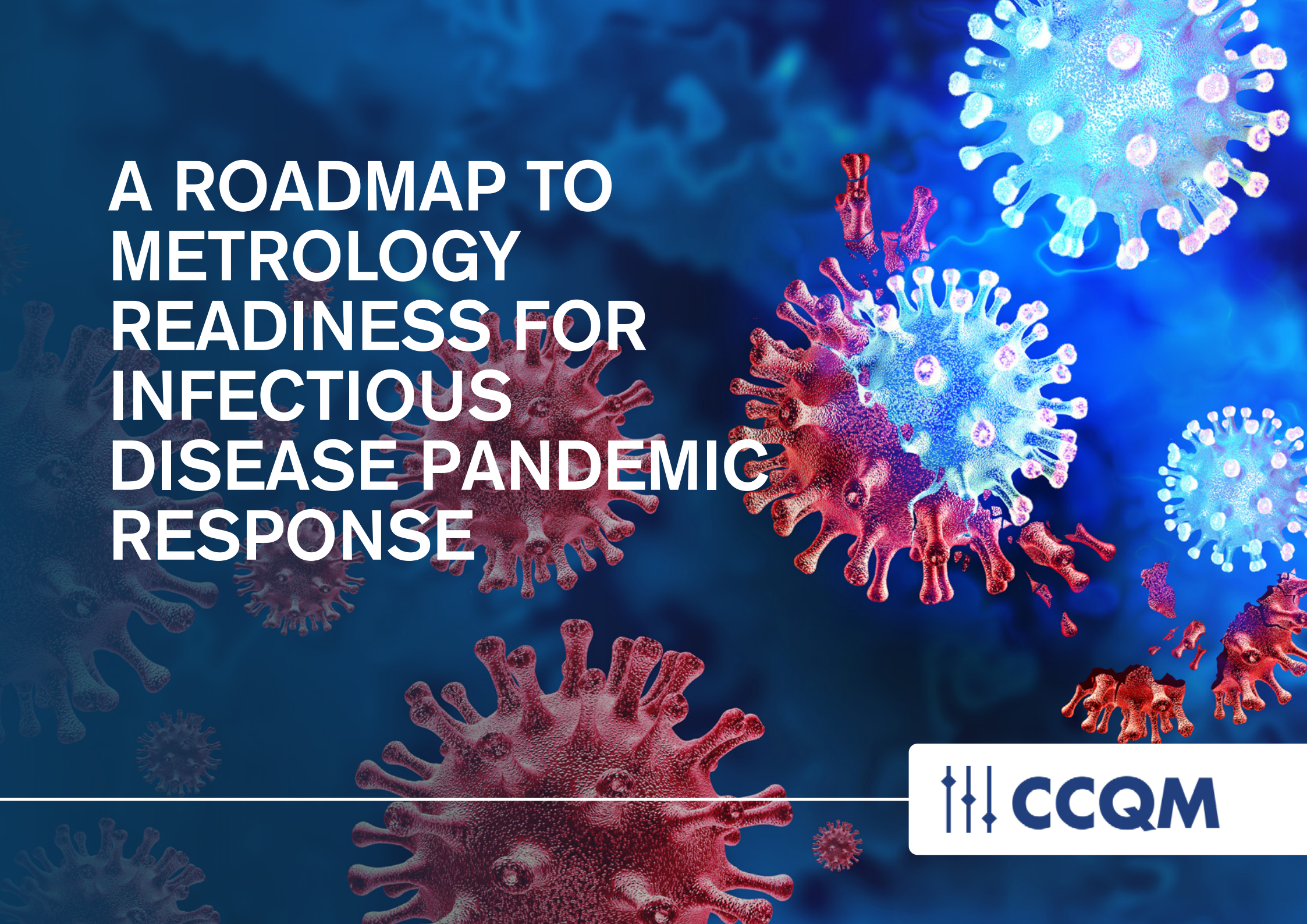
A ROADMAP TO METROLOGY READINESS FOR INFECTIOUS DISEASE PANDEMIC RESPONSE



CCQM

ABOUT THIS ROADMAP

Diagnostic testing for SARS-CoV-2 has been imperative for identifying and managing infected individuals, contact tracing, epidemiologic characterisation and public health decision-making at the national and international level throughout the COVID-19 pandemic.

The unpredictability surrounding the time of onset and the type of pathogen that will cause the next pandemic emphasizes the need for pandemic preparedness plans for future health emergencies and especially for:

- greater understanding of pathogens, their evolution and circulation for early identification of infectious threats - particularly through increased global systemic genomic sequencing capacity and rapid sharing of data, including meta-data
- implementation of standardized test requirements for in vitro diagnostic (IVD) detection assays, including quality requirements and assay design principles
- development of a collection of diagnostics, therapeutics and vaccines (DTVs) for early deployment to infectious threats, demonstrated through a well-rehearsed action plan.

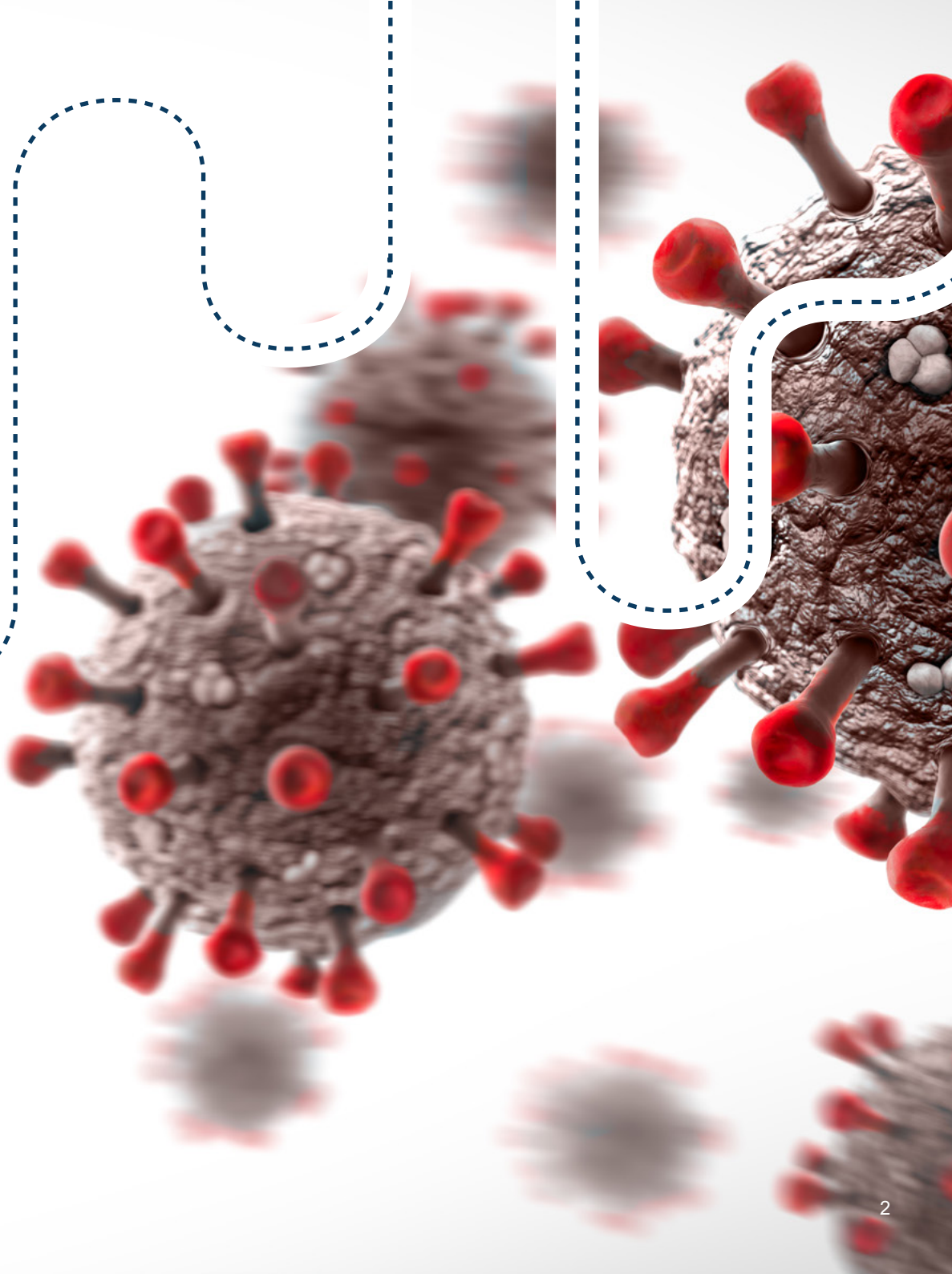
The metrology community successfully provided international reference measurement system procedures and materials for molecular and serological testing within months of isolation of SARS-CoV-2. It also more generally supported sovereign testing capability for wider stakeholders, albeit to different and largely national extents throughout the world.

