Paul Carton (Host):

Hello and welcome to the rapidmicrobiology Podcast, and I'm your host Paul Carton. Today's topic is the surveillance of SARS-CoV-2 in waste water, a microbiology testing service which is now an integral part of many regional public health agencies' strategy in curbing the spread of SARS-CoV-2, the zoonotic virus that has caused the current COVID-19 pandemic.

One of these surveillance programs currently provided by GT Molecular, and which is in widespread use across the U.S., wouldn't be as effective and as popular if not for our guest today. Let me introduce Dave Alburty CEO of InnovaPrep. Hello Dave.

Dave Alburty:

Good Morning

Host:

Rose Nash, director of research and development at GT Molecular.

Rose Nash:

Good morning.

Host:

And Dave Goad, senior scientist also from InnovaPrep.

Dave Goad:

Hello.

Host:

Rose, you developed a waste water SARS-CoV-2 surveillance service that incorporates the InnovaPrep concentrating pipette select, and now provide this service under the GT Molecular brand. You also supply molecular-based tests and give guidance to labs carrying out their own testing. Can I ask you... we know that the data on SARS-CoV-2 collected from waste water is significant. But how does the information on SARS-CoV-2 and waste water you collect and provide to your clients influence their decisions in terms of public health?

Rose Nash:

First, I want to highlight that the beauty of waste water, if you will, is that it gives you a head start on COVID-19. This comes from the unique life cycle of SARS-CoV-2, in which it's seemingly shed from the intestines into human waste a full week before a patient has symptoms and subsequently arrives for positive case result at the clinic.

I like to think of this as the Achilles heel of the virus, and that it throws up a red flag and announces its arrival before it begins to spread.

Our testing service which has been empowered by InnovaPrep since the beginning has helped us stop outbreaks before they begin. To highlight this, I want to discuss some of our work with universities early in the pandemic, in which we helped them monitor their dormitories for COVID-19.

By being able to get a readout on a given dormitory or even the entire community living on campus several times a week, these schools were able to avoid unnecessary nasal swabbing, something I'm sure that we've all experienced at some point in the last year. And also catch outbreaks before they could begin to spread and cause the university to have to close their doors.

We estimate that by doing this testing, using the InnovaPrep concentrating pipette, that we were able to save these universities hundreds of millions of dollars by being able to stay operational while cases were surging across the United States.

Host:

Can you give us an actual example of one of these universities that the client has seen a benefit of this practice?

Rose Nash:

We worked closely with the university in Denver, which is very close to our headquarters here in Fort Collins. What they would do is they'd monitor their dorms three or four times a week, and they would identify a positive dorm, go in and swab everyone within the dorm, and then move the infected or positive individuals into a quarantine dorm. We'd like to describe what this data looked like, and that it was like playing a game of Whac-A-Mole, where you'd see a dormitory, or a dormitory floor become positive. They'd go in, identify the student and then move them into the quarantine dorm, and you'd see those levels drop, and you'd see the quarantine dorm levels go up.

This university benefited from this, in that they were able to not have to swab all of their students every day to be able to catch them, and also we often found cases before anyone knew that they were sick and knew to go to the clinic, which allowed them to have less interactions with other students before they found out they were positive.

Host:

Okay. In terms of outside the campus, how about more of a community-level client. Do you have an example for us?

Rose Nash:

We've always been driven to generate what we call data for action, which means that we want to harness that seven-day head start that wastewater monitoring provides us, and get the data to customers as quickly as possible. The customer I'd like to highlight is Burlington, Vermont in this case. They've been one of my favourite examples of a community that's used the data for action. The Burlington group really goes above and beyond what a lot of communities do, and they actually don't just test at their wastewater treatment plant, they also test it sub-sewer sheds upstream.

What that means is they've actually subdivided their city into several smaller communities. Then they send us samples from these smaller deviations of their community. Then they use the different

levels of COVID by location to direct pop-up testing locations or vaccination locations. My favourite is these large signs that they put over their interstates to warn drivers that they could be exiting into an area undergoing a surge. An absolutely wonderful use of data for action, really getting it, using it to help community members make data-driven decisions.

