Clinical Microbiology and Infection 30 (2024) 582–585

Contents lists available at ScienceDirect

Clinical Microbiology and Infection

journal homepage: www.clinicalmicrobiologyandinfection.com

Narrative Review

Viral epidemic preparedness: a perspective from five clinical microbiology laboratories in Europe

Miguel Julián Martínez [1, 2], Matthew Cotten [3, 4], My V.T. Phan [3], Karsten Becker [5], Mateu Espasa [6], Truls M. Leegaard [7, 8], Gorm Lisby [9], Uffe Vest Schneider [10], Climent Casals-Pascual [1, 2, *]

[1] Department of Clinical Microbiology, CDB, Hospital Clínic of Barcelona, University of Barcelona, Barcelona, Spain
[2] Institute for Global Health (ISGlobal), Barcelona, Spain
[3] London School of Hygiene and Tropical Medicine, London, UK
[4] MRC-University of Glasgow Centre for Virus Research, Glasgow, UK
[5] Friedrich Loeffler-Institute of Medical Microbiology, University Medicine Greifswald, Greifswald, Germany
[6] Department of Clinical Microbiology, UDIAT, Hospital Universitari Parc Taulí, Universitat Autònoma de Barcelona, Barcelona, Spain
[7] Department of Microbiology and Infection Control, Akershus University Hospital, Lørenskog, Norway
[8] Division of Medicine and Laboratory Sciences, University of Oslo, Oslo, Norway
[9] Department of Clinical Microbiology, University of Copenhagen Hvidovre Hospital, Hvidovre, Denmark
[10] Department of Virus and Microbiological Special Diagnostics, Statens Serum Institut, Copenhagen, Denmark

ARTICLE INFO

Article history:
Received 1 January 2023
Received in revised form
16 April 2023
Accepted 22 April 2023
Available online 28 April 2023

Editor: L. Leibovici

Keywords:
COVID
Microbiology
Pandemic
Preparedness
SARS-CoV-2

ABSTRACT

Background: Pandemic preparedness is critical to respond effectively to existing and emerging/new viral pathogens. Important lessons have been learned during the last pandemic at various levels. This revision discusses some of the major challenges and potential ways to address them in the likely event of future pandemics.

Objectives: To identify critical points of readiness that may help us accelerate the response to future pandemics from a clinical microbiology laboratory perspective with a focus on viral diagnostics and genomic sequencing. The potential areas of improvement identified are discussed from the sample collection to information reporting.

Sources: Microbiologists and researchers from five countries reflect on challenges encountered during the COVID-19 pandemic, review published literature on prior and current pandemics, and suggest potential solutions in preparation for future outbreaks.

Content: Major challenges identified in the pre-analytic and post-analytic phases from sample collection to result reporting are discussed. From the perspective of clinical microbiology laboratories, the preparedness for a new pandemic should focus on zoonotic viruses. Laboratory readiness for scalability is critical and should include elements related to material procurement, training personnel, specific funding programmes, and regulatory issues to rapidly implement “in-house” tests. Laboratories across various countries should establish (or re-use) operational networks to communicate to respond effectively, ensuring the presence of agile circuits with full traceability of samples.

Implications: Laboratory preparedness is paramount to respond effectively to emerging and re-emerging viral infections and to limit the clinical and societal impact of new potential pandemics. Agile and fully traceable methods for sample collection to report are the cornerstone of a successful response. Expert group communication and early involvement of information technology personnel are critical for preparedness. A specific budget for pandemic preparedness should be ring-fenced and added to the national health budgets. **Miguel Julián Martínez, Clin Microbiol Infect 2024;30:582**

* Corresponding author. Climent Casals-Pascual, MD, PhD, Department of Clinical Microbiology, CDB, Hospital Clínic of Barcelona, University of Barcelona, Barcelona 08036 Spain.
E-mail address: ccasals@clinic.cat (C. Casals-Pascual).

https://doi.org/10.1016/j.cmi.2023.04.024

Introduction

In 1972, Frank MacFarlane Burnet, the eminent Australian immunologist, Nobel laureate, and virologist wrote: "On the basis of what has happened in the last thirty years, *the most likely forecast about the future of infectious disease is that it will be very dull*" (MacFarlane Burnet & White, 1972, p. 263). At risk of being unfair to a statement taken out of the historical context, it is fair to say that the statement was rapidly disproved by the HIV pandemic, and more recently in the context of SARS-CoV-2. The emergence and re-emergence of viral pathogens in the second half of the 20th century indicate that viral epidemics and pandemics will continue. We have seen the SARS epidemic in 2003, likely transmitted from bats via civet cats [1], the 2009 influenza pandemic (of swine origin) [2], the Middle East respiratory syndrome coronavirus in 2012 (via dromedary camels) [3,4], the Ebola virus Zaire outbreak in 2014 to 2016 (probably originating from bats) [5,6], the Zika outbreak in 2015 to 2016 (insect transmitted, animal origin unknown) [7], the human monkeypox virus [8], and the Sudan Ebola virus in 2022 [9].