However, its contributions were often not pro-actively sought by wider stakeholders and under-represented in decision-making based on measurement, due to an over-arching lack of awareness of the benefits to be offered by the metrology community and the lack of an international cohesive presence of the community in infectious diseases allowing:

- a sufficiently fast provision of metrological recommendations (days to weeks)
- a direct link to IVD producers in the relevant sector
- assistance with rapid expansion of testing capacity and capability.

This document identifies priorities and sets out pathways for pandemic preparedness for the metrology community resulting from the 2021 series of CCQM Webinars and Workshop.

ROADMAP VISION



Identify measurement priorities and outline pathways needed to provide effective contributions in a timeline appropriate for pandemic response and which raise awareness of the metrology community with wider stakeholders

The Roadmap provides:

- the metrology community with a route to accelerate their response – better accelerated measurement leading to improved decision-making and accelerated recovery outcomes during the critical juncture of a future pandemic; and

- wider stakeholders with visibility and guidance on the metrology interventions at the technological, platform and infrastructural level available as part of an integrated response to a future pandemic.

The Roadmap addresses metrology needs to provide comparable/equivalent diagnostic test measurements that result in the same outcome; traceability is an established way of achieving this in other industries/communities.

The Roadmap does not address metrology for the continuous process of data collection and analysis and its dissemination to policy makers and healthcare and other professionals for public health surveillance, priorities for personal protective equipment (PPE), potential pharmaceutical/ medicinal treatments or vaccines.

The Roadmap focus areas:

Understanding pandemic frequency, lifecycles and associated testing challenges.

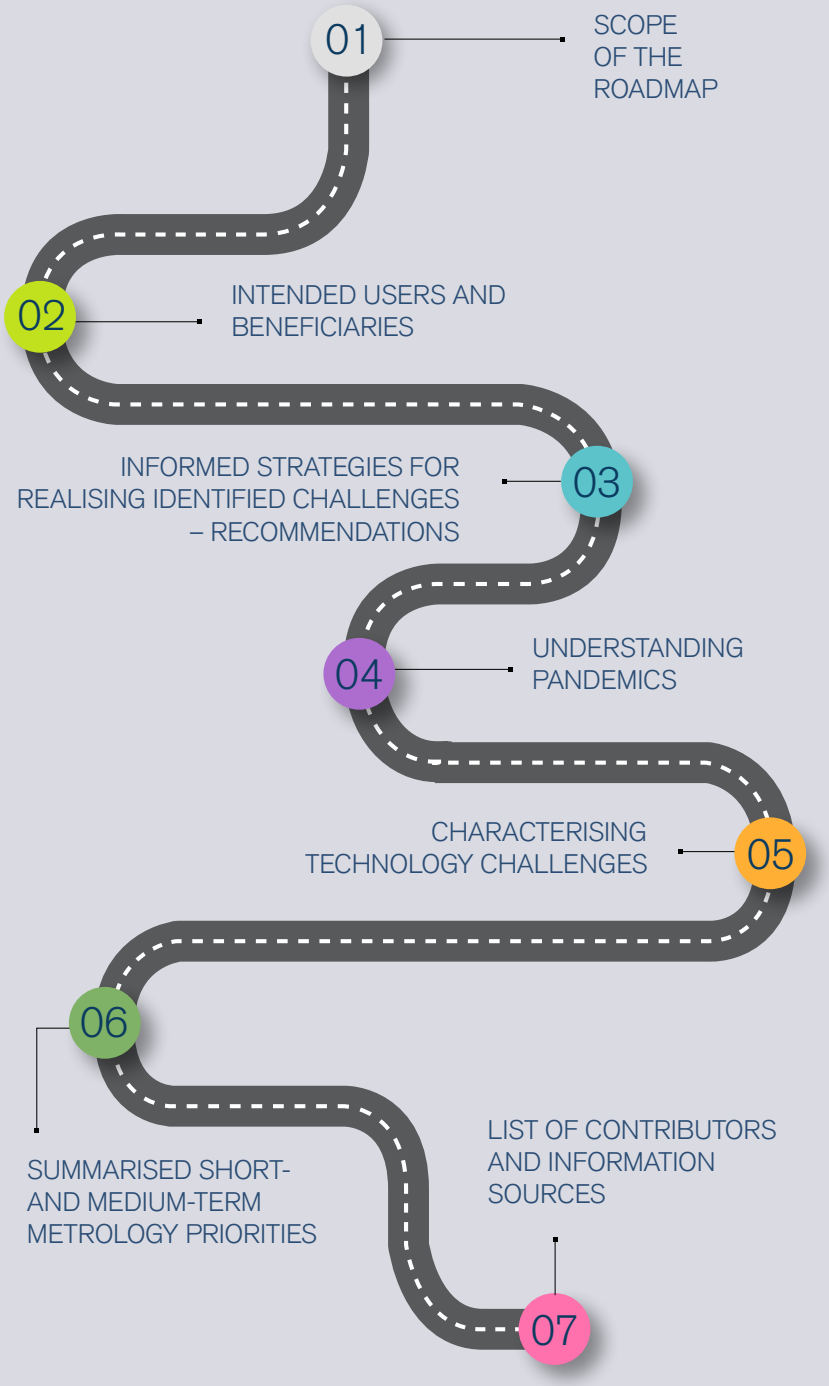
Characterizing technology challenges necessary to improve readiness and drive down response time and improve clinical intervention for the different stages of a future pandemic - identification of materials, technology platforms and infrastructure needs.

Summarized short-term and medium-term metrology priorities.

Informed strategies for realizing identified challenges (based on sound science) that will enable a more rapid metrology response, reduce the impact on everyday life and enhance clinical intervention outcomes for a future pandemic:

- Political
- Collaborative; and
- Technical.

List of contributors and Information sources.



01 SCOPE OF THE ROADMAP

Clinical performance of infectious disease diagnostic testing extends beyond pathogen targets (viral proteins and RNA) and includes:

- clinical characteristics (such as the viral burden of the patient and the time since exposure or onset of symptoms)
- operational testing attributes (such as specimen type, swab technique and transport conditions, as well as the laboratory technique used); and
- analytical test properties (such as sample preparation, signal amplification and data interpretation).

While the race for diagnostic detection of SARS-CoV-2 saw a diverse assortment of technological tests being offered, many of them exhibited varying performance requirements and limitations for clinical utility in specific use settings. The appropriate interactions of relevant expert analytical scientists, clinicians, risk assessors and public health officials were not optimal. The gaps and assumptions in diagnostic testing were poorly explained and not translated into an uncertainty that could be quantified and monitored throughout the pandemic. The creation and re-purposing of existing capacities for deployment against the COVID-19 pandemic were also exposed by the scale, geographies, timeliness, quality, integration of surveillance and diagnostic needs and comparability of associated clinical measurement data, reflecting a need for co-ordinated systems with sufficient robustness and controls to maximize test accuracy.

The G7 countries and global science leaders have since launched and mobilized the 100 Day Mission (100DM), working with the G20 and wider international partners, the World Health Organization (WHO) through their global genomic surveillance strategy, and the life sciences and biotechnology sectors. The intention is to advise the G7 Presidency on how to develop and deploy safe and effective diagnostics, therapeutics and vaccines within the first 100 days of a pandemic.

Early intervention from a strong and organised international metrology framework able to respond at sufficient pace (days to weeks) to the rapidly emerging future pandemic would have been even more beneficial in supporting national readiness for any such crisis. Deployment of ready-made measurement strategies to optimise safety, speed and ease of infectious disease testing (without compromising accuracy) would have provided robust front-line tests and minimum quality requirements able to deliver results, with the right clinical utility at the right time, faster and would have underpinned better public health predictions through provision of detection limits and uncertainty estimations around utilized measurements.

Such a faster and more coordinated international response would likely reduce public health and economic impacts and costs by:

Ascertaining performance of diagnostic tests with respect to identifying which tests are fit for purpose at the different stages of the pandemic and breakthrough infections among vaccinated persons (with or without booster vaccination).

