The unsung virtue of thermostability

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The current vaccination campaign against SARS-CoV-2 has many challenging aspects, one of which is maintaining the cold chain for the distribution, delivery, and storage of available vaccines and guaranteeing that their full titre is retained for administration. Although outstanding technology for vaccine development has enabled products to be put on the market in 1 year, it is difficult to understand why approximately the same length of time is taken to roll out their administration, thus jeopardising the effect of the campaign. Additionally, if a substantial proportion of vaccines lose their potency or safety, or both, because of problems during transportation and storage, they will be less efficacious, and an increase in the overall costs of deploying the campaign will be inevitable. The reason for having to implement the cold chain is that thermostable vaccines do not exist (ie, heat-stable and freeze-stable, so as to be stored at a temperature of >8°C, which is a preferred vaccine characteristic recommended by WHO).[1] No COVID-19 vaccine exists in a format that could be delivered to homes by mail and, ideally, self-administered.

In actuality, high-income countries were not really interested in and committed to developing thermostable vaccines, because this feature was never expected to become a major hurdle in the limited scope of scientists. There was a failure to identify any foreseeable circumstances under which high-income countries would not have enough refrigerating capacity to manage any widespread vaccination campaign. For this reason, developing thermostable vaccines was never a priority or a core requirement for high-income countries. In fact, the real demand and insufficient push for thermostable vaccines, both in veterinary and in human medicine, comes from low-income and middle-income countries and, although supported by international organisations, it was never prioritised to become an essential characteristic sought by vaccine developers, industries, and funding entities.

Perhaps investing in global needs, which include the needs of the poorest people, would have benefited the whole of humanity in tackling the COVID-19 pandemic. Now is the time to reprioritise the urgent improvements in vaccine development that are essential to fully make use of the power of immunisation campaigns even under diverse epidemiological, geographical, and logistical circumstances.

We declare no competing interests.

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Rapid identification and tracking of SARS-CoV-2 variants of concern

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In the past few months, we have seen emergence of clinically important mutations that alter infectivity, severity, or immune susceptibility of SARS-CoV-2.[1] Prominent examples include Asn501Tyr, His69_Val70del, and Glu484Lys mutations in the spike protein that have emerged independently in many global strains, such as those from the UK, South Africa, and Brazil, possibly driving resurgence of the pandemic when it appeared to be coming under control.[2] Some of these variants are likely to be resistant to vaccines and capable of reinfections. Future public health policy and pandemic response will need knowledge of the presence of such variants in the local population and their rapid identification on introduction into communities. This is not possible without local sequencing capacity, which is scarce in many vulnerable parts of the globe, where lockdown regulations are not strictly enforced and movement is unrestricted.[3] Even in those low-income and middle-income countries where such capacity is present and high alert is in place, the delay between positive diagnosis and sequencing results leads to an opportunity for a new variant to become established. Hard quarantine, involving strict confinement and isolation for all people with a positive test for SARS-CoV-2 who are at risk of carrying clinically important new variants, until cleared by sequencing, is a public health measure that is difficult to implement. This difficulty arises because long turnaround times that are associated with sequencing might lead to extra pressure on healthcare authorities for institutional quarantine or follow-up after release in the event of variant detection in an individual. Diagnostic platforms that are based on sequencing and are suitable for use at the point of care, such as pore-based technologies, are anticipated to contribute substantially to this process in the near future, being capable of diagnosis, variant calling, genealogy, and novel mutant detection. Until then, we propose an alternative approach for low-resolution, yet accurate, early detection of specific variants of concern through clustered interspaced short palindromic repeats (CRISPR) diagnostics, which rely on the specific DNA interrogation properties of enzymes, such as FnCas9, Cas12, or Cas13, to identify variants of concern through fluorescence or paper strip-based diagnosis (appendix).[4] Such tests are rapid, inexpensive, and especially suited for low-income countries. Even where sequencing is being done, CRISPR diagnostics can help to isolate variants in the first instance, which can then be sequenced to validate and map coexisting mutations (appendix). We have used this approach to identify the Asn501Tyr variant of concern, starting

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from RNA.[5] The variant detection strategy (ie, rapid variant assay) can be readily combined with a CRISPR diagnostics platform that is already approved as an equivalent diagnostic method to quantitative real-time PCR in India, providing diagnosis and identification of one variant of concern in less than 90 min from sample to result, at a test cost of less than US$15.

