Backstory

Open access methods and protocols promote open science in a pandemic

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This Backstory discusses the development of a SARS-CoV-2 detection method using widely available laboratory equipment. The approach, reported in Cell Reports Methods and STAR Protocols, is intended as a diagnostic tool for COVID-19 that is accessible for resource-limited areas. We describe how the published method and protocols encourage adoption of the detection strategy in different areas and a variety of biological contexts. For complete details on the UnCovid method and protocols, please refer to (Alcántara et al., 2021a; Alcántara et al., 2021b; Mendoza-Rojas, et al., 2021).

The COVID-19 pandemic in Peru

Our Backstory is set in Peru, a nation of approximately 33 million people that has been notably hard-hit by the COVID-19 pandemic, both in terms of total numbers of cases and mortality (https://www.npr.org/sections/goatsandsoda/2021/11/27/1057387896/peru-has-the-worlds-highest-covid-death-rate-heres-why). Peru’s leadership was quick to respond to the COVID-19 threat, instituting stringent lockdown orders at a very early stage of the pandemic: in March 2020, there were only 75 cases confirmed in the country (https://time.com/5844768/peru-coronavirus/). However helpful it may have been, the pandemic clearly continued to spread aggressively.

Mass testing has always been a part of the COVID-19 puzzle, particularly during the early days, before vaccine availability. However, the extent of testing in Peru has remained low throughout the pandemic (see for example https://ourworldindata.org/search?q=daily+covid+19+tests+per+thousand), even compared to regional neighbors such as Uruguay. According to the World Bank, approximately 20% of Peruvians live at or below the nationally adjusted poverty line (https://data.worldbank.org), and there is also notable inequality in terms of where these individuals and communities are geographically distributed. Poverty is comparatively concentrated in relatively remote, inland communities, and the extent of COVID-19 testing has similarly been lower in these areas (reported, for example, in Hernández-Vásquez, et al. 2021).

Figure 1. UnCovid in use at the bench
These were some of the circumstances motivating Pohl Milón, a professor at the Centre for Research and Innovation of UPC University, and his group of junior researchers and undergraduate students in 2020. The researchers wanted to know how to improve testing accessibility for a large, socioeconomically diverse population, including communities that are economically vulnerable and geographically challenging to reach. Milón’s expertise and interest in biosensors and pathogens, as well as a familiarity with the challenges of performing research with limited instrumentation and resources, left him uniquely poised to innovate in this context. Milón’s work also helps to tell larger story about how research communities were repurposed to address a global health crisis as existing public health systems became strained (https://www.science.org/content/article/pandemic-pivot-how-scientists-answered-call-diagnostic-tests).

A new approach to low-cost disease diagnostics

The goal was to develop a SARS-CoV-2 detection method that would utilize the type of standard laboratory reagents and equipment accessible to scientists working in low- and middle-income countries (LMICs). A key consideration was to avoid using specialized reagents typically only available through the international supply chain, which affected their availability in Peru. Sensitive detection strategies for SARS-CoV-2 rely on PCR-based amplification of viral nucleic acids. So for the first step, Roberto Alcántara (a junior researcher), Eva Dueñas and Gabriel Mendoza (both undergraduate students), worked to establish suitable methods for production of PCR enzymatic components as well as Cas12a enzyme for downstream detection steps. Next, Alcántara and colleagues (under Milón’s supervision) worked to optimize primer selection and the conditions of the PCR reactions, ultimately leveraging BEARmix, an open-source RT-PCR protocol that the authors note was instrumental in the project overall (Graham et al. 2021.) Finally, multiple detection strategies, including gel-based and Cas12a-based methods were tested and optimized for use with the optimized amplification protocol.

The authors further extended their commitment to the idea of testing accessibility in LMICs by exploring whether their methods could be implemented using a portable laboratory set-up from Bento Lab. This was motivated by the needs of Peruvian communities located in remote and rural areas, where a portable testing component could be critical to expanding access to testing and utilizing testing results for public health responses in something closer to real-time. As Milón explains, even if antibody testing were widely available, if that test has one to two weeks delay, it doesn’t tell you who’s infected with the virus right now.

The “Un-Covid” method they developed comprises an end-to-end workflow beginning with generation of key reagents and ending with multiple options for clinically validated readouts of viral infection. Of course, like all scientific stories, this one had its share of quirks and twists of fate. Funding for the project originated as a grant focused on CRISPR-Cas methods for detecting leishmaniasis, back in 2019, before the whole world changed. Milón and colleagues had to mount an appeal to the funding agency to secure emergency funds based on research activities in common with the original project.