Addressing the spread of disease through faster identification and sharing of the virus, up-scaling testing, improved data generation for contact tracing, trend analysis, clinical performance assessment and integration of diagnostic testing into routine medical care and enhanced epidemiological prediction tools.

Identifying subsequent changes in the virus within populations through faster identification of viral variants.

Accelerating identification, testing and regulatory review of potential treatments and vaccines through better tools for disease analysis, innovative clinical trial approaches and regulatory approval pathways.

02

INTENDED USERS AND BENEFICIARIES

- Metrologists
- Healthcare providers
- Policy makers
- Raw materials suppliers
- Control material (reference standard) manufacturers
- Instrument and kit manufacturers
- Researchers
- Informaticians
- External quality assurance/ Proficiency testing operators
- Accreditation bodies
- Notified bodies.

03

INFORMED STRATEGIES FOR REALISING IDENTIFIED CHALLENGES – RECOMMENDATIONS

A

The Consultative Committee for Amount of Substance – Metrology in Chemistry and Biology (CCQM),

NOTING

- the importance of internationally comparable and accurate measurements of infectious disease diagnostics for human health
- the response of the international metrology community to the COVID-19 pandemic through the CCQM Nucleic Acid Working Group (NAWG) and Protein Analysis Working Group (PAWG) comparison studies to establish, within months, viral (SARS-Cov-2) and protein (IgG antibody) reference measurement procedures and reference materials
- the technical considerations identified during the 2021 series of CCQM Webinars and Workshop on infectious diseases needed to achieve a centralized strategy for adeptly mobilizing and scaling up appropriate rapidly deployable accurate measurement standards, within days to weeks, which are capable of being deployed to differentiated end-user communities for a future infectious disease pandemic.

RECOMMENDS

- the implementation by CCQM NAWG of 'fire drill' comparison exercises, starting in 2022, to demonstrate the international metrology community's capability for a rapidly deployable response of reference measurement methods and dissemination of equivalent calibration materials for infectious disease diagnostics in support of governments, policy-makers and control material manufacturers and suppliers.

ENCOURAGES

- National Measurement Institutes (NMIs) and associated Designated Institutes (DIs) to initiate collaborative work programmes addressing identified short-term and medium-term technical measurement research activities to establish accurate measurements for a panel of agreed infectious disease pathogens with known uncertainties.

B

The Consultative Committee for Amount of Substance – Metrology in Chemistry and Biology (CCQM),

NOTING

- the lack of pathways and rigorous guidelines and standards for evidencing assay performance testing that will enable in vitro diagnostic (IVD) developers to navigate the regulatory approval process more adeptly
- the need for BIPM and the NMIs to develop more intimate working relationships with the infectious disease community and other stakeholders to ensure that international comparability of infectious disease measurements is realized
- the existing memorandum of understanding (MoU) between BIPM and the WHO,

RECOMMENDS

- the establishment of a Task Group (TG) for infectious diseases with metrology working group representatives and other stakeholders, during 2022, to aid:
  - the community design of the 'fire drill' comparison exercises
  - shape the planned technical work programme of the metrology community
  - identify the desirable characteristics/minimum acceptable product specifications (target product profiles, TPPs) for the development/evaluation of safe and effective in vitro diagnostic assays and provision of (transparent) evidence to regulatory decision-making, for well-defined clinical situations across the lifecycle of a pandemic.

C

The Consultative Committee for Amount of Substance – Metrology in Chemistry and Biology (CCQM),

NOTING

- the BIPM and the NMIs need to ensure knowledge transfer and training such that existing national and regional health response interventions are available in the event of any future pandemic,

RECOMMENDS

- the establishment of a Task Group (TG) including representation from Regional Metrology Organisations (RMOs) to:
  - identify and agree development of eLearning modules on metrology for infectious diseases, deployable via the BIPM eLearning platform
  - agree the mechanisms for education of community users, particularly in clinical settings, to adopt clearly defined measurement units, rather than Ct values, for the purposes of laboratory comparisons
  - facilitate an agile metrology workforce able to pivot to pandemic response when required, e.g. small molecule metrologists able to apply skills to metrology of large protein quantification.

04

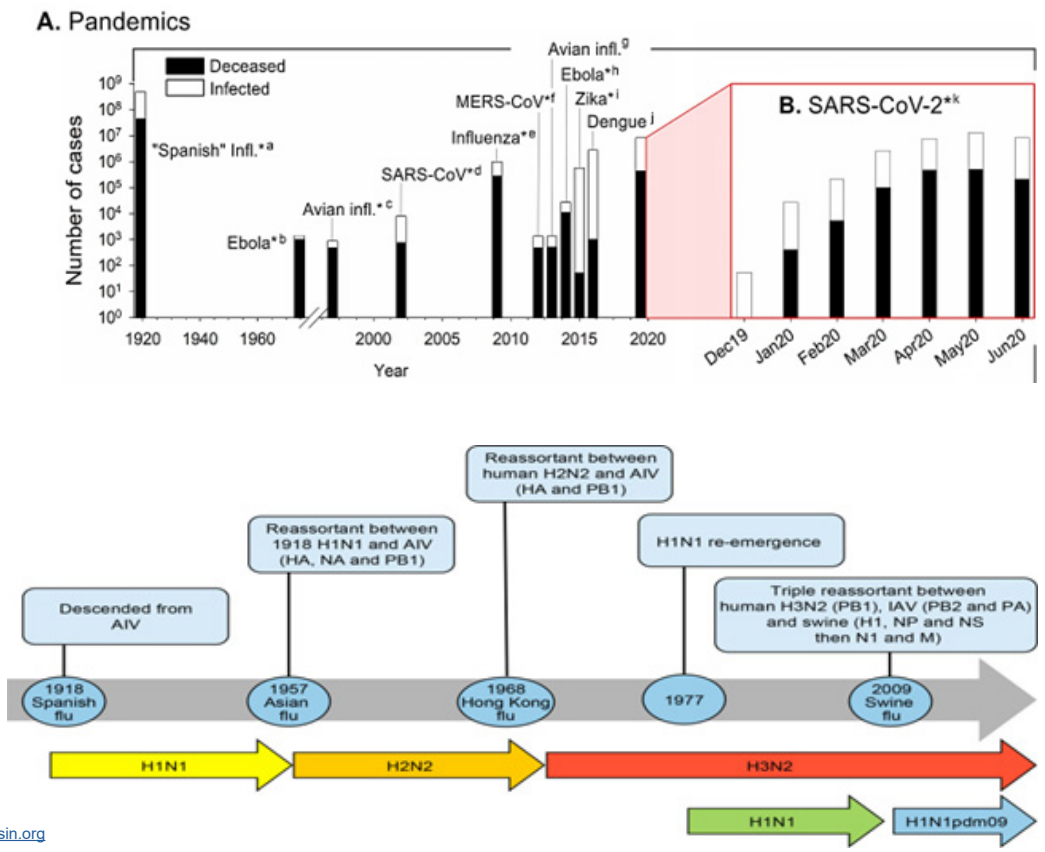
UNDERSTANDING PANDEMICS

– Frequency, lifecycles and associated testing challenges

The frequency of emergence and spread of infectious diseases, such as influenza, severe acute respiratory syndrome coronavirus (SARS-CoV-T), Middle East respiratory syndrome coronavirus (MERS-CoV), Ebola, Zika and Dengue viruses, have been increasing steadily through transmission of zoonotic pathogens to humans as a result of increased contacts with animals and urbanization, land-use and climate change affecting their surrounding environments.

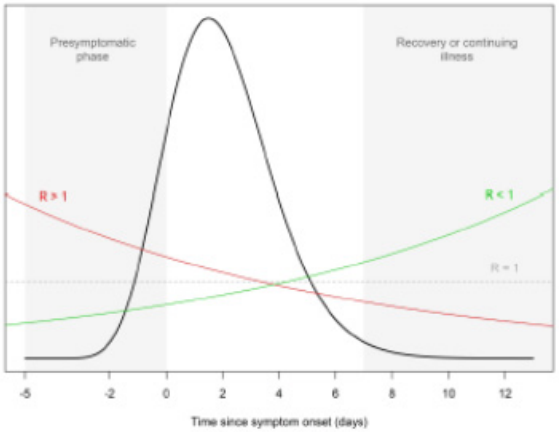
Global surveillance programmes of water-borne pathogens, vector-borne diseases and zoonotic spillovers at the animal-human interface continue to be of prime importance to detect rapidly the emergence of infectious threats. The implementation of public health measures (such as isolation, quarantine and border control) along with technological development and advances in pharmaceutical interventions have helped largely to contain the spread of such infectious diseases – at least until recently. Nonetheless certain classes of infectious diseases, such as flu and others such as malaria, HIV and syphilis, continue to remain endemic in our society and, on occasion, evidence themselves in the form of a pandemic.