If the arrival of SARS-CoV-2 was unexpected, the magnitude of the pandemic surpassed all predictions. As the early months of the pandemic passed, we learned to respond to an ever-increasing demand for tests. Faster and more reliable tests were needed, and then massive sequencing efforts allowed us to track the ongoing evolution of the virus and subsequent pandemic waves that has led to a global death toll of 2.65 million deaths (as of 12 April 2023).

Did our accumulated knowledge impact how COVID-19 was managed? Newly introduced tests and sequencing technology, vaccine strategies, and extensive research funding have been instrumental in the response to the COVID-19 pandemic. Sequence-based monitoring and reporting was rapid and partly expedited because of advances in technology and analysis algorithms. Within weeks of virus discovery, a validated PCR assay was available [10], whereas serology assays were available shortly after that. Vaccines followed much faster than expected. This benefited from the extensive research and funding that had been allocated previously to the study of SARS, Middle East respiratory syndrome coronavirus, Ebola virus, HIV-1, and many other pathogens [2,4,11,12]. The international cooperation of scientists and physicians working on SARS-CoV-2 was unique and led to sustainable structures for the outbreak response.

In this article, the key elements of preparedness and response are addressed from the perspective of a clinical microbiology laboratory with a focus on SARS-CoV-2 diagnostics and genome sequencing. The following sections recapitulate the major issues and challenges we encountered while dealing with the SARS-CoV-2 pandemic.

Pre-analytical considerations

A lot has been learned during the COVID-19 pandemic. At the outset, the priority was to test different sets of primers and probes to optimize and validate the assay conditions and to produce rapid and reliable results. This continued to be necessary, even when predesigned and commercially available assays were available, due to the shortage of tests and consumables.

At different times in the COVID-19 pandemic, laboratories worldwide experienced delivery problems for kits, reagents, and utensils. The shortage was applied to commercial assays as well as reagents and utensils necessary for laboratory-developed assays. There were two main reasons for the universal shortage: 1) an unprecedented level of testing worldwide. For example, in Denmark, at the height of the testing activity in early 2021, a daily testing by nucleic acid amplification tests of 175 000 individuals (3% of the general population) and by rapid antigen tests of 440 000 individuals (7.6% of the general population) was performed, and 2) failure of the widespread "just in time" supply strategy, in which no part of the supply chain maintained more than a minimum stock, as the consumption before the pandemic could be accurately forecasted.

Research supports the notion that most laboratory errors, even when adequate quality controls and standard operating procedures (SOPs) are in place, take place in the pre-analytical stage, especially in the reception of samples. It was crucial to document all sample procedures in SOPs, and that the procedures were followed meticulously to guarantee the traceability of the sample, the quality of the results, and the health of both patients and members of staff.

As the pandemic advanced and test numbers increased exponentially, samples arriving to the clinical microbiology laboratory presented new operational challenges that could not be solved with a simple "all hands on deck" approach or by appropriate SOPs. A lesson learned was that many SOPs were not "scalable". Operational challenges, such as designing clear and agile workflows and training new (or relocated) personnel, became a priority as the number of samples per day increased from hundreds to thousands. This was further complicated by different priorities in the urgency of testing and test validation depending on the objective, either screening of large populations or the detection of individual diseases with varying severity. Thus, several test strategies were carried out in parallel in the laboratory.

Other tasks, such as screening unacceptable samples, became problematic, as the number of samples increased exponentially. Hundreds to thousands of samples needed processing under appropriate biosafety conditions, and initial screening was critical to filter inappropriate samples (i.e. duplicated samples, unnecessary control samples, etc.). Importantly, setting up different workflows for urgent samples and screening samples became crucial to avoid delays. It became clear that training in sample collection, handling, and transport was necessary. Inappropriate samples arriving from understaffed health facilities had to be rejected (e.g. leaking tubes with loose caps, tubes labelled with ink that dissolved with disinfectants, incomplete metadata, poor sample cooling, delays at shipping, lack of secure shipping containers, etc.). These not only posed a risk for the staff but also delayed the testing and could cause patient inconvenience by requiring repeat sampling. This situation was further complicated by the need to hire and train support/temporary staff to handle the sheer volume of testing.

