Testing times: The science of fighting Covid-19

Abigail Salmon, Chief Medical Scientist at St Vincent's University Hospital Dublin, with colleague Aoibheann Sally.

It has been a baptism of fire for the Chief Medical Scientist of Microbiology at St Vincent's University Hospital (SVUH) in Dublin.
Abigail Salmon was appointed in December 2018, taking over the role from a predecessor who had an eight-year stint.

Learn more

Just over one year into the role, she and her team were planning for a possible pandemic. Like a boxing match, she said, all the sparring and training in the world could not prepare a fighter for a match taking place in the dark, with an unseen opponent jabbing and punching from every angle.
“In non-pandemic times, there is a recruitment crisis in medical laboratory science across the board. There are a lot of posts not filled. Prior to the pandemic, we had five vacancies to fill, as well as two maternity leave posts. It is not that SVUH is not letting us recruit, the hospital administration is enthusiastic and competitively trying to do so, but we just couldn’t fill the posts. Graduates are just not staying in the field,” she said.
With not enough graduates staying in the field, it meant all hands on deck for current SVUH staff from January.
“SVUH started multidisciplinary preparedness meetings across the departments back in January -- labs, ED, ICU, procurement, catering, everyone bought in. We had a whole system in place. But you couldn’t truly plan -- it was firefighting from the get go.

Abigail Salmon with Concepta Gallagher, Senior Medical Scientist at the specimen reception area of the microbiology lab in the hospital where swabs for Covid-19 testing are gathered.

"We were having meetings but weren't certain about what could happen. We were planning how we'd get a patient to the emergency department, and planning to send the Covid-19 samples to the UCD National Virus Reference Laboratory (NVRL). But the pandemic took hold like wildfire and the hospital had to test their own patients and staff."

From firefighting to sourcing equipment to maintaining social distancing while testing for Covid-19 and maintaining the routine microbiology testing for a number of hospitals -- such as its own patients, St Vincent's Private Hospital, St Michael's in Dun Laoghaire, St Columcille's Hospital in Loughlinstown, as well as a GP catchment in South East Dublin and North Wicklow -- it has been an all-out team effort, Ms Salmon said.

"The team has really shown what it is made of. They have responded to longer days and manic schedules and lack of equipment with great professionalism and sense of duty. Backed up by the excellent support of the team of consultant microbiologists and registrars in SVUH, they can always be proud as to how they responded to Ireland when their country needed them."

What is involved in the process of testing?

"At the time when we first heard of Covid-19, only the NVRL in UCD was testing for it. We are a bacteriology lab. The only viruses we would test for were Influenza A,B and RSV using GeneXpert, a molecular test that can detect those three viruses within 30 minutes.

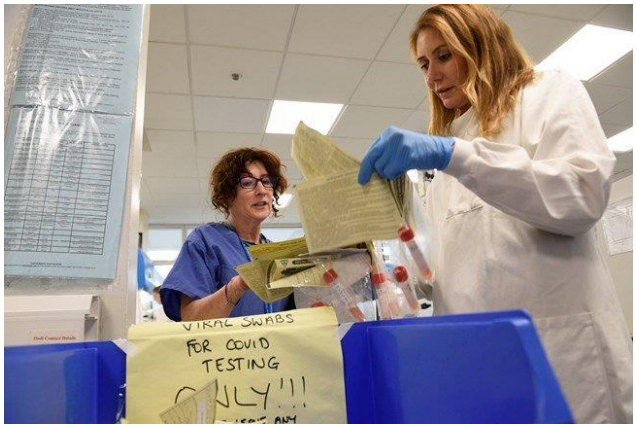
"Around March 17, we were told we would start testing for Covid-19. You swab the patient, and that swab goes into the Viral Transport Media, which goes to the lab.

"We put a little bit into a lysis buffer, the lysis buffer kills the virus and exposes the RNA (takes 30 mins) -- we then want to get that RNA pure, so we put that on the MagNa Pure 96 in order to be extracted -- that takes approx 1.5 hours. We then add that to a 96-well plate with the PCR mix and put it onto a Light Cycler 480 (LC480). We have to turn that RNA back into DNA and then proceed with PCR. That cycling machine does that and it then takes about two hours to get the result. It is not a simple swab-and-see process."

Break down the process step-by-step:

"The patient goes to get tested, the nurse takes a swab, down the throat and up the nose, it's not pleasant. That is why there can be false negatives, if you haven't tested properly. A quick swab up the nose won't do. It should be nasopharyngeal swabs, through the nose into the nasopharyngeal cavity, and down the back of the throat sampling the oropharyngeal cavity. Sputum is thought to be a better specimen, but it can be hard to get sputum samples from a Covid patient or an asymptomatic person.

“The swab is then put into liquid called Viral Transport Media. If there is the virus present in the swab, it should be released into that liquid. The liquid will preserve the virus and prevent degradation.
“We take the sample and need to deactivate it. We add some of that VTM liquid into the tube, and also add lysis buffer. The purpose of the lysis buffer is to deactivate the virus, and also breaks open the protein membrane of the virus. Basically a virus is like an envelope with nucleic acid material inside, either RNA or DNA. Lysing exposes the RNA, that’s what we want.
“After that step, the virus is dead but we need to get the RNA pure. We’ve now got a mix of VTM and lysis buffer. It’s like throwing the egg with the shell, and wanting to get the yolk out. That’s what the MagNa Pure 96 is for –if RNA is present we want to get it isolated and pure. MagNa Pure is fully automated, it will do its job, doing 96 samples in an hour, give or take.”