Schematics showing timelines of historical pandemics over the last 100 years



frontiersin.org

Three parameters are important in describing the dynamics of a pathogen: the basic reproduction number (R) which determines the rate at which the pathogen is likely to spread in the population, the threshold number of hosts required for the pathogen to become established and the mean levels of infection of the pathogen in the host population. On the latter point, no current technology accurately measures the number/quantity of infectious virus particles. This leaves the need to understand the gaps between what can be measured, copies of RNA/DNA/protein and what is intended to be measured, including units of measurement and their comparison. It is also important to be aware that insufficient emphasis placed on the intended use of the different tests raises the risk of the tests being used inappropriately. Furthermore, diagnostic performance may change relative to the effective R number; the disparities between patient populations across time are an important consideration when evaluating or deploying diagnostic tests.



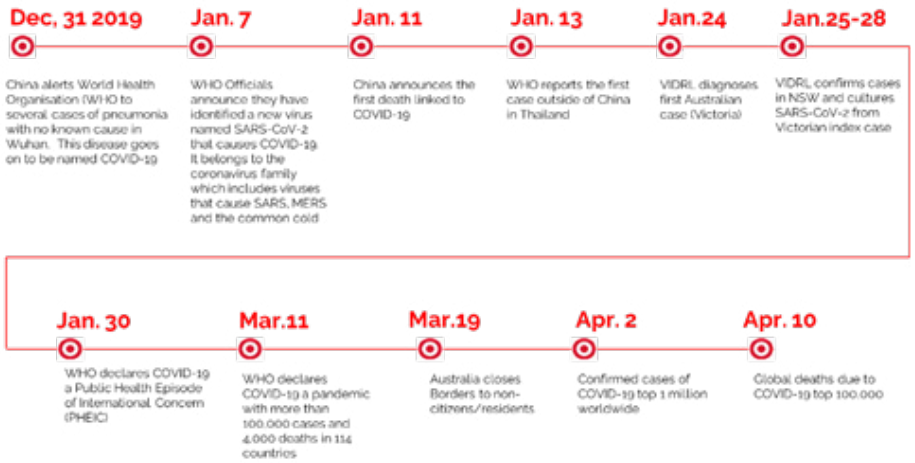
Schematic to illustrate examples of how the proportions of COVID-19 patients are distributed among the different stages of SARS-CoV-2 infection when the effective reproduction number, R >1 (red line) and <1 (green line). The grey dashed line represents an endemic equilibrium state, where R = 1. Also shown is an example of the change in viral burden (black line) with time since symptom onset. When the infection is spreading and R>1, there are more patients of the total who are at an earlier stage in their infection - as high viral burden occurs earlier in the infection there are also more patients with higher viral burden. The reverse is the case when R<1. This will result in a change in diagnostic performance (manifesting as a clinical sensitivity shift) when using quantitative thresholds or analytically less-sensitive methods.

Recently, enveloped RNA viruses that are distributed broadly in animals have been identified in birds and mammals, including humans. These coronaviruses have a genome which is the largest among all RNA viruses. Six coronaviruses have been associated with human disease: SARS-CoV-T, MERS-CoV, human coronavirus 229E (HCoV-229E), human coronavirus OC43 (HCoV-OC43), human coronavirus NL63 (HCoV-NL63), and human coronavirus HKU1 (HCoV-HKU1).

The latest observed coronavirus, SARS-CoV-2, is a beta-coronavirus. The cell surface receptor for this virus is the angiotensin-converting enzyme 2 (ACE2), involved in regulation of cardiac function and blood pressure and expressed in epithelial cells of its primary lung and small intestine targets, as well as in heart, kidney and other tissues. SARS-CoV-2 replication in the upper and lower respiratory tract allows transmission by droplets and aerosols. The median incubation period for the original virus was 5.7 (range 2–14) days, similar to SARS-CoV-T and MERS; albeit the latest predominant variant of the virus has reduced this significantly. Most infections have been uncomplicated, but 5–10% of patients needed hospitalization, mainly due to pneumonia with severe inflammation. Severe respiratory and multi-organ failure complications were observed in a number of cases, their likelihood being based on a number of risk factors, including immunodeficiency.

Hence, while only 3 weeks elapsed from visualization of the SARS-CoV-2 virus to elucidation of its genetic sequence, compared to the 5 months for its predecessor SARS-CoV-T, the emergence, spread and effect of SARS-CoV-2 was felt worldwide, with confirmed cases topping 1 million and deaths 100,000 within 3-4 months of initial reported pneumonia cases (later named COVID-19) in Wuhan.

Schematic of the key initial events in the COVID-19 pandemic



At the time of drafting this Roadmap (9 June 2022), global confirmed COVID-19 cases according to the World Health organization (WHO) have reached ~531.6 million with ~6.3 million attributed deaths.

Viral diagnostic approaches can be divided into two broad categories, clinical diagnostics and in vitro diagnostics. Clinical diagnostics include symptoms, laboratory markers not specific to the virus of concern and imaging technology, all of which may be indicative of the virus, but do not provide definitive evidence. In vitro diagnostics are essential for patient management and outbreak control. Acceptable design, development and establishment of quality in vitro diagnostic detection methods is critical to ensuring control of the spread and impact of the pathogen and remains the preferred confirmatory diagnostic in the early stages of a pandemic.

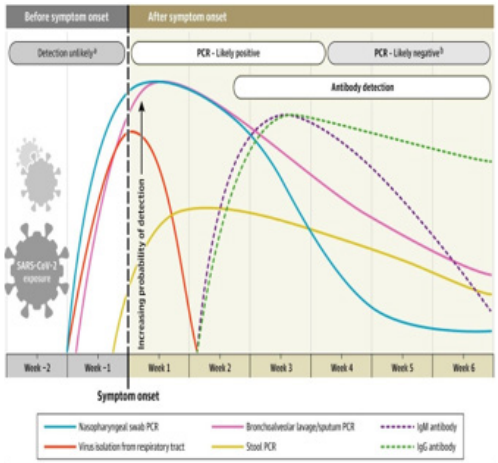
The lessons learned from the SARS-CoV-T outbreak clearly guided development of SARS-CoV-2 detection strategies, with researchers able to adapt established diagnostic technologies rapidly to this pandemic.

Laboratory confirmation testing for pandemics typically use nucleic acid amplification tests (NAATs) (more specifically real-time polymerase chain reaction (RT-PCR) assays), with 'sample-to-answer' platforms (including high-throughput systems and rapid point of care assays) being employed to increase testing capacity. As NAATs are highly sensitive and accurate, they can remain positive for some weeks after infection, whereas viral culture studies (at least for SARS-CoV-2) suggest replication of the virus for only a week or two after symptom onset.

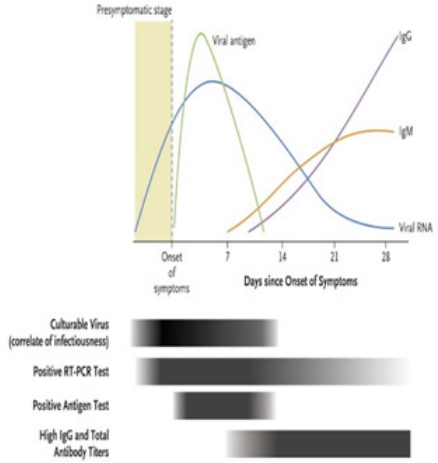
Antigen- and antibody-based assays can complement NAATs for certain resource-limited settings. Antigen-capture methods are routinely used for viral assays, such as HIV and hepatitis B virus, and were employed for SARS-CoV-T and MERS. They detect protein fragments on or within the virus, rather than viral nucleic acids. Antigen-based assays offer rapid identification (15-30 mins) of highly infectious cases (typically 5-12 days after onset of symptoms) and so can provide information about potential transmissibility, enabling targeted isolation and tracking of infectious cases and contacts, and correlation with disease severity and death. Experience from their performance during the H1N1 pandemic suggests sub-optimal sensitivity is not to be unexpected, given the lack of pre-amplification of the target analyte and higher limit of detection of these tests. Their different performance in manufacturers' trials than in the real-world merits further comparative evaluation with a standardized validation protocol and reference material at known concentration.

