THE SCIENTISTS’ FORUM

Transforming a research institute into a COVID diagnostic centre – scientists step forward to protect public health during the coronavirus pandemic

Daniela Ruffell
FEBS Letters Editorial Office, Heidelberg University Biochemistry Center, Heidelberg, Germany

Let us face it: COVID-19 caught us unprepared. Virologists had abundantly described the high mutational rate of viruses and their potential infectivity. And yet, mankind was surprised by the sudden appearance of SARS-CoV-2. We saw what happened in China and watched the coronavirus spread through countries, but we were slow in reacting.

However, we do have the knowledge and the technology to help stop the pandemic, and scientists around the world are giving their all to make this happen.

COVID-19 is treacherous because of the lag time between viral infection and the appearance of symptoms: 14 days during which an infected individual can inadvertently spread the virus to many other people. Early detection of the infection is key to preventing further contagion. Diagnostic centres normally have the capacity to run a few dozen samples a day, even a few hundred in hospitals, but this proved to be insufficient to control a pandemic.

The positive aspect, if there is one, is that diagnosing COVID-19 is quickly and simply achieved by PCR. Easy peasy... All molecular biology laboratories are equipped with a PCR machine, so many research institutes around the world came to the rescue by setting up COVID diagnostic pipelines within their premises.

We describe here the implementation of an automated high-throughput COVID diagnostic pipeline at the Centre for Genomic Regulation (CRG) in Barcelona, Spain, one of the hotspots of the pandemic in Europe, to provide useful guidelines for other organisations that may wish to embark on what has turned out to be quite a formidable enterprise.

‘I contacted the Spanish health authorities back in early March to offer the collaboration of the biomedical institutes belonging to the Association of Institutes of Excellence in Spain, SOMMa’, says Luis Serrano, Director of the CRG and Chair of SOMMa [1]. ‘However, the situation appeared to be under control at the time, and the offer was not picked up’. Only a few weeks later, infection had spread, and the hospitals could no longer cope with the high demand for tests. The local government of Catalonia realised that the scientific institutes of Barcelona were a valuable resource and asked for their help. The agreement gave birth to the Orfeu Programme, a task force coordinated by the CRG, with the participation of the Institute for Research in Biomedicine (IRB), the Institute for Bioengineering of Catalonia (IBEC) and the the National Centre for Genomic Analysis affiliated to the CRG (CNAG-CRG) to set up mass PCR testing across Catalonia. The aim of the programme is to screen the elderly in nursing homes and the local police, to reach a total of 170 000 tests. The CRG alone is running 2200 samples a day on average.

Repurposing manpower

One of the first problems to solve in the whole set-up was where to get the manpower to run such a large-scale project during the peak of the pandemic. The CRG made an open call for volunteers among institute workers who were confined at home as their laboratories had been shut down. Despite the difficulties related to the lockdown, such as lack of safe...
transportation and added responsibilities of childcare and homeschooling, the response was tremendous. Over 130 people, including staff scientists, PhD students, postdocs and members of administration, offered to help! ‘I feel in a position of privilege to have skills that can contribute to tackling this pandemic’, says senior graduate student Róisín-Ana Ní Chárthaigh, one of the volunteers. ‘In research, we can often feel removed from the real-world implications of our work and their effects on society. The Orfeu Programme at the CRG allowed us to play a direct role in protecting public health, so I felt it was my duty’.

A crew of 60 volunteers was selected, progressively incorporated in three waves. All the volunteers were given a 5-h training in biosafety and were assigned to different working groups, where they received several days of on-site technical training. While they were all familiar with the molecular techniques involved in the testing process, they required training to implement these at high throughput. Each trained set of volunteers then helped to train the following wave of volunteers.

Preparing the pipeline

Scientists at the CRG held teleconferences with other institutes around the world, such as the VIB in Belgium, Universities in Israel, the Crick Institute in the UK and the Broad Institute in the United States, that were setting up similar pipelines, to compare workflows and identify pitfalls.

The Protein Expression and Purification Team, led by Carlo Carolis, Head of the Biomolecular Screening and Protein Technologies Unit, took care of the basic preparations, producing large stocks of enzymes, such as the reverse transcriptase and the DNA polymerase, required for the PCR tests.

A second team, led by Guillaume Filion, focused on the optimisation of the PCR tests in order to establish a robust and efficient quantitative PCR to be used for tens of thousands of samples. In particular, they studied ways to multiplex the assays, and they explored alternative methods of RNA purification and assessed isothermal amplification tests.

The third and largest team is dedicated to the actual running of the PCR tests.