Also, once a sample was accepted by the diagnostic unit, it had to be registered and labelled for traceability. At the beginning of the pandemic, many laboratories experienced difficulties with laboratory requests from peripheral health posts with insufficient metadata, sometimes hand-written, or mismatched batches of samples and sample request forms. Clearly, the laboratory information system (LIS) needed to be compatible with laboratory request forms employing barcoding or a quick response system that can be read by the LIS, which expedites the patient sample registration while simultaneously minimizing clerical errors. This was a key determinant for the number of samples that had to be processed daily. Once the samples were ready to be processed, they had to be prioritized according to the urgency of the test, so different circuits were created according to the time of response (TOR) for routine samples and urgent samples.

The diversity of sample tubes used by collection laboratories was a challenge. Few vendors could meet the demand, resulting in the simultaneous use of multiple different sample tubes. Most instruments require a specific sample tube, and as sample tube variety and alternative buffer systems increased, it became necessary to sort samples prior to analysis to send samples to the correct

laboratory workflow and instrument. To ensure full use of all platforms, it was necessary to transfer patient specimens to correct tubes and manually relabel these samples to shift to workflows with a shorter time. The lack of compatibility of collection tubes and inactivation buffers across platforms put laboratory workflows at a difficult crossroad.

At the top of a wish list, one would have easy-to-use sample collection material (primary tubes) that can be labelled at the point of collection, automatically registered and compatible with the LIS in place. Importantly, the primary tubes and transport medium must be compatible with the variety of assay platforms in use so that the labelled samples can be immediately processed. In addition, sample collection, transport, and correct interim storage, also decisive for test success, required specific attention.

Analytical considerations

The analytical phase begins in the laboratory, when the patient sample is ready for testing and ends when the result of the test is interpreted, validated, and communicated to the end user. The three major considerations in the analytical phase are staff, equipment capacity/scalability, and reagent availability. Scalability and the ability to quickly increase capacity when needed is a challenge even for the most robust equipment.

Anticipate: what should we be preparing for and how?

Viruses have been responsible for major epidemics/pandemics and are likely to account for the next ones. The Danish physicist and Nobel laureate Niels Bohr said, “prediction is very difficult, especially about the future”. Predicting which virus will cause the next epidemic/pandemic is complex for a number of reasons, including the following: i) the list of candidates is ample and it is not possible to be prepared for all; ii) the emerging virus could be a novel species, as happened with SARS-CoV-2; iii) the conditions suitable for efficient virus transmission and spread may not be well characterized and unexpected (i.e. monkeypox spread in 2022), or conditions may be known but not possible to alter (i.e. temperature and rainfall); iv) adaptation and spill over events may occur over time in areas not accessible to routine surveillance systems. Nevertheless, several lessons can be extracted from the history of emerging viruses that may guide preparations:

Origins: Experience points to the zoonotic origin of the next viral threat. Most pandemic agents are of animal origin. The One Health approach, combining animal, human, and environmental health, has become necessary to understand the emerging potential of viruses.

Routes of transmission: Pandemics are likely to be caused by viruses against which the population has no immunity. With high disease severity, the response will require proper biosafety measures (personal protective equipment and cabinets) to handle the clinical samples. Thus, clinical microbiology laboratories should be prepared to handle many samples under these conditions or be able to expand their sample processing facilities within a short time.

The involvement of other species: Zoonotic viruses may be transmitted from animals to humans (and vice versa) either directly or via vectors, such as arthropods in the case of arboviruses. Therefore, drivers of vector emergence and the vector's ecological plasticity, the range of animals that can host an emerging virus, and all the ecological conditions affecting transmission dynamics will play a role that will be difficult to anticipate or prevent. As an example, while malaria was declining over the past decades, the decrease in vector control activities during the COVID-19 pandemic resulted in an upsurge of cases. In addition, new players may jump into the transmission court, such as *Anopheles stephensi*, an urban-adapted species that may impact malaria transmission and prevention in some African countries.

These considerations apply mainly to the global aspects of pathogen emergence. In this sense, sequencing has turned into one of the main surveillance pillars. Next-generation sequencing protocols can overcome RT-PCR primer mismatches and provide valuable information on virus variants that would otherwise be missed in routine testing. Routine metagenomics surveillance, especially in hot spots of pathogen emergence in low- and middle-income countries, may provide insights on the coming pandemic viruses. Routine sequencing allows investigators to modify measures depending on sequence data (“track and adapt”), contributing to the following:

(i) Validating diagnostic tools by comparing sequences used in diagnostic assays with current circulating strains. Sequence data can validate and improve both genomic and antigen detection methods.
(ii) Monitoring drug resistance and antibody/vaccine-escape variants. In addition to treatment efficacy, this is highly relevant for the cost-effectiveness of expensive therapies.
(iii) Understanding viral spread through phylogeography. This is a particularly important issue in preparedness, if virulent or treatment/vaccine-escape variants appear. At the early stages of an epidemic, this can inform public health measures for controlling virus movement.
(iv) Identify viral mutations that may influence viral persistence or increased transmission to adapt to public health measures.
(v) Develop novel approaches to viral surveillance, such as the monitoring of sewage, wastewater, and dust.