Serologic antibody testing is particularly useful in the later stages of disease, as PCR positivity decreases. Measurement of specific antibodies can be used to assess prior viral exposure and infer potential immunity to the virus. As a diagnostic tool, antibody serology is particularly useful for patients with delayed clinical presentation. Accurate interpretation of serologic testing depends on both antigen specificity and the antibody isotype detected. In general, IgG is more specific (although there is a wide mixture of IgGs) and may appear later in infection, but specific humoral responses are dominated by IgA antibodies. However, in a pandemic context where early diagnosis is essential for patient management and outbreak control, antibody assays are sub-optimal due to the delays in seroconversion and variability in performance – due to the kinetics and durability of antibody presence, and their unclear correlation with immunity. Antibody specificity and kinetics, particularly in the setting of re-infection, merits further investigation.

Schematic showing timings for onset of COVID-19 symptoms



Schematic showing timings for application of antigen- and antibody-based assays



Preliminary findings suggest that a person's genetic makeup may play some role in determining their risk of getting, or having severe outcomes, from COVID-19. However, any direct correlation has yet to be validated.

Genomic sequencing techniques are needed for the identification and monitoring of variants of concern (VOC) and variants of interest (VUI) for SARS-CoV-2. However, while recognizing its specialized contribution, genomic surveillance comes at a high cost technically, especially in resource-limited settings. Nonetheless, there is the opportunity to embed genomic sequencing capacity at the national level and to integrate it with other disease surveillance systems for future broader zoonotic disease surveillance, evaluation of changes in the viral genome over time and for tracing patterns of transmission.

Despite current approaches for the ever-expanding array of available tests, the SARS-CoV-2 pandemic has highlighted the differences between theoretical sensitivity/specificity values for many of the available diagnostic tests and diagnostic sensitivity/specificity values in given patient populations; the majority requiring further validation around these values, speed, ease of use and, importantly, their deployability.

05

CHARACTERIZING TECHNOLOGY CHALLENGES

Virus and viral variants

Virus

Robust viable culture of 'wild-type' or variant pathogen material is reliant on correct storage of the original samples, re-sampling or early detection and prompt collection of retained material, particularly in high and low prevalence scenarios. Such material cannot be shared readily, in a timely manner, across borders, their successful culture is not guaranteed and yet their management is a priority for clinical and epidemiological control of the pathogen. Future resilience through established and maintained distribution networks to ensure widespread dissemination of relevant material is essential. Resultingly, where inactivation procedures are necessary or contrived samples employed, assurance of comparability with 'live' material is essential. With frequent horizon scanning, it should be possible to culture and produced variant materials in advance of those variants becoming widespread, although successful culture is not guaranteed.

Sequencing protocols based on Sanger and next-generation sequencing (NGS) (e.g. Illumina and MinION/Nanopore) are increasingly being applied to generate genome sequences rapidly, with the promise that data will inform diagnostic development, epidemiologic investigations, host-virus interactions, viral evolution (mutation with associated transmissibility and/or virulence effect), pathogenesis, and prevention and treatment targets. NGS can also be used to evaluate the host microbiome and co-infection with certain pathogens, but its cost to date has limited its use to research purposes rather than clinical management.

Complimentary to sequencing, genotyping also offers rapid and cost-effective data.

Standard material requirements:

- Rapid access to 'wild type' and variant materials for genotyping VoC performance monitoring and sequencing, and external quality assurance and accreditation purposes
- Clinical access to circulating strains is dependent on prevalence, public co-operation and testing strategies
- Availability of agreed reference strains (including sequences) for control and validation and verification materials, and in silico analysis
- Availability of materials/methods appropriate for viral genetic diversity (fast production)
- Reference measurement procedures for quantity and identity
- Current variation in laboratory use of a range of construct contrived or surrogate commercial synthetic controls for validation, verification and assessment of molecular and antigen assays (samples range from pure proteins to synthetic RNA constructs) prevents accurate comparison of products on the market
- Need for samples that reflect the heterogeneity of clinical samples in the format that has been validated by manufacturers for regulatory purposes.

Identified challenges:

- Rapid access to standardised, characterised and quantified cultured material in formats that are suitable for the end-user
- Some tests are manufactured and sold solely nationally so that, internationally, VoC/VUI performance is unknown
- Internationally standardised methods to validate, verify and assess assays for true comparability; comparable materials in a range of units suitable for multiple technologies laboratory and extracted molecular assays, antigen assays copies/ml, PFU/ML
- Remove reliance on cycle threshold [Ct] values for indicative viral loads, performance comparison and selection of samples for genotyping and sequencing, rather than true limit of detection
- Standardised regulatory expectations for, and granting of market access to, commercial assays.

Molecular and antigen technologies

Nucleic acid amplification methods

Unlike DNA, RNA is highly susceptible to degradation - sample storage, handling and RNA isolation must follow optimized protocols to minimize degradation at each step. Targets should be highly conserved and abundantly expressed genes of the virus. Use of multiple PCR targets helps to avoid false negatives associated with mutations in the primer site region, but they require regular review and possible updating as is the case with influenza.

Furthermore, viral RNA detection by combining reverse transcription (RT) (to generate a complementary DNA copy of the viral RNA sequence) with qPCR (RT-qPCR) does not necessarily demonstrate the presence of infectious virus, as patients who have recovered from infection can be more persistently PCR-positive.

RT-qPCR enables (typically) a fluorescent detection of labelled target nucleic acid sequence. The output is the quantification cycle (Cq) as termed by the Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MIQE) guidelines, but also referred to as Ct or crossing point (Cp). Quantification is performed following a user-applied calibration step involving a reference calibrator of known concentration for the RT-qPCR – the quantitative relationship between Cq value and calibrator copies being established based on the gradients of each standard setting the standard curve. Reported associations between Cq values and ability to correlate SARS-CoV-2 virus and patient outcome, along with other factors such as symptoms, have led to Cq values being used by physicians to guide decisions pertaining to individual patients. However, Cq is an arbitrary unit, not transferable without access to the same reagents, protocols and calibrators; there is reported variability in Cq between the same devices, variability in Cq between different devices, an increasing view from manufacturers that the 'user' calls the Cq, some devices do not report Cq and there is observed irregularity in calling 'positive' between test sites (based on one or two targets being reported 'positive'). Thus, while the concept of using Cq as a threshold for risk based on viral burden is logical such variation, combined additionally with low viral copy number at stages in the disease progression and undetermined potential errors surrounding sampling collection, may engender differences in absolute Cq values (Cq is log base 2 and so small changes give large concentration changes).

Consequently, it would seem using Cq thresholds to quantify SARS-CoV-2 burden, stratify risk and aid patient management would be challenging, while also problematic if used to discuss broader analytical performance or as a measure to guide the development of IVDs.

Interestingly, Cq has not typically been applied in such a way when managing other viral infections, despite clinical virology arguably representing the medical field that most broadly applies accurate molecular quantification for managing patients based on nucleic acid quantity.

Reverse transcription digital PCR (RT-dPCR) uses the same reagents as RT-qPCR, but applies limiting dilution, end-point PCR and Poisson statistics to give a direct estimate of concentration. Consequently, such quantification overcomes normalization and calibrator issues, being less affected by bias resulting from repeatability and reproducibility errors. This technology has been used for analysis of absolute DNA copy number (and RNA copy number with knowledge of the efficiency of the reverse transcription step) from clinical samples and has been demonstrated to provide improved assay reproducibility when compared to quantitative RT-PCR for detection and quantification of SARS-CoV-2 RNA from purified RNA and crude lysate samples. Further guidance on the use of digital PCR can be found in ISO 20395.

Calibrated copy-based units (used elsewhere in virology) clearly offer a more reproducible alternative to Cq values.

Isothermal amplification methods (isoPCR) offer an alternative strategy for detecting virus-infected patients, as they do not require use of expensive thermocycling equipment.

While PCR-based approaches are most widely used for confirmatory diagnosis, loop-mediated isothermal amplification (LAMP) combined with reverse transcription (RT-LAMP) needs only heating and visual monitoring and has a sample-to-result time of around 1 h, making it suitable for low-cost field deployment.

