I mean, in our lab, we've performed mostly that ARTIC amplicon sequencing, and we've seen a lot of contamination. And the contamination we've seen, COVID genomes where you don't want to see COVID genomes. So in negative controls, you'll see contamination popping up.

And yeah, we see it from-- so in the ARTIC amplicon sequencing, there's 35 cycles of PCR. And that's specific to the SARS-CoV-2 virus. And it means that you're amplifying trillions and trillions of SARS-CoV-2 amplicons.

A lot, yeah.

And these can just go everywhere. And you just have to be so, so careful because you can get cross-contamination between the samples because of this. And you get that aerosolisation of it, and evaporation during the PCR, as well. I mean, it just goes everywhere. So you just have to be--

Yes, and I guess also-- because a lot of the plates are sealed, aren't they? There's a lot of the processing we did because it was high throughput. It was in 96 of our plates, which are sealed. So also, the possibility of-- so when you're taking off a seal, to get contamination between wells, or just general pipetting. Because as you say, there are so many copies of the amplicons when you are processing.

And also, we were at least doing something like 96 samples a day, sometimes more, if we had to.

That's it, yeah. The more samples you have, the more likelihood you have of the contaminations, yeah.

Absolutely, multiple factors, yeah.

We include a negative control on every processing play or every batch of samples we process.

Now, what is a negative control?

So we use nuclease-free water. Always make sure we use like a fresh aliquot. It's clean and-- yeah, we'll UV that in the hood, as well, just to make sure that's completely free of anything. And then, that's what we use is our negative control.

So we take it throughout the entire sequencing process. So it goes through everything that the samples go through. And at the end, it's sequenced, and we will check and see if we've got any new SARS-CoV-2 genomes in there, or any amplicons in there.

Yeah, yeah. And what about placing, where you place it on the plate? Is that important at all?

It is. So we always make sure it's in a different location each time. So it needs to be random, and you definitely don't want it as the last sample on a plate. You want it in between all your other samples.

So it depends because each lab will have their own sort of threshold of how many reads or how many amplicons you would allow to go through. Because sometimes you're always going to see a tiny bit. Yeah, different labs make their own thresholds depending on they're processing, like how they're processing samples, and then also depending on what they want from the data. Because it actually will vary, what that threshold is going to be.

But if you do the analysis and you find that you've got a genome coverage of SARS-CoV-2 in your negative control that is like over your preset threshold, this means that there has been contamination and you need to just scrap the run, to be honest. You can't use that data because you can't trust that there hasn't been contamination in the samples, as well as the negative control. And you can't trust the results that you're getting from the samples.

So you just can't use that data. Then it just has to-- it has to go, and you have to repeat it.

So we have two areas, two separate areas. So we have the pre-PCR area and the post-PCR area. So, to avoid contamination in the pre-PCR area, we have two separate hoods. So the master mix only hood, and the template addition hood. In the first one, the first one includes all master mixes and reagent plating.

The template addition, so is the RNA, samples RNA, must only occur in the template addition hood. And both hoods and the dedicated pipettes must be cleaned with RNase AWAY, followed by ethanol, and then UV for 30 minutes prior to use. Also, any plasticware and water aliquots required during the processing should be placed into the hood prior to UV decontamination.

And as a good measure, the removal of pipettes, pens, plate holders, and other contents of each hood should be kept to a minimum. And the use of sweety jars in hoods instead of open Bio-bins, and making single-use aliquots. So don't need to dip back into all aliquots. And of course laptops are not allowed in the pre-PCR area.

And in addition, we have lab coats for specific areas. So we have one lab coat for pre-PCR area, and another one for the post post-PCR area. And we must change gloves between both areas, between the areas, and between the hoods.

So what about in the post-PCR area? Is there any other measures there?

It's sort of similar, in a way. You just have to be really, really careful and be really mindful of the amplified product, as we've already said. There is so much of it that you just want to be really careful. So making sure that you spin down your amplicon plates, making sure those little droplets come off the seal before you take the seal off. Again, removing the seal really, really carefully. Don't want to be ripping it off and the droplets flying everywhere.

And then, yeah, after you're handling those amplicon plates, clean your gloves or replace your gloves. There's also other things that we do in the pre and post PCR areas. So things like having those single-use aliquots and recording lot numbers of reagents.

And again, just keeping everything really clean. So just cleaning all your pipettes before and after use, and using fresh water. And I mean, we've--

And I guess cleaning benches.

Exactly.

Before use and after use. But it is very much the same as what you would do in the pre-PCR.

Exactly. And the key thing is just keeping those areas separate and never going from one to the other with any items. So gloves, lab coats, and-- yeah.

So the contamination we saw, I guess because we were high throughput, did it did it affect about four plates? Because we were processing before really getting the results from-- like the bioinformatician weren't we?

Yeah, yeah.

So it must have been about four plates that we had contamination. And then, at that point, we stopped because we realised we didn't want to waste any more reagents or time and everything. And Charlotte, what did we do?

Yeah, we needed to investigate the cause of this contamination and find out where it was coming from. So basically, we suspected already that it would have been something happening during the pre-PCR stage as we've got all this amplified product. So we designed a little controlled experiment just to determine where our source of contamination was coming from.

So we set up the ARTIC PCRs with all negative controls. They're all just nuclease-free water. And we used different combinations of our old reagents that we would already have been using that we did the PCR with. So the Q5 master mix and the ARTIC primers. And then, also used brand new stocks of these that we hadn't opened yet just so that we could find out where the contamination was.

So we ran the PCRs and we cleaned up the products, and we did some Qubit quantification on them, and we found the aliquots that were actually contaminated because they gave readings with the Qubit results, whereas the new reagents-- obviously, brand new-- didn't give any readings. So we found out exactly where the contamination had come from, which was actually really, really good and really helpful.

So obviously we threw those aliquots away. This is where we started to learn our lessons and change how we were working. So after this, we implemented things like tracking of reagents and recording lot numbers and aliquoting dates. And actually, this is when-- because it was our Q5 that had become contaminated. This is when we decided actually we were going to pre-aliquot our Q5 into single-use reactions because previously we were dipping back into old tubes had already been used.

Yeah, basically, because we were getting through them so fast, we were high throughput, we thought it would be all right, didn't we? But no. We did learn a lesson there. That's all single-use aliquots. But I think we have a very good tracking system in place now.

And we haven't really had problems with contamination since then, have we? But I think if we did, we most probably now wouldn't need to set up that experiment, that we could go back on the reagents used, and most properly be able to work out the problem just by looking at the logs that we keep.

Yeah, I think that's true. And also, we do record the equipment used and the personnel who process the plates. So all these things will tie in, and we can track these things. And avoid these issues.

Yeah. And what did we do? I know we found out that the Q5 was the problem, but what else did we do? I think Luz Marina at the scene sort of that time.

Yeah, so after contamination is found, we must keep UV and clean the pre-PCR area and hoods, and before and after use. And of course we need to redo processing the samples. And yeah, we learn from mistakes and take on board lessons.

Yeah, I think that is just the most important message, really, that you can take steps, as much as you can, to avoid contamination. But that contamination, at some point, will happen, and it's more learning through your mistakes.