

# Biomarker Research and Development for Coronavirus Disease 2019 (COVID-19): European Medical Research Infrastructures Call for Global Coordination

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An effective response to the coronavirus disease 2019 (COVID-19) pandemic requires a better understanding of the biology of the infection and the identification of validated biomarker profiles that would increase the availability, accuracy, and speed of COVID-19 testing. Here, we describe the strategic objectives and action lines of the European Alliance of Medical Research Infrastructures (AMRI), established to improve the research process and tackle challenges related to diagnostic tests and biomarker development. Recommendations include: the creation of a European taskforce for validation of novel diagnostic products, the definition and promotion of criteria for COVID-19 samples biobanking, the identification and validation of biomarkers as clinical endpoints for clinical trials, and the definition of immune biomarker signatures at different stages of the disease. An effective management of the COVID-19 pandemic is possible only if there is a high level of knowledge and coordination between the public and private sectors within a robust quality framework.

**Keywords.** COVID-19; biomarkers; European Research Infrastructures; SARS-CoV-2 pandemic.

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic has generated a fast response from the global scientific community, governmental organizations, the life sciences industry, and healthcare providers. With unprecedented speed, a number of laboratory tests have been developed with the aim to facilitate easy and efficient detection of virus infection [1–4], and tests are emerging for the measurements of antibodies for identifying past SARS-CoV-2 infections.

As the pandemic evolves, it is becoming clear that there is a gap between the ambition and the usefulness of these tests. Evidence continues to accumulate on the limitations of the currently available diagnostic and prognostic approaches [2–6].

Coronavirus disease 2019 (COVID-19) assays can be distinguished in (1) virus detection assays (nucleic acid and imaging based), (2) immunity assays (serological or immune cell based), and (3) prognostic assays reflecting severity of disease, complications and degree of recovery (miscellaneous biomarker testing) [4, 7].

In particular, serological tests are becoming more relevant as they are able to detect past COVID-19 infections [8]. However, many open questions remain around each test's specificity and sensitivity, which represents its validity and usefulness in a clinical setting. The value of these tests, as with many other biomarker tests in healthcare and patient management, is one of today's major challenges.

In addition to SARS-CoV-2 detection and testing of immune response, there is an urgent need to predict which patients will develop specific disease characteristics. Indeed, some individuals develop mild symptoms and others very severe ones for unknown reasons, and patients can differ dramatically in the degree and speed of their response following hospitalization [7]. Recent studies showed how COVID-19 patients with comorbidities, such as hypertension or diabetes mellitus, are more likely to develop a more severe course and progression of the disease [9]. Differences in the immune response [10] or prior coronavirus infections could also affect the COVID-19 clinical course [11].

This heterogeneity of manifestations of SARS-CoV-2 infection constitutes one of the greatest challenges in managing the clinical consequences of the pandemic. Biomarker profiles are of vital importance to clinicians when evaluating treatment options, for defining the clinical course, and for close monitoring and support of patients in their disease management and remission trajectory.

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Tools should enable population screening and the identification of high-risk patients. Given the large interindividual heterogeneity, this can be achieved using biomarker signatures, composed of multiple analytes. Given their relevance in this context, robust and well-validated biomarkers are crucial to enable effective decision-making.

### **SARS-COV-2 TESTING: CURRENT SITUATION**

SARS-CoV-2 and COVID-19 testing kits are designed to be used in routine laboratories and also at the point-of-care setting, with the ambition of shortening the diagnostic time window and thereby facilitating rapid identification of COVID-19 positive patients and contacts. In order to be effective, these kits must be based on validated biomarkers and biomarker assay formats that yield high sensitivity and specificity results, for instance, to distinguish an infected person from a noninfected one.

SARS-CoV-2 diagnostic tests are based on the detection of the viral genome (eg, reverse transcription polymerase chain reaction (RT-PCR)-based methods, isothermal amplification assays and CRISPR [12–16]), viral proteins (eg, antigen-based test) [17, 18], or antibodies against the virus (eg, serological test) [8].

Methods based on the viral genome detection, with their large range of applications, high sensitivity, and high sequence specificity, have become a routine and reliable technique for detecting [16].