However, RT-LAMP is challenged by low specificity due to presence of multiple pair primers that may increase non-specific byproduct formation and, where viral RNA is low, inefficient amplification of the target sequence leading to false-negative results. Sensitivity and specificity of such assays needs to be evaluated against a range of viral loads for validation and optimization. They are therefore increasingly being applied in combination with other novel detection strategies, such as Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)- or DNA sequencing-based detection platforms to boost accuracy and performance. Nonetheless, such emerging methods require careful validation and field testing.

Antigen methods

Antigen testing is a serologic assay that is attractive as a potential point of care diagnostic. Antigen-based diagnostics detect protein fragments on or within the virus (rather than viral nucleic acids) within 15mins (compared to hours by RT-PCR). Viral proteins detected by antigen-capture methods (such as antibodies or aptamers) are used routinely for other viral assays, such as human immunodeficiency virus (HIV) and hepatitis B virus. When their performance is acceptable, rapid antigen tests can reduce transmission through early detection of highly infectious cases, enabling implementation of targeted isolation and tracking of infectious cases and contacts.

However, for SARS-Co-V-2, swab samples showed excellent specificity but varying overall sensitivity, with higher viral loads associated with better sensitivity. This is analogous to the performance of the influenza antigen test in the H1N1 pandemic, where specificity was excellent but sensitivity low.

Further studies are needed to determine the performance of these assays and their clinical utility, especially with regards to emerging variants.

Identified challenges for deployment:

- Fast production (days to weeks) of a standard control material: does not need to be perfect, but what are acceptable limitations?
- Synthetic target panels and other routes for quality assurance (including failure)
- Quality assurance for pre-examination stages (e.g. swabs, different specimens etc.): comparison studies
- Panels for specificity
- Materials for internal controls
- Routes for improved guidance: standardised methods, minimum validation criteria etc.
- Materials to support mass testing (e.g. point of care, high throughput etc.)
- Routes to allow in vitro diagnostic developers/physical standard providers/regulators to interact.

Identified challenges for measurement research:

- Improved accuracy of viral load measurement methods, with clearly defined units and an idea of how they compare (education)
- Date uncertainty needs to be accounted for, particularly to inform more robust evidence-based policy-making
- Routes to understand the differences between detecting viral presence (RNA vs protein) and infectivity (replicative intermediates, viral/disease progression)
- Impact of VOC and VUI on testing
- Commutability of reference/standard materials
- Routes to simplify test assurance (without skilled workers)
- AI companion apps
- Routes to ensure safety.

Antibody detection and response technologies

Serologic measurement of specific antibodies can be used to assess prior exposure to virus and to infer potential immunity. Antibody serology as a diagnostic tool is particularly useful for patients with delayed clinical presentation. Serological data is particularly useful for epidemiologic purposes and to evaluate the impact of control measures (lockdowns, broad testing, and other policies). Antibody evaluation can also facilitate identification of plasma donors and assessment of vaccine immunogenicity, especially in elderly or otherwise immunocompromised people.

Cross-reactivity between antibodies to the virus and other endemic human viruses may enable design of pan-viral therapeutics or vaccines.

Serological surveillance may identify potential zoonotic disease transmission from wildlife reservoirs, such as bat-borne coronavirus and influenza virus (e.g. H1N1).

For coronaviruses, the spike (S) and nucleocapsid (N) proteins are the primary viral antigens used for antibody assays. The N gene is reportedly more conserved and stable than S, and available S-based assays measure total binding antibodies rather than only neutralizing antibodies. Neutralising antibodies are measured conventionally by the plaque reduction neutralization test (PRNT), requiring specialized containment facilities and 2–4d to complete. Whilst a pseudovirus neutralization test (pVNT) offers a more practicable alternative, utilizing genetically-modified pseudovirus that mimics the real virus requires comparative study with the PRNT assay and the optimal class of pseudovirus is needed to ensure suitable validation of the pVNT assay.

Several point mutations have demonstrated the ability of pathogens to escape neutralization by convalescent sera and monoclonal antibodies. Hence further studies are needed to characterize antibody dynamics and determine specific antigen suitability for monitoring and surveillance purposes.

Therefore, in a pandemic context where early diagnosis is essential for patient management and outbreak control, antibody assays are sub-optimal due to delayed seroconversion and performance variability.

Identified challenges:

- Sourcing good quality material of the target proteins (spike and nucleocapsid protein) to produce high quality antibodies
- Determining which systems are most suitable to produce different antibodies? - for the N-protein bacterial cells are suitable; for the highly glycosylated S-protein, human cells are required
- Ensuring calibration of enzyme-linked immunosorbent assays (ELISA) for serology
- Determining what kind of samples can be measured? - dried blood spots (convenient for screening) or plasma samples
- Development and harmonization of quantitative serological assays for COVID-19 antibodies (using conversion factors for assay unitage) pivotal in evaluating immunization status
- Determining the differing behaviours of neutralizing assays and binding assays as they may have different targets
- Identifying a commutable physical standard for all assays that target the same measurand: use of a standard has been shown to improve the comparability of neutralization assays for SARS-CoV-2 but not of binding assays.
- Standardisation of high-quality target proteins, antibodies or whole virus material, which may be too time consuming in the midst of a pandemic but must be available to ensure constant quality of measurements during any pandemic
- Determining, when antibodies are measured in human plasma, what the result really means in terms of immune response to the virus?

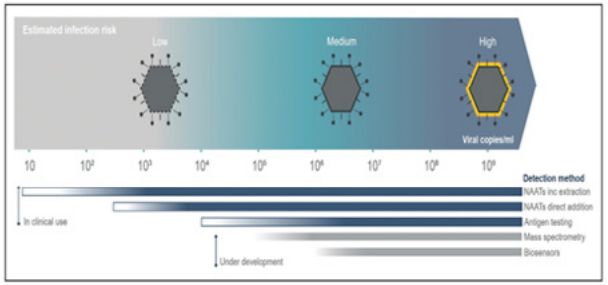
Emerging prognostic technologies

Biomarkers play a pivotal role in the early detection of disease etiology, diagnosis, treatment and prognosis. Clinical biomarkers are the measurable biological indicators of the presence, severity or type of disease in medical settings. Prognostic biomarkers are a clinical or biological characteristic that provides information on the likely patient health outcome, irrespective of treatment, and frequently serve as useful predictors for development of severe disease.

The use of proteomics for the study of various aspects of infectious diseases worldwide is relatively new, although this approach identified and described proteins and antigenic proteins of the SARS-CoV-T and the ACE2 protein as the main receptor for SARS-CoV-2 in humans. Recently, the technique has been used in the study of COVID-19 disease through profiling of virus-infected human cells, comparative profiling of infected cells versus normal cells and clinical proteomics of COVID-19-positive patients. The approach has become readily automatable, sensitive enough to detect infectious patients (on similar amounts of sample compared to RT-qPCR) and can easily be multiplexed to detect several viruses in a single run. The potential targeting of multiple viruses and blood biomarkers simultaneously makes it a promising early warning technique.

Sensing technologies too are changing the landscape of infectious disease detection. Immuno-biosensing is based on the microfluidic flow of biological fluid with detection of any antigen-antibody interaction by way of a change in capacitance. Other types of biosensors use carbon nanotubes to enhance the signal and sensitivity levels of biomarker disease detection. Electrical immuno-sensing helps with sensitivity issues. Many such assays provide only a qualitative method of biomarker detection through an observable colour change.

One of the key problems (medically and in terms of long-term economic burden) is understanding the immune-metabolic underpinnings of Long-COVID and creating clinically actionable strategies for its mitigation. Common laboratory findings amongst COVID-19 patients have included leukopenia, lymphopenia and elevated levels of aminotransaminase, lactate dehydrogenase (LDH) and inflammatory markers (e.g. ferritin, C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR)). High D-dimer levels, severe lymphopenia, increased neutrophil to lymphocyte ratio, marked thrombocytopenia, hypoalbuminemia, elevated interleukin IL-6, procalcitonin, cardiac troponin I, and serum amyloid A have been associated with critical illness or mortality in COVID-19. Nonetheless, just three biomarkers have showed sensitivity and specificity values of >50%: a decrease in the lymphocyte count and increases in the inflammatory markers CRP and IL-6.



Schematic comparing viral concentration detection levels for COVID-19 assays

While future test developments may provide more quantitative measures of viral concentration at a given time and address such measures in patients with low viral loads, the same non-specific biomarkers may also be elevated in other infectious diseases such as dengue fever, typhoid fever or influenza.