Host:

Okay. They're actually providing real-time information, showing this on billboards on highways, sort of like a missing person sort of action alert

Rose Nash:

Absolutely. One of my favourite things is that the mayor of Burlington actually regularly tweets our data. When they saw a spike following thanksgiving, he called it a call to action for all Burlingtonians. We just really love them getting that data in front of people so that they can make their own data-driven decisions on whether to wear a mask or leave the house to run an unnecessary errand back when the pandemic was surging.

Host:

Although I do have problems with our own politicians tweeting updates. I do actually like the idea of a community alert being sent to everyone's phone instantly, especially during a pandemic.

In the early days of the pandemic, there was long lead times for clinical testing, which causes all sorts of problems with retesting and false negatives. But this was solved simply by the expansion of testing centers and automation. However, a wastewater sample is different to your nasal sample. It's a difficult matrix to work with, and contains inhibitory elements that can interfere with molecular detection methods. Long lead times was the norm for wastewater epidemiology.

Can I ask you Rose, how was GT Molecular able to reduce its turnaround times for its wastewater surveillance program?

Rose Nash:

Primarily, through process optimization. When we were originally developing the molecular workflow actually for our home state of Colorado state-wide program, we needed to be able to turn around data within 24 hours of sample receipts. Again, to guarantee that concept of data for action and really exploit that seven-day head start that wastewater can give us.

We heard of communities around the nation that were waiting weeks to get back their wastewater data. In my scientific opinion, that just really nullifies the impact that this sort of testing can have. As we were researching all of the protocols that were out there, we really identified the viral concentration step to be the most tedious part of the workflow, it was very likely resulting in many of these bottlenecks that had been reported up to that point. We reached out to this really fantastic and incredibly supportive wastewater research group that has formed on Slack. We talked to this network, and we heard through the grapevine that InnovaPrep had a solution, and that it could concentrate virus in just a couple minutes per sample.

Of course, this was highly attractive to us, as the alternatives included these two-hour long centrifugation steps, and we all know people only have so many centrifuges. This was a really large bottleneck in the process.

By using InnovaPrep through a rapid viral concentration technique, we combined that with some very strict quality controls, which allowed us to catch failed samples very quickly. We also developed a streamlined data analysis and reporting software. With these things all together, our average sample to result time over the last 7000 samples has been 24 hours, which is something that we're really proud of.

Host:

You heard about InnovaPrep through the Slack working group on wastewater. But before actually adopting the concentrating pipette select, did you do any sort of a comparison or evaluation studies with other methods out there?

Rose Nash:

When we were choosing a concentration method, we looked for three major attributes. First and foremost was speed and throughput. I'm going to sound redundant here, but this was really driven by that idea of harnessing the seven-day window of opportunity. We really needed to be able to turn data back quickly.

Secondly, we evaluated all of the contenders for their supply chain. We all know that supply chain has been a major hurdle for everything COVID related. We needed to make sure that the manufacturers of every reagent and every consumable in our workflow could keep up with our demand.

Lastly, but just as importantly, we needed high performance. We were out to develop the most sensitive technique possible. We needed a high degree of viral concentration and viral recovery, which is something we absolutely found with the InnovaPrep concentrating pipette.

Host:

Over the past year, there has been several papers published on SARS-CoV-2 surveillance in wastewater. They do show a variety of methods for concentrating the virus. Many of these methods such as PEG, skimmed milk, they require multiple steps and reagents. The InnovaPrep concentrating pipette, as far as I know, was based on an ultra-filtration method, like some others on the market.

May I ask, Dave Goad, the senior scientist at InnovaPrep. Can you explain how it works, and how different it is to other similar ultra-filtration methods on the market?

Dave Goad:

First off, to echo some of the stuff that Rose was talking about, the comparison to other methods that have been used. The concentrating pipette gives us significant advantages in terms of time and speed, and again, as Rose said, throughput, in comparison to methods like PEG precipitation, for anyone that's actually doing that, or has done that, they're familiar with the fact that that takes actually multiple days to get done. It takes an overnight precipitation step. Throughput is just very, very poor with that system. It also requires a lot of hands-on steps and so forth.

Other methods, like electronegative filtration processes, those require some significant hands-on steps, they're cost-efficient, but they require some hands-on steps in terms of adjusting pH and adding salt and filtration. Our system has an advantage on all these in terms of speed and efficiency, and above all, automation.