Logistics

While running a PCR may sound simple, working out the logistics for thousands of samples taken from places up to 200 km away can be very complicated. ‘One of the first things we did, in collaboration with the local health authorities, was to change the way that the health workers were collecting the samples’, explains Dr Serrano. ‘They had been collecting the nasal swabs and suspending them in viral transport medium, which meant that the sample that reached the diagnostic centre was still infectious. We instead decided to send them collection tubes prefilled with lysis buffer (from the Zymo extraction kit), which inactivates the virus immediately’. This has the advantage of strongly reducing the risk of infection for all those involved in the diagnostic pipeline, while also eliminating a step in the workflow and concentrating the sample, increasing the sensitivity of the method. Once the tubes return to the institute with the nasal fluids, they are sprayed with alcohol to destroy any potential remaining virus on the outside of the containers. Thanks to this procedure, the samples can be processed in an S2 laboratory rather than an S3.

Registration of the tubes, in connection with patient names, is another rate-limiting step which needed to be circumvented in a high-throughput pipeline. To do this, the scientists chose to use Micronic tubes that have a 2D barcode on the bottom. The nurse performing the swab sticks a second barcode onto the side of the tube and a matching one on the patient form. When the samples reach the institute, the barcodes on the bottom and side of each tube are scanned and linked, and finally registered on the Laboratory Information Management System (LIMS), a program developed by Sergi Beltran and the Production Bioinformatics Group at the CNAG-CRG with the help of the CRG Bioinformatics Facility. The LIMS platform is used throughout, from registration, to data collection and transmission of the test results to the virologists of the national healthcare system. See infographic steps 1 to 5. Infographic by Fundamentium. Kindly provided by the CRG.

Running the PCR tests

One of the typical problems of doing diagnostics in times of pandemic is getting sufficient consumables and reagents. The sudden high demand caught the providers off guard. ‘So, one of the first things I did’, explains Jochen Hecht, Head of the Genomics Facility at the CRG, ‘was to call Tecan to make sure they had enough robot tips to cover tens of thousands of PCR tests on our Tecan PCR robots. As it turned out, Tecan not only had enough tips, but also said they had four robots for nucleic acid
A STEP BY STEP GUIDE TO THE TEST

1. **Sample collection**

   A cotton swab is put up the nose. The swab is rubbed in the tube for ten seconds so that the biological material dissolves into the solution.

   - Solution with chemical products that disarm the virus and conserve its genetic material (RNA)
   - Coronavirus
   - Solvents that break the membranes of the virus and human cells
   - Salts that stabilise both human and viral RNA

   - The swab stick is discarded in a biological waste container
   - Tube barcode

2. **Transport and distribution**

   The health service collects the sample containers and distributes them to where the swabs will be taken.

   - 100,000 tests
   - Centre for Genomic Regulation (CRG)
   - Parc Científic de Barcelona (PCB)
   - 70,000 tests
   - IBEC
   - CNAG
   - CRG

3. **CRG receives the samples**

   Samples registered and conserved to 4°C

4. **Sterilisation**

   When the samples are ready to be analysed the tubes are sterilised to avoid infecting lab personnel.

   - Tubes are sprayed with alcohol

5. **Registration**

   The barcode on the sticker is linked to the barcode on the tube as a security measure.
transformation in Europe, one of which they could provide for free (apart from the shipping and installation costs) as their contribution towards fighting the pandemic. The robot was delivered in only 12 days. However, it was originally set-up on a program that could process one 96-well plate at a time in about 1 h and 50 min, which was unsuitable for high-throughput screening. In the end, Dr Hecht succeeded in reprogramming the robot to run four plates simultaneously in just 1.5 h. Thanks to this modification they have been able to test up to 3000 samples in less than 10 h.

Here is how it is done. The Micronic tubes containing the samples are arranged on a 96-well plate. The plate is inserted in a Micronic decapping machine to open the tubes and then transferred to the Tecan Fluent DreamPrep NAP for RNA extraction using the Quick-DNA/RNA Viral MagBead Kit from Zymo Research. Another Tecan Fluent is used to transfer 5 μl of suspended RNA in the PCR Master Mix, which contains both the reverse transcriptase and the DNA polymerase. Finally, the quantitative RT–PCR is run using the Luna Universal Probe One-Step RT–qPCR Kit from New England Biolabs in two separate machines following the CDC protocols [2]. See infographic step 6.

In every tube, the amplification of a stretch of human RNA is used as internal control. The rate at which viral genetic material is amplified helps determine the viral load of the sample. If a single molecule of viral RNA is present in the sample, it will be amplified, but the cycle threshold (Ct) will be rather high (around 30–32), indicating a low viral load in the patient. Cts larger than 40 are considered negative. In contrast, a sample containing many molecules of virus will have a low Ct, corresponding to a high viral load. A patient with a high viral load is at the peak of infection. To give an idea, a patient with a Ct of 12 has 2^{20} more viral particles than a patient with a Ct of 32. See infographic steps 7 to 8.