To complement the viral genome tests, viral antigen tests have been developed. These tests allow the virus detection early in infection but display limitations on sensitivity and potential cross-reaction with other coronaviruses [17, 18].

Despite the fact that COVID-19 is a severe pandemic, many governments are leaning toward “mitigation” and “containment” as strategies. The overarching goal is for all countries to control the pandemic by slowing down the transmission and reducing mortality associated with COVID-19. Indeed, in the absence of a vaccine, reaching group immunity is no straightforward path with major ethical considerations as the societal consequences of achieving it are devastating [19]. Mobility and travel restrictions, social distancing, and the use of personal protective equipment have been introduced in order to reduce human-to-human transmission. The use of face masks in particular is enforced widely within the general population, together with hand hygiene.

Stopping the spread of COVID-19 requires finding and testing all suspected cases so that confirmed cases are promptly and effectively isolated and receive appropriate care. It is important that the close contacts of all confirmed cases are rapidly identified, quarantined, and medically monitored for the virus incubation period of up to 14 days.

Next to the need for well-validated and reliable diagnostic tests, this scenario demands high quality and reliable serological tests, measuring the immune responses induced by past and new viral infection, in combination with tests addressing T-cell

activity. These assays are important for understanding the prevalence of COVID-19 and whether the development of a humoral immune response to SARS-CoV-2 protects against the disease.

As the World Health Organization (WHO) clearly underlined, “Laboratory tests that detect antibodies to SARS-CoV-2 in people, including rapid immunodiagnostic tests, need further validation to determine their accuracy and reliability” (<https://www.who.int/news-room/commentaries/detail/immunity-passports-in-the-context-of-covid-19>). Addressing these issues is crucial, as serological assays are critical for the patient care pathway and for the management and surveillance of the virus.

Limitations to the use and development of the tests described above include poor test sensitivity due to sample collection [14], poorly described reference material, low specificity, and lack of technical validation, and therefore a threat of false disease diagnosis.

Uncertainty in test sensitivity that lead to false-negative cases of COVID-19 likely constitutes a serious threat to the control of the pandemic. Indeed, false negative results are more weighty, because unrecognized infected persons may not be isolated and can infect others [4]. Because of this, some governments require RT-PCR test and quarantine for people who are considered close contacts of positive cases, with additional testing and isolation in case of negative results. Moreover, in presence of a strong epidemiological link to COVID-19 infection, paired serological tests (in the acute and convalescent phase) could support diagnosis [20].

Testing limitations are likely a result of combining several unknowns such as the lack of understanding of the biology of the disease, in particular its natural history and associated immune response, a relatively low number of samples, and the use of novel laboratory test kits whose quality and accuracy has not been rigorously tested. Furthermore, the lack of rigorous study design and methodology to robustly validate the tests before deployment affects the tests’ reliability and ultimately the correctness of the clinical assessment.

### **URGENT NEED FOR VALIDATED BIOMARKERS**

A collaborative global response for diagnostics, therapeutics, and vaccines development as well as the future management of the pandemic called the new Access to COVID-19 Tools (ACT) global accelerator has been launched in April. The ACT accelerator will require additional molecular tools to identify relevant COVID-19 related biomarkers that will have a critical role in: (1) assessing the efficiency of future vaccines and/or therapeutics; (2) preventing and identifying clinical complications, in particular those related to the deadly immunological storm reaction, vascular activation and hemostasis control, and, (3) stratifying patients to define therapy targets and identify individuals at risk of infection, suitable for preventive interventions. Due to the complexity of the immune response, in-depth phenotypic analysis is necessary in order to identify specific biomarker signatures, integrating omic and clinical data.

According to the GlobalData's Biomarkers database, a large number of different biomarkers have been utilized for COVID-19 trials for different purposes such as monitoring treatment response, predicting and monitoring treatment safety. However, only a few of them are validated for clinical application, with the risk that the results produced are not reliable and are not of much use for medical decision making.