Clearly, advances in assay development, laboratory instrumentation, and automation have markedly improved the quality of laboratory results and significantly reduced error rates [13]. However, the validation of new assays (even commercial) takes time. Some of this time will be spent trying to find suitable samples to validate assays, and time can be saved by using sample remnants to prepare collections of anonymized respiratory, serum/plasma, faecal, or cerebrospinal fluid samples and stored at −80 °C ready to use for validation. Another consideration is to secure institutional review board approval to create a representative sample collection for further testing of anonymized samples. This may be a good investment of time because this process may take from weeks to months depending on the institution. Also, this is a good opportunity to describe the standard analytical plan; i.e. the statistics of diagnostic performance, which can be easily scripted to run the analysis automatically. However, it is difficult for a single laboratory or institution to map comprehensively the genetic diversity within a given species represented by different clonal (sub-)lineages. Thus, the availability of (inter-)national collections that are easily accessible to professionals is crucial to quickly gain well-characterized strains and/or extracted nucleic acids covering at least the most frequently circulating and emerging microorganisms.

Post-analytical considerations

As the COVID-19 pandemic continued, challenges in procuring test reagents emerged, leading to inevitable delays in the TOR. Clinical frustrations because of delays in diagnostic results were partially ameliorated by managing expectations and actively communicating with all clinical services. Indeed, TOR is the single most important determinant of how the laboratory work is perceived by other clinical services in a hospital in emergencies. This is determined by several factors. TOR depends on the diagnostic test being used (PCR or molecular serological based tests vs. rapid diagnostic tests), the personnel available to run these tests,

and how well the equipment that runs those tests communicates with the LIS. Essential for the diagnostic laboratory is the involvement of information technology specialists to speed up connectivity of equipment to automate results delivery. Smartphone applications in some countries have helped deliver results, not only to physicians, but also to patients and the public health care system and contributed to a better management of positive cases (detection, isolation, and treatment). The alignment of laboratory and clinical services can also be anticipated through periodic meetings in pre-pandemic preparedness plans.

Make it happen before it happens: how to fund preparedness and the challenge of the *in vitro* diagnostic medical devices (IVD) regulation

It is worth emphasizing that from a laboratory perspective, any plan of preparedness that is not financially supported is likely to become a futile exercise. Taking into consideration the global economic crisis that has followed the 2020 lockdown, which resulted in a 3% drop in gross domestic product (data from the International Monetary Fund), the governments should seriously consider ring-fencing emergency funds to be released in case of a new epidemic/pandemic. Developing local laboratory networks led by reference centres or hospitals could help peripheral hospitals to become quickly prepared to process samples and implement diagnostic tests in the case of a new pandemic.

Some regulatory aspects could delay the implementation of laboratory-developed tests (also known as “in-house” tests). According to the current European Regulation (EU 2017/746) concerning *in vitro* diagnostic medical devices (the IVD Regulation), laboratories are no longer allowed to develop “in-house” PCR tests. If a new need arises, such as a pandemic, the laboratories will no longer be able to develop new tests or troubleshoot existing commercial tests. This limitation must be revisited considering new pandemics because this severely restricts the ability of clinical microbiology laboratories to respond promptly (see Supplementary material for further information).

Conclusions

A pandemic, such as COVID-19, is likely to occur again, and we should seriously consider increasing the readiness level for infrastructure, organization, technology, funding, and personnel as follows:

1. Maintain metagenomics viral sequencing capacity, including methods and databases.
2. Apply regular metagenomics-based assessment of clinical samples to provide a catalogue of local pathogen diversity of medically important viruses and, if necessary, other agents [14].
3. Apply surveillance of potential animal reservoirs (bats, rats, common farm animals and species frequently exposed to humans; e.g. raccoons, skunks, foxes, and deer).
4. Maintain and support scientists and research groups that can design and troubleshoot PCR and sequencing reagents and methods as scalable laboratory-developed testing.
5. Maintain a supply strategy for commercial kits as well as reagents and equipment needed for laboratory-developed assays.