Hence, their use remains challenging and no individual biomarker can yet be used reliably to rule an infectious disease in or out. Nonetheless, there are emerging indications that mass spectrometry and nuclear magnetic resonance (nmr) can evaluate quantifiable immuno-metabolic signatures associated with progression of severity of the disease or even risk factors for death. Correlation between laboratory findings, disease severity, co-morbidities and complications therefore needs to continue to be investigated.

Identified issues:

- More academic attention to driving technology development with little consideration to what question is being asked and what is fit for purpose for subsequent translation, e.g. diagnosis, rapid screening, patient stratification, prognosis, surveillance, deployment etc
- Lack of definition of the measurand and measurement units (number virus particles, number of infectious virus particles, copies of RNA, Ct, PFU) and comparison between different units.

Metrology challenges:

- Early engagement of the metrology community in the production of matrix reference materials and method validation during transfer of developing methods from academic settings into routine clinical laboratories
- Internal quality controls for clinical trial type biomarker discovery studies seem to be functioning well, even in multi-platform centres: focus should lie with standardization of the relatively small number of emerging biomarker targets, assuming their long-term use is predicted (e.g. lipoproteins, lipids, peptides, volatiles etc)
- Support increasing standardisation and harmonisation of practices for routine use (especially for breath analysis).

06

SUMMARIZED SHORT- AND MEDIUM-TERM METROLOGY PRIORITIES

Short-term priorities:

- Establish a legacy reference measurement infrastructure for prioritized infectious disease diagnostics, through clear definition of the measurand, (SI) units of measurement (and their comparison with current practice), limits of detection, sensitivity and specificity, and estimation of measurement uncertainty, with associated common data collection and comparable reporting standards across technology platforms, geographies or demographic groups (further information on metrological traceability can be found in ISO 17511:2020)
- Establish reference method procedures and (commutable) matrix control panels (RNA and protein) against common viruses to facilitate smooth workflows through the lifecycle of a pandemic, and their correlation with infectivity levels
- Establish characteristics to guide development and evaluation of diagnostic testing approaches with high analytical sensitivities, and investigate clinical sensitivity shifts on diagnostic performance benchmarks
- Support continual review of the impact of VoC and VUI on applicability of diagnostic tests.

Medium-term priorities:

- Establish measurement variation with different specimen types and routes to control error from pre-analytical stages of testing
- Develop statistical models using clinical reference data sets to provide alternative routes for diagnostic evaluation
- Prioritise emerging biomarker targets for reference/standard material production, where long-term use can be predicted
- Ensure workflows established to enable rapid transfer of emerging technologies from research to routine clinical laboratories
- Identify routes to simplify assurance of infectious disease diagnostic tests (without skilled workers).

07

LIST OF CONTRIBUTORS AND INFORMATION SOURCES

Contributors to production of the roadmap

Steering Committee Members

Prof. Julian Braybrook - Chair, Steering Committee & Director, National Measurement Laboratory at LGC, UK
Prof. Jim Huggett (Chair, CCQM NAWG)
Dr Jeremy Melanson (Chair, CCQM PAWG)
Dr Jonathan Campbell (Chair, CCQM CAWG)
Dr Daniel Burke (NMIA)
Dr Bernd Guettler & Prof. Gavin O'Connor (PTB)
Dr Liana Dong & Hongmei Li (NIMC)
Dr Young Bae (KRISS)
Dr Michael Tarlov, Katrice Lippa & Dr Carlos Gonzalez (NIST)
Dr Elena Kulyabina (VNIIMS)
Dr Ralph Paroli (NRCC)
Dr Robert Wielgosz & Dr Ralf Josephs(BIPM).

Additional contributions from:

Webinar and workshop participants and broader consultation process.

CCQM Webinars

25 MARCH 2021

CCQM Webinar (3): Ensuring reliability of measurements in response to the Covid-19 Pandemic

Dr Naomi Park
Setting up high throughput SARS-CoV-2 surveillance sequencing at the Sanger Institute

Dr Thierry Rose
Developing novel high-throughput tests for SARS-CoV-2 antigens and standardization challenges

Prof. Jo Vandesompele
Addressing the challenges of high-throughput SARS-CoV-2 RT-qPCR testing.

10 DECEMBER 2020

CCQM Webinar (2): Ensuring reliability of measurements in response to the COVID-19 pandemic

Prof. Maria Zambon
Molecular testing for SARS-CoV-2; standardization, challenges and needs

Dr Corine Geurts van Kessel
Validation and implementation of rapid antigen testing for detecting SARS-CoV-2 infection

Dr Hans Vissers
Experiences from MS-based projects for SARS-CoV-2 detection.

7 JULY 2020

CCQM Webinar (1): Ensuring reliability of measurements in response to the COVID-19 pandemic

Prof. Jacob Moran-Gilad
COVID-19 Diagnostics: from measurement to policy

Prof. Dr Heinz Zeichhardt
Molecular Diagnostics: International proficiency testing as basis for standardizing genome detection of SARS-CoV-2

Prof. Michael Neumaier
SARS-CoV-2 Antibody testing and the challenges in standardization of measurements: What we can learn from interlaboratory studies and what are the implications?

Dr Michael A. Drebot
The development and assessment of Covid-19 serological platforms for serosurveillance and potential immunity correlation

Guest Panel Member: Prof. Ian Young (JCTLM/IFCC).

Organizing Committee for webinar series: J. Huggett (LGC), J. Melanson (NRC), B. Guettler & G. O'Connor (PTB), J. Campbell (LGC), R. Wielgosz (BIPM), S-R. Park (CIPM).

Additional contributions from Webinar participants.

CCQM Workshop

5-7 OCTOBER 2021

18 senior policy or metrology officials from UK, Europe, US, Canada, Asia and Australasia led the workshop presentations and discussion on the technological issues and lessons learned from the COVID-19 pandemic.

160 workshop participants from 21 countries.

General observations:

- Availability of automated RT-PCR for high-throughput platforms remains critically limited, especially for low- and middle-income countries
- Performance against blinded panel of spiked clinical materials, given that implementation of multiple platforms to increase processing capacity resulted in inadvertent inefficiencies with multi-tasking between platforms and increased turnaround times (TAT)
- Optimization of analytical sensitivity across specimen types remains one of the greatest challenges; interest in alternatives may be at the expense of diagnostic accuracy, as comprehensive comparative performance data is currently unavailable
- Correlations between specimen types in which SARS-CoV-2 is detected and organ system manifestations is required
- Different institutions rely on varying numbers of SARS-CoV-2 gene targets and different target regions
- False-negative results might be addressed by adjusting the timing of specimen collection and repeat testing
- Specimen pooling has been used as a large-scale testing strategy, but pooling is only most efficient when infection incidence is low.

Take-home messages:

- Best is the enemy of (very) good! Emergency response requires 'off the shelf' response. QA/QC support is lagging behind operational response for an emergency situation. Can the community anticipate? What arrangements need to be in place? Invest in optimisation of laboratory methods in "peace" times. Create generic approaches for ensuring accurate measurement in future scenarios. Define scenarios, benchmarks, assay performance against stored pre-pandemic materials (specificity), dilution series of different control materials (probit analysis)
- Devise a global common terminology language, especially around measurement units
- Identify level of infectiousness.

Gaps and solutions to be considered for identified QA needs:

Organizing Committee for workshop:

J. Braybrook, J. Huggett & J. Campbell (LGC), J. Melanson (NRC), D. Burke (NMIA), B. Guettler & G. O'Connor (PTB), L. Dong and Hongmei Li (NIMC), Y. Bae (KRISS), M. Tarlov, K. Lippa & C. Gonzalez (NIST), E. Kulyabina (VNIIMS), R. Paroli (NRCC), R. Wielgosz & R. Josephs (BIPM).