Hematology laboratory and routine coagulation tests have made a significant contribution in the identification of useful prognostic markers as well as in predicting outcomes and recovery [21, 22]. Moreover, in the era of personalized medicine, biomarkers can enable the selection of appropriate treatment for COVID-19 infected patients. Biomarkers of inflammation such as interleukin (IL)-6 and IL-10 [23], of cardiac injury [24], of liver and kidney function [25, 26], as well as of coagulation measures [27], are significantly elevated in patients with both severe and fatal forms of COVID-19. Moreover, it has been assumed from studies in severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS) patients that memory T cells, induced by the contact with previous pathogens from the coronavirus family, may have the potential to recognize SARS-CoV-2. Hence, features and distribution of preexisting T cells could be used as markers for explaining some of the differences in infection rates or pathology observed during this pandemic [11].

The mentioned markers could support patient stratification and represent objective and standardized criteria to guide therapy and allocate resources.

Although the postrecovery course of the COVID-19 disease is not clear yet, limited observations demonstrated that they are at risk of psychological and physical complications of the disease itself, as well as treatment-related lung damage and other organ injuries [28–30]. Biomarker signatures can play a key role in the management of the post-COVID-19 patients, predicting medium and long-term clinical outcomes.

More insights on the biological processes and replication studies are crucial before the adoption of any molecule and parameter in the clinical setting.

The coming months will be critical for accelerating a COVID-19 biomarker pipeline that will enable diagnostic and prognostic profiles, provide reliable end points for clinical trials, assess treatment response and allow vaccine candidate selection, together with supporting healthcare systems with tailored strategies and patient-centred interventions.

## **HOW TO OPTIMIZE THE RESEARCH PROCESS: THE POINT OF VIEW OF EUROPEAN MEDICAL RESEARCH INFRASTRUCTURES**

In order to structurally address the issues above, a long-term vision is necessary. The optimization and the acceleration of the research process requires a high level of knowledge and coordination, as well as the application of standards and quality to

reduce uncertainty along the biomarker pipeline [31]. This is possible only if there is an effective interaction between private-public networks. In Europe this collaboration is facilitated by research infrastructures. In particular, the Alliance of Medical Research Infrastructures (AMRI, <https://bit.ly/2FitLu9>), including EATRIS-ERIC (focused on translational medicine, <https://eatris.eu/>), ECRIN-ERIC (focused on clinical research, <https://www.ecrin.org/>) and BBMRI-ERIC (focused on biobanking <https://www.bbmri-eric.eu/>) provides resources and services for the medical research communities to conduct research and foster innovation. During the present health crisis, substantial public funding has become available for research in diagnostics, treatments, and vaccines for the new coronavirus disease, and AMRI has worked to accelerate and manage international research collaborations on COVID-19, acknowledging that the challenge is a global one. The medical research infrastructures have made a significant effort to share knowledge and to ensure robustness of the COVID-19 related project outcomes [32]. In addition, thanks to its expertise as well as its culture in quality standards and reproducibility, the Alliance identified several action lines (Table 1) to optimize the research process and to address issues related to diagnostic/prognostic tests and biomarker development. Their main strategic objectives are: (1) to establish a European taskforce for validation of novel diagnostic products; (2) to define and promote criteria for COVID-19 sample handling, data collection, and biobank management including ethical considerations; (3) to validate novel diagnostic approaches; (4) to identify and validate biomarkers as clinical endpoints for clinical trials; and (5) to define the biomarker profile determining the innate and acquired immune response to the infection and establish immune signatures at different stages of the disease. These actions are highly relevant for an effective response from the research community to the COVID-19 pandemic. Only with close collaboration in these key areas will we be able to efficiently support the biomarker R&D process, helping to understand antigen response mechanisms, inform vaccine development, and enable antiviral drug design.