What is the Magna Pure 96?

“Professor Paddy Mallon kindly loaned us his MagNa Pure 96 from his UCD CEPHR lab and engineers from pharmaceutical firm Roche, which makes it, had to come in and set us up. MagNa Pure 96 is a system that can extract nucleic acid from 96 samples at a time.
When we commenced testing, we used MagNa Pure 24 but we were only able to run 40 samples a day using this. Once the engineers set up the MP96 and trained staff on it, it allowed us to do two test runs a day - 96 and 96, or the capacity to test 192 a day. The training and verification was done in record time.”

What was causing the backlog in testing around the world and Ireland?

“The initial problem was running out of swabs and VTM, and then there was a huge shortage of lysis buffer. That was a big problem. Some of the academic institutes are now making them, should there be a shortage again. A recipe for lysis buffer has actually been released.
“But when we bring in the commercial lysis buffer, there have been tests done on it, we know it fully deactivates the virus. The ones made in non-commercial labs, we have to be really careful. We’d much prefer commercial ones.
“The third shortage were the PCR kits. PCR (polymerase chain reaction) basically detects if the virus is there, by looking for and amplifying a part of a gene specific to the virus. We put it onto the Light Cycler 480, which heats and cools for around 40 cycles. If the gene is there, every round of heating/cooling will double the number of copies of the gene, a fluorescent probe binds to it and we start to see an amplification curve. That is a positive sample.”

What are PCR kits, and what is a reagent?

“PCR kits are commercial kits with primers, fluorescent probes, enzymes and reagents. A reagent is an ingredient, you need lots of reagents in the process, which are usually ready

for us in what is called a master mix. You then add the RNA from the MagNa Pure 96 onto a plate the size of a Post It note. There's 96 little holes, you put one sample per hole, which are called wells.
"You add RNA from sample One into a well, then you would add your PCR master mix. You repeat this for each sample. Once you've added all RNA and master mix into your plate, then this is loaded onto the Light Cycler.
"Ideally we like to use CE-IVD marked PCR kits, which means already approved by the EU. With this marking, the company who make it already have put the product through rigorous testing so then the lab must perform a verification to ensure it works as it should. We could make our own PCR mixes, but this requires extensive validation, which is a huge undertaking and very time consuming. We would not have been able to make our own PCR mixes."

There was a shortage of many reagents?

"There was a shortage of swabs, VTM, lysis buffer and then the PCR kit. A kit made by Altona was one of the first on the market. The NVRL was using it, so all labs began to use it. Altona couldn't keep up with the worldwide demand.
"SVUH is a public-voluntary hospital and we order through our own system, but the HSE really stepped up, with weekly Zoom calls among all the labs. They began to centrally allocate supplies to all labs as per their needs. As different PCR kits started coming on the market, the labs used different kits so we weren't all pulling from the same resources."
We also had a shortage of extraction kits/consumables for the automated extractors. SVUH uses MagNa Pure, as do some other labs. Through the Zoom calls we were able to ascertain who used what and going forward who could use other alternative instrumentation. The HSE then procured automated extraction systems and their associated kits from China. SVUH was provided with one and this is now used as a back up.

Abigail Salmon with a GeneXpert SARS-CoV-2 cartridge. Using this cartridge a result of a Covid 19 test can be reached in 50 minutes. So lysing, extraction and RT-PCR occurs onboard. However it can only test one at a time. St Vincent's have two GeneXpert instruments to test 20 cartridges at the same time. It is not conducive for high throughput batch testing.

Why can't we make our own reagents?

"As explained earlier the academic institutes made lysis buffer. But we can't use generic consumables on all our instrumentation. Take for example the MagNa Pure 96. It is a closed system, all it requires is made and supplied from Roche. There are a lot of plastic consumables associated with it and there was a shortage. However we can't just use generic plastic moulds made by another company on the instrument. It's like a Nespresso machine, if you use the supermarket capsules and your coffee machine breaks, Nespresso won't cover it. Likewise Roche won't cover a breakdown of one of its instruments if we were using non-

Roche plastics. We couldn't take the chance. This is not just a Roche thing -- it is the same for all companies.

The coordination and team effort across the country helped speed up the process?

"When we were really short of swabs, we put out an appeal to our local GPs and the response was astounding. They saved the day. I spent a day sorting through the boxes of swabs. We are really grateful. That's the kind of effort and team effort we needed, and we got it."

Are there any positives to come out of this pandemic?

"Because we were so short-staffed, I was mainly working in the lab myself. This is the first time I have been able to do real managerial work associated with my grade, coordinating how this was to be done.

"We are now getting equipment we never had, that we can keep, and we will be able to use for other tests, which will really improve outcomes. This will save money in the long run, because we will save time and bed days by running tests on this new equipment. If another pandemic happens, we should be able to hit the ground running. Staff will be trained on the equipment, it'll have already been validated.

"I've been able to network with many other labs in the country, as well as the HSE so it has been very good that way. That cooperation and coordination can only be a positive."