Gaps and solutions discussed during the CCQM Workshop and considered as identified QA needs:

QA NEEDS	GAPS	FUTURE PLANNING
Calibrated virus materials Synthetic RNA covering all targets	Pace & agility to meet massive expansion Distribution network	Rapid production of synthetic RNA
Diagnostic samples for evaluation work	Diagnostic samples for evaluation work Commutable materials Regulatory recognition	Preparation of simulated clinical samples
Sample types & sites	Inventories Performance characteristics	Comparison studies Pilot surveillance
EQAs	Rapid distribution Low copy number controls SMART analysis/middleware programmes for LIMS	Open-source software development
Proficiency panels	Rapid feedback	Panel preparation
Material suitable for mass testing outside laboratories	Easily distributed materials	Commercial providers linked to standards
Materials which account for viral diversity	Rapid provision of international standards	Rapid scale-up

References

Stegeman I, Ochodo EA, Guleid F, Holtman GA, Yang B, Davenport C, Deeks JJ, Dinnes J, Dittrich S, Emperador D, Hooft L, Spijker R, Takwoingi Y, Van den Bruel A, Wang J, Langendam M, Verbakel JY, Leeflang MM. Cochrane COVID-19 Diagnostic Test Accuracy Group. Routine laboratory testing to determine if a patient has COVID-19. Cochrane Database Syst Rev. 2020 Nov 19;11(11): CD013787. doi: 10.1002/14651858.CD013787. PMID: 33211319; PMCID: PMC8078159

Dinnes J, Deeks JJ, Berhane S, Taylor M, Adriano A, Davenport C, Dittrich S, Emperador D, Takwoingi Y, Cunningham J, Beese S, Domen J, Dretzke J, Ferrante di Ruffano L, Harris IM, Price MJ, Taylor-Phillips S, Hooft L, Leeflang MM, McInnes MD, Spijker R, Van den Bruel A. Cochrane COVID-19 Diagnostic Test Accuracy Group. Rapid, point-of-care antigen and molecular-based tests for diagnosis of SARS-CoV-2 infection. Cochrane Database Syst Rev. 2021 Mar 24;3(3):CD013705. doi: 10.1002/14651858.CD013705.pub2. PMID: 33760236; PMCID: PMC8078597

Scheiblauer Heinrich, Filomena Angela, Nitsche Andreas, Puyskens Andreas, Corman Victor M, Drosten Christian, Zwirglmaier Karin, Lange Constanze, Emmerich Petra, Muller Michael, Knauer Olivia, Nubling C. Micha. Comparative sensitivity evaluation of 122 CE-marked raid diagnostic tests for SARS-CoV-2 antigen, Germany, September 2020 to April 2021. Euro Surveill. 2021;26(44):pii=2100441. https://doi.org/10.2807/1560-7919. ES.2021.26.44.2100441

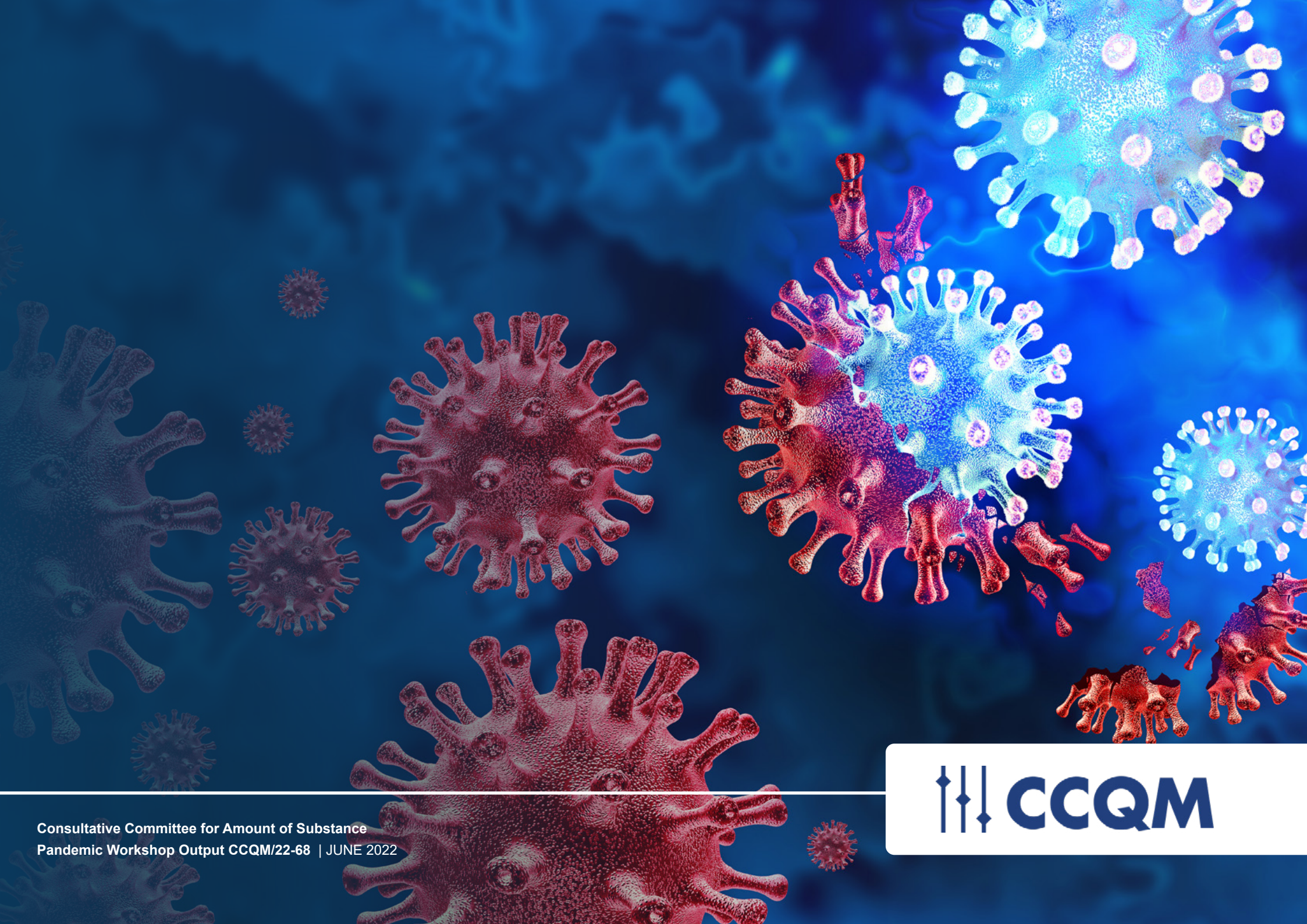
Puyskens Andreas, Krause Eva, Michel Janine, Nubling C. Micha, Scheiblauer Heinrich, Bourquain Daniel, Grossegesse Marica, Valusenko Roman, Corman Victor M, Drosten Christian, Zwirglmaier Karin, Wolfel Roman, Lange Constanze, Kramer Jan, Friesen Johannes, Ignatius Ralf, Muller Michael, Schmidt-Chanasit Jonas, Emmerich Petra, Schaade Lars, Nitsche Andreas. Establishment of a specimen panel for the decentralized technical evaluation of the sensitivity of 31 rapid diagnostic tests for SARS-CoV-2 antigen, Germany, September 2020 to April 2021. Euro Surveill. 2021;26(44):pii=2100442.https://doi.org/10.2807/1560-7919. ES.2021.26.44.2100442

Subali AD, Wiyono L. Reverse Transcriptase Loop Mediated Isothermal Amplification (RT-LAMP) for COVID-19 diagnosis: a systematic review and meta-analysis. Pathog Glob Health. 2021 Jul;115(5):281-291. doi: 10.1080/20477724.2021.1933335. Epub 2021 Jun 4. PMID: 34086539; PMCID: PMC8182821

Macedo ACL, Prestes GDS, Colonetti T, Candido ACR, Uggioni MLR, Gomes AC, Silva FR, Ceretta LB, Grande AJ, da Rosa MI. A systematic review and meta-analysis of the accuracy of SARS-COV-2 IGM and IGG tests in individuals with COVID-19. J Clin Virol. 2022 Mar;148:105121. doi: 10.1016/j.jcv.2022.105121. Epub 2022 Feb 23. PMID: 35245882; PMCID: PMC8863416

Ghasemi S, Harmooshi NN, Rahim F. Diagnostic utility of antigen detection rapid diagnostic tests for Covid-19: a systematic review and meta-analysis. Diagn Pathol. 2022;17(1):36. Published 2022 Apr 13. doi:10.1186/s13000-022-01215-6

WHO. Global genomic surveillance strategy for pathogens with pandemic and epidemic potential 2022-2032. ISBN: 9789240046979 (electronic version) 9789240046986 (print version). https://www.who.int/publications/i/item/9789240046979



CCQM

Consultative Committee for Amount of Substance
Pandemic Workshop Output CCQM/22-68 | JUNE 2022