The qPCR results are deposited in the LIMS, where they are immediately available to the virologists of the healthcare system, who take care of the final diagnosis. After the tests are run, the remaining volumes of sample are frozen and will be sent to a local Biobank, where they will be used for an epidemiological study, involving sequencing of the virus, analysis of their genome and genotyping of the patients.
‘The high throughput pipeline is very innovative and proves to be ideal for mass testing, although it poses some problems for microscale analysis, such as when we need to repeat the test for a single sample, in the rare cases when a doubt remains over the result’, comments Alba Vilas Basil, one of the virologists of the Catalan Health System in charge of analysing the data and validating the results. ‘On the other hand, the sensitivity of the tests is excellent and the reproducibility of the results, which was tested by the CRG scientists, is extremely high, to the point that we can diagnose the patients with very low viral loads as positives quite confidently’, she adds. ‘Finally, the rapidity of the results means that we are able to detect COVID patients in a timely manner. This allows the health service not only to isolate positive patients, but also to systematically monitor all those who have been in direct contact with him/her, which is so far the only means we have to control the pandemic’.

The high sensitivity of the PCR tests can create a number of shortcomings as well. A sample with a very high viral load (i.e. Ct of ~11–12) can easily contaminate neighbouring samples via aerosol on the robot tip. A single molecule of viral nucleic acid will be amplified and result in a false positive. ‘When there is a sample with a very low Ct in a plate, we are forced to take the sample out and rerun the whole plate’, explains Dr Serrano. ‘Unfortunately, there is no
standardization across hospitals and diagnostic centres regarding the sensitivity that protocol and reagents should achieve’, he continues. ‘We have found that the sensitivity largely depends on the protocols, robots and RT-PCR machines, as well as the amount of sample used. Just changing the kit for RNA extraction or decreasing the amount of sample can change the sensitivity by 40%, which means that up to 40% of the weak positives may be missed. No one seems to be working on setting the standards at European level. This bears the risk of leaving a number of infected people with low viral load undetected, as well as making infection rates in different areas not comparable’.

Who ever thought that a qRT–PCR could be so complicated? See infographic steps 9 to 11.

**Problem solving**

Setting up and managing a high-throughput pipeline during a pandemic are not just a scaling-up exercise. A number of decisions must be taken under time pressure and problems solved without prior testing of the options. Logistics are complicated due to the large number of samples. The shortage of consumables and reagents must be circumvented, and the compatibility of protocols, reagents and machines has to be guessed. The procedures and protocols used are constantly being tested, optimised and updated while running the pipeline. ‘There were weeks at the beginning when we were purifying viral RNA using the Qiagen 96-well plate centrifugation system set up on our in-house robotic platform, in parallel to using the microbeads on the new Tecan DreamPrep, in order to keep up the pace of the pipeline’, says Dr Carolis. ‘Only after Dr Hecht tweaked the machine to run four plates at the time, could we settle on the new robot and protocol. We could not know it all in advance, and some decisions were gambles, but finally it all worked out well’.

All the staff involved in this pipeline has been working incessantly, including weekends, and even nights in some cases. ‘I did not feel the lockdown, as I kept going to work throughout. However, I frequently got stopped by the police patrolling the city while walking home at 3 or 4 in the morning’, admits Dr Hecht, who, as Head of the Genomics Facility, felt particularly responsible for the smooth running of the pipeline. ‘Delivering the results as soon as possible makes a difference not only to the patient involved, but also to the people around him/her who are at risk of infection. It is a strong source of motivation’.

**Future perspectives**

The pandemic is slowly subsiding in Spain, and people are gradually resuming their daily activities. Epidemiologists, however, warn of a second wave yet to come. ‘As restrictions in Spain and beyond begin to lift, I can’t help but feel apprehensive at the disconnect
between the test results we see every day and the apparent feeling on the streets that the lifting of some lockdown restrictions represents a resolution of this crisis’, says Róisín-Ana.

The diagnostic pipeline set-up at the CRG and collaborating institutes will be kept alive at least until the autumn, or longer if more testing will be necessary. It would be perhaps wise for the government to consider extending mass testing to other critical parts of the population and places that are more likely to trigger a new wave, such as among tourists and social workers, in medical centres, in schools or universities (once they open again), and in all workplaces that require direct contact with people.

Hopefully, for future pandemics (as they will happen no doubt), we will be able to react more readily in terms of identifying the population at risk, establishing safety measures and implementing mass diagnostics.

References

1 https://www.somma.es/.
2 https://www.fda.gov/media/134922/download.

Correspondence

D. Ruffell, FEBS Letters Editorial Office, Heidelberg University
Biochemistry Center, Heidelberg, Germany
E-mail: ruffell@febs.org