The coming months present a challenging scenario: tracking and controlling the spread of such variants and simultaneously understanding their effects on the pandemic. Large-scale sequencing efforts and tailor-made diagnostic solutions, such as CRISPR diagnostics will be crucial.

DC and SM have filed patents relevant to the work and are inventors of a CRISPR diagnostic licensed to Tata Medical and Diagnostics. AA declares no competing interests.

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WHO International Standard for anti-SARS-CoV-2 immunoglobulin

The development timeline of COVID-19 vaccines is unprecedented, with more than 300 vaccine developers active worldwide.[1] Vaccine candidates developed with various technology platforms targeting different epitopes of SARS-CoV-2 are in the pipeline. Vaccine developers are using a range of immunoassays with different readouts to measure immune responses after vaccination, making comparisons of the immunogenicity of different COVID-19 vaccine candidates challenging.

In April, 2020, in a joint effort, the Coalition for Epidemic Preparedness Innovations (CEPI), the National Institute for Biological Standards and Control (NIBSC), and WHO provided vaccine developers and the entire scientific community with a research reagent for an anti-SARS-CoV-2 antibody. The availability of this material was crucial for facilitating the development of diagnostics, vaccines, and therapeutic preparations. This effort was an initial response when NIBSC, in its capacity as a WHO collaborating centre, was working on the preparation of the WHO International Standards. This work included a collaborative study that was launched in July, 2020, to test serum samples and plasma samples sourced from convalescent patients with the aim of selecting the most suitable candidate material for the WHO International Standards for anti-SARS-CoV-2 immunoglobulin. The study involved 44 laboratories from 15 countries and the use of live and pseudotype-based neutralisation assays, ELISA, rapid tests, and other methods. The outcomes of the study were submitted to WHO in November, 2020. The inter-laboratory variation was reduced more than 50 times for neutralisation and 2000 times for ELISA when assay values were reported relative to the International Standard.

The International Standard and International Reference Panel for anti-SARS-CoV-2 immunoglobulins were adopted by the WHO Expert Committee on Biological Standardization on Dec 10, 2020.[2] The International Standard allows the accurate calibration of assays to an arbitrary unit, thereby reducing inter-laboratory variation and creating a common language for reporting data. The International Standard is based on pooled human plasma from convalescent patients, which is lyophilised in ampoules, with an assigned unit of 250 international units (IU) per ampoule for neutralising activity. For binding assays, a unit of 1000 binding antibody units (BAU) per mL can be used to assist the comparison of assays detecting the same class of immunoglobulins with the same specificity (eg, anti-receptor-binding domain IgG, anti-N IgM, etc). The International Standard is available in the NIBSC catalogue.

Initiatives have been launched for the harmonisation of immune response assessment across COVID-19 vaccine candidates, including the CEPI Global Centralised Laboratory Network.[3] CEPI centralised laboratories will achieve harmonisation of the results from different vaccine clinical trials with the use of common standard operating procedures and the same crucial reagents, including a working standard calibrated to the international standard.

The basic tool for any harmonisation is the global use of an International Standard and IU to which assay data need to be calibrated with the use of a reliable method. It is therefore crucial that the International Standard is properly used by all vaccine developers, national reference laboratories, and academic groups worldwide, and that immunogenicity results are reported as an international standard unit (IU/mL for neutralising antibodies and BAU/mL for binding assay formats).

In this manner, the results from clinical trials expressed in IU would allow for the comparison of the immune responses after natural infection and induced by various vaccine candidates. This comparison is particularly important for the identification of correlates of protection against COVID-19; should neutralising antibodies be further supported as a component of the protective response, the expression of antibody responses in IU/mL is essential


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For the **WHO International Standard for anti-SARS-CoV-2 immunoglobulin** see https://www.nibsc.org/products/brm_product_catalogue/detail_page.aspx?catid=20/136

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