and the Smorodintsev Research Institute of Influenza (Russian Federation). Results are due to be reported in Autumn 2020 and will enable the comparability of potential reference measurement procedures (RMPs) to be evaluated.

The availability of proven RMPs to SARS-CoV-2 RNA gene targets will allow high accuracy quantification of the biological reference standards that can support diagnostic manufacturers in their test development and ensure routine testing quality as it is expanded across hospitals and laboratories around the world. Ensuring international standardization will support defined test performance criteria such as limit of detection, providing more confidence and better comparability of diagnostic test results for COVID-19 related molecular testing. This will enable meaningful exchange of information between countries and government agencies and ensure its maximum value in contributing to their decision-making.

National Measurement Laboratory (NML) at LGC

As the National Measurement Laboratory (NML) at LGC (UK), our role is to ensure confidence and quality in the chemical- and bio-measurements made in the UK. We are using this expertise, working with healthcare providers, industry, universities and the global measurement and standards communities to help support efforts against the COVID-19 pandemic.

International standardisation

We are developing SI-traceable reference measurement procedures based on digital PCR (dPCR) to support COVID-19 diagnostic testing using our nucleic acid measurement expertise.

In our role as a nominated expert *laboratory for external quality assurance provider INSTAND eV*, we are supporting their new Proficiency Testing (PT) scheme for SARS-CoV-2 genome detection (600+ participants). This involves assigning reference values for virus quantification and evaluating material homogeneity. In addition, we are contributing to international guidance documentation for assay design and standards assessment, through active involvement in the steering committee of the newly formed "*Coronavirus standards working group*" (Joint Initiative for Metrology in Biology, USA).

Our previous work on the validation of reference measurement procedures for the counting of biological and molecular entities developed in a European project (EMRP Bio SI-trace) has been instrumental in developing international *(ISO) standards relevant to the diagnosis of respiratory infections and identification of microbial pathogens*. One of these (ISO 20395:2019 Requirements for evaluating the performance of quantification methods for nucleic acid target sequences – qPCR and dPCR) has been made freely available by ISO https://www.iso.org/obp/ui#iso:std:iso:20395:ed-1:v1:en to support the development and implementation of effective Covid-19 testing and has, amongst others, already been central in the implementation of the quality assurance for field labs testing for coronavirus.

Supporting the UK healthcare system

We are providing regular support to the NHS around validation of molecular diagnostic viral method performance, e.g. collaborating with local UK NHS hospitals and testing laboratories (*Great Ormond Street NHS Foundation Trust (UK), Health Services Laboratory (UK)*) where we have assisted in the clinical development and utility of COVID-19 testing to ensure the robustness of different assays for increased volumes of patient samples at the front line.

We are a partner in the new *COVID-19 National DiagnOstic Research and Evaluation Platform (CONDOR)*, funded by the National Institute for Health Research and UK Research and Innovation. CONDOR will create a single national route for evaluating new diagnostic tests in hospitals, GPs and care homes. Led by Manchester University NHS Foundation Trust and the University of Oxford, in collaboration with four NIHR Medtech and In vitro diagnostics Co-operatives and co-led by the Chief Medical Officer, Prof. Chris Whitty, CONDOR is one of a number of COVID-19 studies that have been given *urgent public health research status by the Department of Health and Social Care*. In our national role we will provide independent validation of the new molecular point of care tests and of

emerging serological tests.

Collaborating with academics and supporting research

We are partners in multiple *joint collaborative academic research proposals* that will use the NML's mass spectrometry and molecular biology resources and expertise to help with the development of improved molecular and antibody testing for the COVID-19 pandemic. This includes the Mass Spectrometry COVID-19 coalition, a global initiative led by the University of Manchester (UK) set up to inform serological testing, support vaccine and therapeutic development (mapping viral proteins and their interactions) and develop methods to determine disease prognosis and the lifetime of infectious particles in the environment (1, 2).