We do use a type of an ultra-filtration process; we use what's called a dead-end filtration process. Rather than using, for example, a flat filter, which most people are familiar with, filtering things through just a flat membrane type filter, our system actually uses a bundle of fibres, hollow fibres, and each fibre actually acts as its own filter. These filters actually give us a lot better surface area compared to say, flat filters.

The comparison I'd like to make is directly to, for example, an HA filter the electronegative filter, which seems to be popular. Our surface area is up to five-fold or sixfold higher, just because we're using bundles of hollow fibres and each fibre acts as its own filter.

For our system, the way our system is set up, if you can envision essentially a serological pipette size plastic device that's about six to seven inches long and it has a bundle of hollow fibres inside. Each of these fibres, as I said, acts as its own filtration device.

Liquid is pulled up through these hollow fibre filters and is captured in the lumen of these fibres.

The key advantage of our system is, we use a proprietary expanding foam technology where once the retentate is captured on the inner surface of these hollow fibres, we shoot an expanding foam down through the bore, and it essentially wipes the surface of the inside of these hollow fibres, and allows us to elute the captured particles in a very small volume. There's a lot of factors that influence this, obviously, there's like a squeegee effect or wiping effect on the surface.

Then the foam, as the tiny bubbles start to pop, there's some turbulent effect on the surface, which also helps release things from a surface, and elute those into a small volume.

Host:

Sounds like a very sophisticated piece of instrumentation.

Dave Goad:

Yeah, and the wonderful thing is our volume size that we end up with. The key for detecting things like virus or even bacteria, or other things, is in a concentration process. The critical thing when you're concentrating virus, or even bacteria, is the elution volume. You want a low volume, so that you maximize the concentration of your target in that volume. An example I like to use is the, again the HA filtration which seems to be very popular. Those require typically a larger volume to elute the sample in. One typical process is those flat filters are folded and they're put into a liquid.

In our system, since we're shooting our elution fluid essentially through the middle of these fibre bores, we're able to get our elution volumes down in the 350 to 200 microliter range. If we start with, for example, 50 millilitre sample, and push that to 200 microliters, you can imagine the level of volumetric concentration we get.

Host:

I know that average size samples go from six litres to perhaps 50 mil. That's right. But with your concentrating pipette, what's the sample size you start with?

Dave Goad:

For wastewater-based epidemiology, we typically start with a sample size of around 50 millilitres.

We do have some customers who are using more than that. But again, if you imagine the difficulty of this matrix, it varies quite a bit, and larger volumes tend to be difficult to process.

Host:

Is it composite sampling that you would recommend to most of your clients?

Dave Goad:

Well actually, our system is suitable for influent from sewage treatment plants, as well as samples that come from sewer access points, i.e., manhole covers. This is where Rose has had tremendous success, as she mentioned the work that they've done in the university in Denver where they were able to isolate dorms. That was actually facilitated by the ability to take samples from manholes and be able to identify hotspots of infection.

For our system that we recommend about 50 millilitres, and that's concentrated down into generally we recommend about 350 microliters. It depends on the user, how much volume they want, that's totally adjustable in the system. But the most important part, if there are other ultra-filtration type systems out there, for example, centrifuge-based ultra-filtration, our system it performs better in terms of time, it takes less hands-on time, it takes less processing time. Our typical runtime in the instrument, if you have a sample that, for example, has been pre-filtered to remove the large particles, is a matter of minutes. Rose could certainly validate that for me. Our actual processing time in the concentrating pipette is on the order of five minutes or less.

Host:

What sort of recovery rate do you get?

Dave Goad:

Well, that's a really good question.

Rose Nash:

I'm happy to talk about that if you'd like.

Host:

Yes, that'd be great.

Rose Nash:

Yes, so because we spike in an internal process control, which is a bovine Coronavirus

Host:

That's your control. Okay.

Rose Nash:

Yeah, we spike that into every sample at a known concentration to be able to adjust our data for the process. How much virus we may have lost throughout the entire process. Over the last 7000 samples that we've processed with the InnovaPrep concentrating pipette, our average viral recovery has been above 20%. To someone who might be tuning in, that maybe isn't processing a lot of

wastewaters, this might sound low. 100% sounds good. But really, the average out there that's been reported has been around 5% or lower.