## **CONCLUSIONS**

COVID-19 research is still in its early stages, and we need further research worldwide to better face this pandemic. We still need to learn about the biology of the disease and the variable response that patients display in their disease manifestation and recovery. We expect that the process of biomarker discovery and validation will largely guide an accelerated translational strategy to address this global health crisis. A standardized pathway approach toward the biomarker validation process is thus becoming increasingly important. Quality and reproducibility are essential for translating basic findings into concrete clinic interventions and only following

**Table 1. Overview of Actions Recommended by Alliance of Medical Research Infrastructures (AMRI)**

Strategic Objective	Recommended Actions
<b>(1) Establish a European taskforce for validation of novel diagnostic products</b>	<ul style="list-style-type: none"> <li>• Definition of joint evaluation of novel testing methodologies</li> <li>• Definition of recommendations for statistical study design, including appropriate end points</li> <li>• Focused efforts on stratification of patients to be included based on harmonized diagnosis criteria and standardized diagnosis biomarkers</li> <li>• Promotion of cross-border multicentric diagnostic studies</li> <li>• Creation of standardized protocols so that independent studies can generate interoperable data to increase statistical robustness</li> <li>• Include regulators early in the process and update constantly on progress</li> </ul>
<b>(2) Define criteria for COVID-19 sample handling, data collection and biobank management including ethical considerations</b>	<ul style="list-style-type: none"> <li>• Development of guidelines on the ethical aspects for sampling and data sharing in COVID-19 patients</li> <li>• Definition of specific protocols for protection measures in the context of samples handling</li> <li>• Definition of appropriated samples (origin and source) to be used for diagnostic techniques</li> <li>• Provide guidelines on relevant clinical data collection</li> <li>• Definition of technical controls for samples before the use in each application</li> <li>• Provide guidelines for long-term storage of biological samples (including autopsy samples), including minimization of RNA-degradation</li> <li>• Definition of specific protocols for COVID-19 autopsy samples</li> </ul>
<b>(3) Validate novel diagnostic approaches</b>	<ul style="list-style-type: none"> <li>• Development of appropriate control materials from diverse biological matrix</li> <li>• Definition of standards for amplification specificity</li> <li>• Use of preamplification controls</li> <li>• Improve accuracy of CRISPR-Cas cleavage and read-out system</li> <li>• Improve sensitivity of nonamplification-based technologies</li> <li>• Provide easy and accurate novel point of care portable detection devices</li> </ul>
<b>(4) Identify and validate biomarkers as clinical endpoints for clinical trials</b>	<ul style="list-style-type: none"> <li>• Development of tools for clinical data analysis for identifying prognosis biomarkers and potential endpoint biomarkers</li> <li>• Development of easy, reliable, and standardized detection methods of biomarkers to be used in clinical trials</li> <li>• Development of consensus guidelines for use of biomarker-based patient stratification across Europe in multicentric studies</li> <li>• Identification of individual immunoprofiling signatures for personalized therapies</li> </ul>
<b>(5) Define the biomarker profile determining the innate and acquired immune response to the infection, establish immune signatures at different stages of the disease</b>	<ul style="list-style-type: none"> <li>• Development of quantification methods for serum immunoglobulins: reference values definition</li> <li>• PBL immunoprofiling by appropriated cell markers: reference values definition</li> <li>• Cytokines profiling: SNPs in relevant cytokines (IL-6): reference values definition</li> <li>• Complement cascade and hemostasis regulators quantification: reference values definition</li> <li>• Identification of cofactors/comorbidities affecting immune response: autoimmune diseases, suppressor treatments</li> <li>• Development of predictive immunoprofiling signatures for the identification of patients at risk of cytokine storms</li> </ul>

Abbreviations: COVID-19, coronavirus disease 2019; IL, interleukin; PBL, project-based learning; SNP, single nucleotide polymorphism.

this approach is an effective response to the pandemic guaranteed. Significant efforts and resources have been invested in the development of biomarkers for COVID-19 and AMRI urges that research must be of good quality, providing robust, ethical evidence that stands up to scrutiny and can be used to inform policy making. For COVID-19 management, structural use of the relevant research infrastructures is strongly advised, as they play an important role in centralized management of biomarkers R&D pipelines, biobanking, and clinical trials. The collective efforts of AMRI and collaborative actions of the scientific community will create high-quality knowledge that is openly available and will bring a better understanding of SARS-CoV-2, with benefits for all.

## Note

**Potential conflicts of interest.** The authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

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