National institute of Standards and Technology (NIST, USA)

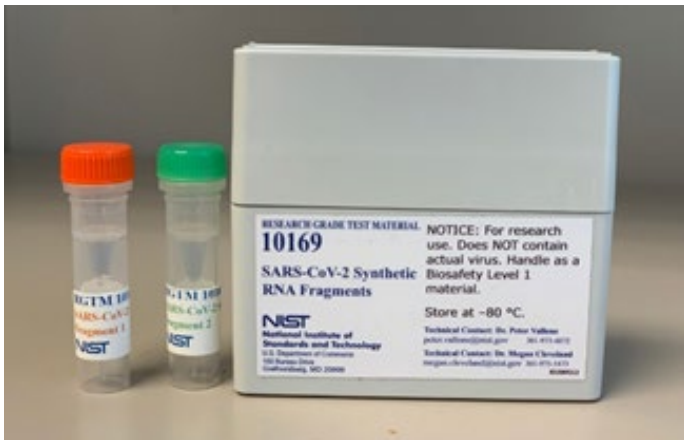
Peter Vallone and Megan Cleveland

RNA material to support SARS-CoV-2 measurements

The National Institute of Standards and Technology (NIST, USA) is supporting the response to the COVID-19 pandemic with the release of a new RNA material to aid measurement and detection of the SARS-CoV-2 virus. This research-grade test material (RGTM 10169) consists of synthetic fragments of the SARS-CoV-2 virus RNA, which is the target of molecular diagnostic tests. The SARS-CoV-2 RGTM differs from a NIST SRM in that it is not as highly characterized or traceable to the SI but is homogeneous and continually tested for stability. We envision the development of a SARS-CoV-2 SRM from the data and feedback we receive from users of the early release RGTM.

These RNA fragments are intended to assist in the development and validation of RT-qPCR assays for the detection SARS-CoV-2. The concentration of the RGTM was measured using digital PCR. It can be used to assess limits of detection for SARS-CoV-2 assays and may be used to calibrate other SARS-CoV-2 controls.

The material is comprised of two separate synthetic RNA components from the SARS-CoV-2 genome individually bottled in a background of 5 ng/µL human Jurkat RNA (stored at −80 °C, BSL-1). The sequence of each fragment was confirmed with next generation sequencing methods. The fragments act as targets for N, E, and ORF1ab regions with the specific sequence ranges described below.



Details on the RNA fragments:

Fragment 1 – Total length: 3985 nt, Includes SARS-CoV-2 sequence: 25949-29698 of isolate USA-WA1/2020

Fragment 2 – Total length: 3790 nt, Includes SARS-CoV-2 sequence: 12409-15962 of isolate USA-WA1/2020

The RGTM is currently being distributed at no cost to National Metrology Institutes, commercial manufacturers, academic research laboratories, and others involved in SARS-CoV-2 diagnostic test development. To obtain RGTM, please visit the following website and fill out the request form https://www.nist.gov/programs-projects/sars-cov-2-research-grade-test-material

National Institute of Metrology (NIM, China)