In conclusion, the preparedness for a new pandemic should focus on zoonotic viruses. Laboratories across various countries should establish (or re-use) operational networks to communicate and respond effectively, ensuring the presence of agile circuits with full traceability of samples. Regional/local sequencing facilities with the ability to process thousands of samples should be in place with trained personnel ready to process and analyse samples. Importantly, governments must understand that preparedness is not an academic exercise but rather a political responsibility, and thus, additional funding for preparedness is critical if we are to respond in a timely and effective manner.

Transparency declaration

The authors declare that they have no conflicts of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cmi.2023.04.024.

References

[1] Poon LL, Guan Y, Nicholls JM, Yuen KY, Peiris JS. The aetiology, origins, and diagnosis of severe acute respiratory syndrome. Lancet Infect Dis 2004;4(11):663–71. https://doi.org/10.1016/S1473-3099(04)01172-7.
[2] Smith GJ, Vijaykrishna D, Bahl J, Lycett SJ, Worobey M, Pybus OG, et al. Origins and evolutionary genomics of the 2009 swine-origin H1N1 influenza A epidemic. Nature 2009;459(7250):1122–5. https://doi.org/10.1038/nature08182.
[3] Memish ZA, Cotten M, Meyer B, Watson SJ, Alsahafi AJ, Al Rabeeah AA, et al. Human infection with MERS coronavirus after exposure to infected camels, Saudi Arabia, 2013. Emerg Infect Dis 2014;20(6):1012–5. https://doi.org/10.3201/eid2006.140402.
[4] Cotten M, Watson SJ, Kellam P, Al-Rabeeah AA, Makhdoom HQ, Assiri A, et al. Transmission and evolution of the Middle East respiratory syndrome coronavirus in Saudi Arabia: a descriptive genomic study. Lancet 2013;382(9909):1993–2002. https://doi.org/10.1016/S0140-6736(13)61887-5.
[5] Arias A, Watson SJ, Asogun D, Tobin EA, Lu J, Phan MVT, et al. Rapid outbreak sequencing of Ebola virus in Sierra Leone identifies transmission chains linked to sporadic cases. Virus Evol 2016;2(1):vew016. https://doi.org/10.1093/ve/vew016.
[6] Dudas G, Carvalho LM, Bedford T, Tatem AJ, Baele G, Faria NR, et al. Virus genomes reveal factors that spread and sustained the Ebola epidemic. Nature 2017;544(7650):309–15. https://doi.org/10.1038/nature22040.
[7] Faria NR, Azevedo RDSDS, Kraemer MUG, Souza R, Cunha MS, Hill SC, et al. Zika virus in the Americas: early epidemiological and genetic findings. Science 2016;352(6283):345–9. https://doi.org/10.1126/science.aaf5036.
[8] Nuzzo JB, Borio LL, Gostin LO. The WHO declaration of monkeypox as a global public health emergency. JAMA 2022;328(7):615–7. https://doi.org/10.1001/jama.2022.12513.
[9] Kiggundu T, Ario AR, Kadobera D, Kwesiga B, Migisha R, Makumbi I, et al. Notes from the field: outbreak of Ebola virus disease caused by Sudan ebolavirus - Uganda, August-October 2022. MMWR Morb Mortal Wkly Rep 2022;71(45):1457–9. https://doi.org/10.15585/mmwr.mm7145a5.
[10] Corman VM, Landt O, Kaiser M, Molenkamp R, Meijer A, Chu DK, et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Euro Surveill 2020;25(3):2000045. https://doi.org/10.2807/1560-7917.ES.2020.25.3.2000045.
[11] Gire SK, Goba A, Andersen KG, Sealfon RS, Park DJ, Kanneh L, et al. Genomic surveillance elucidates Ebola virus origin and transmission during the 2014 outbreak. Science 2014;345(6202):1369–72. https://doi.org/10.1126/science.1259657.
[12] Gangavarapu K, Latif AA, Mullen JL, Alkuzweny M, Hufbauer E, Tsueng G, et al. Outbreak.info genomic reports: scalable and dynamic surveillance of SARS-CoV-2 variants and mutations. Res Sq [Preprint] 2022. https://doi.org/10.21203/rs.3.rs-1723829/v1.
[13] Carraro P, Plebani M. Errors in a stat laboratory: types and frequencies 10 years later. Clin Chem 2007;53(7):1338–42. https://doi.org/10.1373/clinchem.2007.088344.
[14] Phan MVT, Agoti CN, Munywoki PK, Otieno GP, Ngama M, Kellam P, et al. Identification of missed viruses by metagenomic sequencing of clinical respiratory samples from Kenya. Sci Rep 2022;12(1):202. https://doi.org/10.1038/s41598-021-03987-1.