The logistics of physically doing the research provided another challenge. As the pandemic worsened in Peru, curfews and a lack of public transportation made it difficult for lab members to physically travel to the university to conduct their experiments. Milón describes living in a city of 10 million people with no traffic looking “like a postapocalyptic movie.” Fortuitously, one lab member happened to live close enough to the lab that they could keep the project going during curfew times.
and they had to obtain special permits to transit so that experimental workflows would not be disrupted by observing the curfew hours.

**How open-access methods and protocols publishing advanced the project’s goals**

In considering a publication strategy, Milón was motivated by a common feeling of frustration: being fascinated by a new scientific publication and excited to try the new approach in his own lab but ultimately being disappointed to realize that the methods reporting wasn’t quite robust enough to faithfully recreate the experiment. Milón sees this as not only an inconvenience for himself but a broader challenge for research reproducibility. To help prevent challenges to other groups adopting their method, their results were therefore reviewed, polished, and packaged as three freely available scientific documents (Alcántara et al., 2021a; Alcántara et al., 2021b; Mendoza-Rojas, et al., 2021). The development of the method, including detailed reporting of the various optimizations and analytical comparisons that informed each component of the assay was described in *Cell Reports Methods* (Alcántara et al., 2021b). The methods paper provides the empirical justification for each step of the method and serves as both a general blueprint for future open-source diagnostic methods development and as a more specific template from which future modifications to any given step can be explored.

However, the goals of this work ultimately come down to hands-on utility. And for this, Pohl Milón and his colleagues felt that a complementary approach would be critical. In particular, “if you want this to be useful,” he believes, the experimental procedure should also be in a protocol format. In Milón’s opinion, having a standalone protocol with step-by-step instructions makes it more likely that researchers from outside his own lab—perhaps even in another country or working on a different problem—will use his method too. Milón and his colleagues therefore decided to take a modular strategy and publish two separate but related protocols (Alcántara et al., 2021a; Mendoza-Rojas, et al., 2021). One describes the general approach for low-cost production of proteins for molecular detection assays, and the other applies specifically to enzymatic detection of SARS-CoV-2 using RT-PCR amplification and CRISPR-Cas visualization. Together, the complementary methods development and protocol publications have the potential to better ensure the reproducibility and usability of the method than either publication would have alone.

**Future applications in Peru and beyond**

The possibilities of the general approach go far beyond COVID-19 diagnostics. Alcántara and his colleagues described several potential applications particular to South America, where they believe the technology can make an important impact. Proximity to the equator means that parasitic and other under-studied tropical diseases are not uncommon. Low population density in some places means that viral outbreaks can occur without anyone knowing what the virus is. Peru experienced a significant and unexpected cholera outbreak a few decades ago. And antibiotic resistance is a big problem throughout South America due to poor control in livestock and pharmacy. If you want this to beings. Early understanding of the causative agent of a disease during an outbreak, or identifying resistance genes in a known bacterial pathogen, can help local officials understand what they’re dealing with and create a response in real time. The generalized protocol can be used to detect any of these pathogens simply by changing the CRISPR RNA.

Together, the complementary methods development and protocol publications have the potential to better ensure the reproducibility and usability of the method than either publication would have alone.
Further improvements to the technology described in these papers could see it developed into a portable, low-cost diagnostic panel that could be implemented in remote locations. It’s not feasible to bring an entire molecular biology lab to places far from universities or other research centers for on-site detection, so in Peru, patient samples are often acquired in these remote locations and then taken all the way back to Lima. Paired with the Bento Lab, Milón and Mendoza’s protein production method could be used to test every person in a village or other remote location over a few days with material costs far less than 1 USD. Side-by-side tests with full-scale equipment in Milón’s own lab have produced similar results; the next step is to validate the approach directly in the field.

Aside from benefits to human health, Milón hopes his work will inspire other researchers in Low- and middle-income countries. He explains that protein production and purification, considered a routine task for a biology lab in the United States or Europe, is considered difficult and specialized work in LMICs, and he hopes that the protocols will make the process more accessible. Several other researchers have already been in touch to adapt the protocol into an assay for antibiotic resistance genes, for example. Milón even envisions using the approach as a bio-prospecting tool to better understand Peru’s underappreciated biodiversity or for ecological impact studies.

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