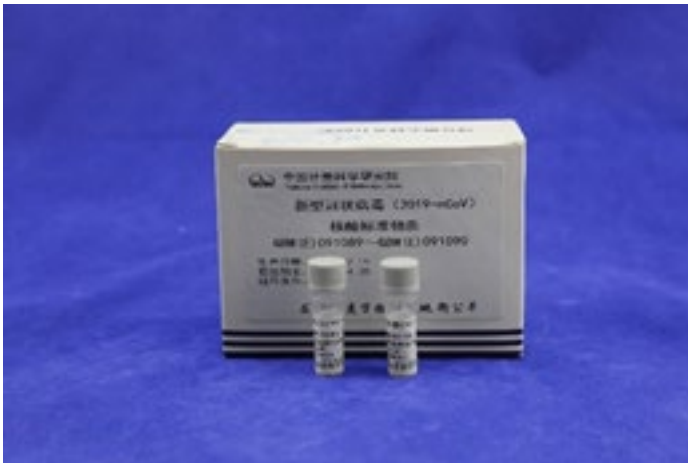
Lianhua Dong and Xinhua Dai

Nucleic acid CRMs to support SARS-CoV-2 testing

In order to support the development of new assays and the evaluation of existing diagnostic kits, NIM has developed *In-vitro* transcribed and genomic RNA CRMs and pseudovirus reference materials for SARS-CoV-2 testing. To obtain the CRMs, please visit the following website: https://www.ncrm.org.cn/Web/MaterialEn/HomeList?pageIndex=1&type=1&term=COVID-19

In-vitro transcribed RNA CRM (GBW(E)091089-GBW(E)091090)

The *In-vitro* transcribed RNA CRMs consist of three *In-vitro* transcribed RNA targets: nucleocapsid (N) (full length), envelope (E) (full length), and the open reading frame 1ab (ORF1ab) gene fragment (genome coordinates: 13201-15600). Each unit consists of two tubes, each containing three synthetic RNA fragments from the SARS-CoV-2 genome. The copy number concentration of three gene targets is determined by digital PCR.



Genomic RNA CRM (GBW(E)091098-GBW(E)091099)

The genomic RNA CRM is purified from inactivated SARS-CoV-2. Each unit consists of two tubes that contain SARS-CoV-2 genome. The copy number concentration of three gene targets is determined by digital PCR.

Pseudovirus reference material

To help evaluate the pre-analytical process of SARS-CoV-2 testing, NIM is developing pseudovirus reference materials, which consist of three *In-vitro* transcribed RNA targets: N (full length), E (full length), and ORF1ab gene fragment. Pseudovirus reference materials can be used to validate the pre-analytical and the analytical process.

Protein CRMs to support SARS-CoV-2 Testing

To support the development of new serological tests and the evaluation of existing serological test kits, three different types of protein Certified Reference Materials (CRMs) were developed

by the National Institute of Metrology (NIM), China.

Nucleocapsid (N) protein CRM (GBW(E)091097)

According to the N gene sequence (28274-29533) published in the NCBI GenBank, a plasmid containing a full-length N gene was constructed. Recombinant N protein is purified from *E. coli.* The protein molecular weight is 45 kD. SDS-PAGE and high effective gel exclusion liquid chromatography showed no obvious hetero proteins.

Human IgG monoclonal antibody (GBW(E)091109-GBW(E)091110)

The two IgG monoclonal antibody CRMs contain humanized monoclonal antibodies against the SARS-CoV-2, Spike protein and Nucleocapsid protein, respectively. Standard values of the two CRMs are determined by Isotope Dilution Mass Spectrometry (IDMS) based on amino acid analysis.

IgM CRM

The ongoing IgM monoclonal antibody CRM contains the humanized IgM monoclonal antibody against Nucleocapsid protein. The standard value of the IgM CRM is determined by Isotope Dilution Mass Spectrometry (IDMS) based on amino acid analysis.

TÜBİTAK UME – National Metrology Institute of Turkey

SARS-CoV-2 Virus RNA Reference Material Production for RT-qPCR Measurements

The Reverse Transcription Quantitative Polymerase Chain Reaction (RT-qPCR) method is one of the most efficient standard molecular diagnostic methods currently used for SARS-CoV-2 virus detection. On molecular-based diagnostic systems, the analysis of the virus starts with the reverse transcription of viral RNA to complementary DNA (cDNA), and in the second step, cDNA is multiplied during PCR. The use of only DNA-based reference materials will not include reverse transcription step. The objective of the project is the production of SARS-CoV-2 virus RNA Reference Material to control all steps of virus detection. The RNA-based Reference Material is in high demand by testing laboratories and kit manufacturers for method validation, as well as for its use as an internal quality control material. Additionally, RNA reference material will be used in proficiency testing schemes for external quality control purposes.

Korea Research Institute of Standards and Science (KRISS, Republic of Korea)

Developing the KRISS SARS-CoV-2 RNA reference material

Young-Kyung Bae[1], Hee Min Yoo[2], Da-Hye Lee[1], Seil Kim[2,3,4]

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dPCR-based SARS-CoV-2 detection

Current diagnostic tests are based on the reverse transcription-quantitative polymerase chain reaction (RT-qPCR) method, the gold standard that involves amplification of targeted regions within the viral RNA after its conversion to DNA.

Our preliminary research suggested a demand for the SARS-CoV-2 reference materials that are accurately quantified and compatible with widely-used commercial kits. We previously tested the potential of using reverse transcription-digital polymerase chain reaction (RT-dPCR) and found that dPCR-based SARS-CoV-2 detection was robust as sub-optimal primer-probe sets performed adequately without a significant loss in sensitivity. Additionally, we have applied a pilot scale RM along with the clinical samples (3).

SARS-CoV-2 RNA reference material

Recently, the Korea Research Institute of Standards and Science (KRISS) has developed the SARS-CoV-2 RNA reference material (KRISS 111-10-506). This RM contains five in vitro transcribed RNA fragments covering approximately 90 % of the SARS-CoV-2 RNA genome. To assign the RNA copy number concentration of each fragment, we utilized RT-dPCR. This calibration-free digital PCR-based technique produces highly reproducible and sensitive results compared to the conventional reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Importantly, our RNA RM was compatible with five Korean Emergency Use Authorized (EUA) diagnostics kits and known assays developed by national reference laboratories. These results assure that this KRISS RM is readily applied to validate current diagnostic kits and develop upcoming ones. Additionally, we provide the RT-qPCR results using these kits as the informative values for our RM.

This SARS-CoV-2 RNA reference material was developed by a synergistic collaboration between two KRISS teams: the Microbiological Analysis Team and the Biopharmaceutical Analysis Team. In the near future, these teams plan to develop other COVID-19 related reference materials. The list includes packaged SARS-CoV-2 RNA, viral antigens, and SARS-CoV-2 antibodies. Furthermore, we will continue to seek areas in need of reliable reference materials such as other emerging commutable diseases.

Reference laboratories

Referenzinstitut für Bioanalytik - RfB

Proficiency testing

The Reference Institute for Bioanalytics (RfB) has run a number of COVID-19 related proficiency tests.

The basis of the first regular survey was a pilot survey in April 2020. Within only 10 days the RfB organized together with the University Hospital Mannheim the pilot survey among 15 selected participants from Germany. Each participant received eight liquid serum samples from Covid-19 patients or patients with other respiratory infections. The patient samples had to be determined for presence of IgG, IgA and IgM antibodies against SARS-CoV-2. The results of all participants were compared with the patient's case history and the result of the virus neutralization test carried out at the Institute for Microbiology of the Bundeswehr in Munich. Due to the lack of a reference system the virus neutralization test was integrated into the study and is used to determine whether a patient sample contains antibodies that prevent cell infection by SARS-CoV-2 *in vitro*.

Seven test systems from different companies were represented in this pilot survey. The results show good specificity and sensitivity in the measurement of IgG and IgA antibodies. Minor problems were observed for the weak-positive sample regarding the sensitivity of some test systems. A significant lack of sensitivity could be seen for the determination of specific IgM antibodies in all test systems.

Since the pilot study confirmed the structure of the EQA scheme, the first regular survey started in May 2020. 180 laboratories, mainly from Europe, were registered as participants. Each laboratory received four samples of patients for the investigation of IgG and IgM. Evaluation of this survey revealed a good specificity for specific IgG testing in all test systems. The sensitivity was limited with serious differences between the different test kits. As in the pilot survey, this was particularly evident in the case of weakly positive samples. For anti-SARS-CoV-2 IgM testing no sensitivity could be determined because no positive samples were sent. However, the specificity testing remained below expectations. For this reason, and because testing for specific IgA antibodies is of greater clinical interest, the determination of IgM antibodies will be excluded from future surveys and the parameter IgA antibodies will be added.

Further surveys are planned for the third and fourth quarter of 2020. Information about the organization of the surveys as well as all results of the past surveys and the following surveys are freely available and published on the RfB website (www.rfb.bio).

Research Centre for Metrological Traceability in Laboratory Medicine (CIRME), University of Milan

Elena Aloisio and Mauro Panteghini

Promoting laboratory result harmonization in COVID-19 avoids confusion and permits unambiguous interpretation of study conclusions

In collaboration with the Clinical Pathology Unit of the 'Luigi Sacco' academic hospital, one of the two Italian reference centres for infectious diseases, CIRME has recently supervised studies on hospitalized COVID-19 patients in order to evaluate the role of laboratory tests as clinical predictors of disease severity. The evaluation of biomarkers was carried out in relation to two major clinical outcomes: death during hospitalization and admission to an intensive care unit (ICU). The focus was on identifying which markers had the best predictive power and, subsequently, on defining interpretative criteria (i.e. cut-off values) that may aid clinicians in evaluating COVID-19 severity. Optimum biomarker cut-offs were specifically selected to have a high rule-in ability in detecting patients at risk of in-hospital death and a high rule-out ability in identifying patients at very low risk of ICU admission (4, 5). At the multivariate analysis, high concentrations of lactate dehydrogenase (LDH) and low concentrations of albumin in serum were significantly associated with higher odds of death, while only low LDH activities remained associated with lower odds of ICU admission. The best cut-offs for death prediction were >731 U/L for LDH and ≤18 g/L for serum albumin, while an LDH activity <425 U/L was associated with a negative likelihood ratio of 0.10 for intensive treatment.

One of the major strengths of the published results was represented by the use of methodologies for which analytical selectivity and standardization had been verified and validated, enabling the universal application of results obtained in our clinical studies and permitting their unambiguous interpretation, providing that institutions implementing them also use standardized assays. Particularly, serum albumin was measured with an immunoturbidimetric assay, which is fully specific for the protein measurement, made traceable to the ERM-DA470k/IFCC reference material, and LDH was measured with a system of which the optimal alignment to the IFCC reference measurement procedure was recently validated (6)

(*Note that both the ERM-DA470k/IFCC reference material and the immunoturbidimetric method (ID no. C1RMP_P4) for serum albumin, and the IFCC reference procedure for LDH (ID no. NRMeth 66) are listed in the JCTLM database*).

These data provide a good example to show that the implementation of assay standardization is an absolute priority for optimizing healthcare, with the example given for COVID-19 patients. Only the use of assays providing standardized results allows the application of common decision limits, as those defined in our COVID-19 studies, worldwide and the comparability of clinical studies performed in different institutions.

Accreditation organizations

ILAC

ILAC has not been engaged in the development of diagnostics tools, but has explained and promoted the role of accredited conformity assessments services in the COVID-19 pandemic, published on the ILAC website:

https://ilac.org/publications-and-resources/videos/

https://ilac.org/latest_ilac_news/iso-casco-covid-19-statement/

Professional organizations

IFCC Taskforce and Online Information Guide on COVID-19

Khosrow Adeli, President, International Federation of Clinical Chemistry and Laboratory Medicine

In response to the current pandemic, the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) has established a global taskforce on COVID-19 as well as an online resource called the IFCC information Guide on COVID-19. The taskforce and the online resource are helping to provide the latest evidence and up-to-date information on population screening, diagnosis, biosafety guidelines for clinical laboratories, and biochemical monitoring of hospitalized patients with COVID-19.

In addition to the online information guide, the taskforce has reviewed the latest evidence and has started publishing comprehensive reviews including an expert opinion article on *Biosafety Measures for Preventing Infection from COVID-19 in Clinical Laboratories: IFCC Taskforce Recommendations* (Lippi et al. 2020) and a critical review on *Molecular, Serological, and Biochemical Diagnosis and Monitoring of COVID-19* (Bohn et al. 2020). These articles not only review the current evidence but also provide practical recommendations on both laboratory biosafety as well as diagnostic/serological/biochemical markers used in infection control and monitoring. The taskforce will continue to review the latest evidence and publish new guidelines and high-quality reviews to ensure a wide-ranging coverage of the role that clinical laboratories play in the fight against this unparalleled and unfortunate pandemic. IFCC will also be releasing specific guidelines on laboratory testing in support the diagnosis and monitoring of COVID-19 infection and associated disease.

International Society for the Advancement of Cytometry (ISAC)

Virginia Litwin, Bruce Davis, Sindhu Cherian, Pratip Chattopadhyay

A consensus understanding of COVID-19 disease is beginning to emerge even in these early days of the pandemic. A tipping point for severe disease appears to be associated with a shift in the host immune response (7, 8). Thus, to understand disease pathogenesis, an in-depth evaluation of the immune system of patients with asymptomatic, mild, moderate, and severe disease will be important.

For over 40 years, cytometric technologies - beginning with flow cytometry - have been critical tools in elucidating the functions of the immune system and its impact on immune-related diseases ranging from autoimmunity to HIV/AIDS. Recently, newer tools for single cell analysis have emerged as well, including imaging, mass, spectral and molecular cytometry (9). Cytometrists around the world now recognize the power of evaluating immune responses both at the single-cell level and in multidimensional space. In the coming months and years, multidimensional single-cell analysis will translate to a better understanding of SARS-CoV-2 infection, the identification of prognostic biomarkers, and a deeper ability to evaluate new vaccines, anti-viral and supportive therapies.

Professional societies that support cytometry can play an important role in the scientific response to COVID-19. In May 2020, the International Society for the Advancement of Cytometry (ISAC) formed its COVID-19 Workgroup (https://isac-net.org/page/COVID-19). The group acts as a focal point, bringing together scientists working on SARS-CoV-2, or otherwise interested in the role of cytometry technologies and methodologies in the pandemic response. This group includes significant representation from the International Society for Clinical Cytometry (ICCS), because the COVID-19 work performed in research settings will need to be translated rapidly into clinical laboratory settings.

The ISAC COVID-19 Workgroup will assist in these areas, providing a centralized source for biosafety guidelines, a forum to discuss experimental design, a networking directory to establish collaborations, and a connection to data repositories and analysis algorithms. In today's climate, it is more important than ever that laboratories generate high quality, reproducible data with traceable, calibrated measurements; COVID-19 Workgroup will help shape that process.

Review paper

Biomarkers associated with COVID-19 disease progression

Tomris Ozben, Department of Clinical Biochemistry, Faculty of Medicine, Akdeniz University, Antalya, Turkey

The coronavirus disease 2019 (COVID-19) pandemic is a scientific, medical, and social challenge. The complexity of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is centred on the unpredictable clinical course of the disease that can rapidly develop, causing severe and deadly complications. The rapid disease spread necessitates to determine risk categories following COVID-19 diagnosis, to ensure an optimal resource allocation and to improve clinical management and prevention of serious complications. Novel biomarkers are needed to identify patients who will suffer rapid disease progression to severe complications and death. The identification of effective laboratory biomarkers able to classify patients based on their risk is imperative for prompt treatment. The scientific community is in urgent need for reliable biomarkers related to coronavirus disease 2019 (COVID-19) disease progression, to stratify high risk patients. Therefore, we analysed the recently published studies to identify haematological, inflammatory, immunological, biochemical, and potential new biomarkers for screening, clinical management, and prevention of serious complications. Our manuscript has been published recently in the journal "*Critical Reviews in Clinical Laboratory Sciences*". Link to our article:

https://www.tandfonline.com/eprint/7PEJJ3UBXUCW9SNS-RCG2/full?target=10.1080/10408363.2020.1770685

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