We have had an incredible level of sensitivity being able to get really great baseline measurements when there's very low COVID-19 in communities, because of this high virus recovery, which translates into a very low limit of detection. We can detect very low baseline levels, because of just how good this instrument is at concentrating virus and recovering a lot of the virus in the process.

Host:

You're using the bovine Coronavirus as the control of your recovery, but you're only getting 20% of SARS. Why is SARS such a difficult viral agent to work with, in your experience?

Rose Nash:

Well, I just want to point out you're saying only 20%. But in a recent publication from the Water Research Foundation, they set their cut off for the different methods at, I believe, 0.1% viral recovery. We're talking orders of magnitude better than that. 20% is really, really great for sensitivity. Why it's not 100% is because, again, this is a very complicated matrix. Samples are filtered through 0.2-micron filters to remove solids. A lot of viruses would rather stick with human waste then go into the aqueous phase. You automatically lose virus at that step.

Host:

Okay.

Rose Nash:

Then, the rest of the processes like extracting RNA, they're not perfect, so you lose a little bit along the way at every step.

Dave Goad:

I presented a talk at the recent World Society of Virology Conference. I demonstrated data using our system and using a 30-minute relatively low speed,. I think it's 3500g clarification step for wastewater influent, and it was spiked with bovine Coronavirus, and I have been able to demonstrate consistent recoveries on the order of 70% bovine Coronavirus and in wastewater samples.

Host:

70% of bovine Coronavirus?

Dave Goad:

70%. A recent publication from the University of Colorado, actually the pre-print, I believe, and I'll ask Dave Alburty to chime in on this as well, if he could clarify, but I believe their recovery rates were averaging around 54% for spiked in bovine Coronavirus.

The system does work well. But one of the keys here is I'm using a pre-centrifugation step, which takes about 30 minutes. Rose is using a much more rapid method, which uses pre-filtration. As she

said that there's probably some loss there. The trade-off is the speed, and she's able to process many, many samples in a short period of time.

I've actually visited her facility and her system is extremely well-designed and very efficient.

Host:

Okay. Any of these methods, the pre-centrifugation step, they can be done by any lab, and it's just the normal centrifugation step.

Dave Goad:

Yes, that's right. If I could, this is a good segue to a product that we anticipate releasing this fall, it's a pre-filter that's custom designed for the concentrating pipette, and this is one that I'm personally excited about.

This pre-filter design is such that it fits on the end of the concentrating pipette, allows filtration of a sample before it enters the actual hollow fibres.

Host:

What's the purpose of that?

Dave Goad:

The purpose is you can remove a lot of particulate material and so forth, and you can do the steps that would involve for example, what Rose talked about with pre-filtration, or if you're doing centrifugation. Those steps disappear, and those are done at the same time that you process the sample with a concentrating pipette. The sample is pulled through the pre-filter and then into the concentrating pipette. It's designed such that once it's fitted to the pipette, you don't need to touch it again. You can process the sample without handling it, without handling the filter or anything else. You end up with the same same type of sample at the end when it's eluted. It's just gone through the pre-filtration and sample processing steps at the same time.

I was able to show actually, again, about 70% recovery with bovine Coronavirus. The actual processing time was on the order of three minutes for 50 millilitres.

Host:

Okay. You've basically replaced your centrifugation step with this pre-filter apparatus for the select pipette, yeah?

Dave Goad:

That's right. We're excited about this, because we have applications even beyond wastewater analysis environmental samples. We anticipate having a range of pore sizes in these pre-filters, but ideally for wastewater it's perfectly suited. If you're familiar with turbidity measurements in water, the turbidity of my samples is around 400 NTU, which is very cloudy, you can't see through it. Again, these are influent samples processed directly with the pre-filter and the concentrating pipette using an ultra-filtration tip.

The processing time was less than four minutes. I believe my results were about 60 to 65% recovery of spiked in bovine Coronavirus.

Host:

Is this a proprietary filter that InnovaPrep have patent on, or is there anything like this on the market?

Dave Goad:

Yes, it is proprietary, and it's custom designed for our system for our concentrating pipette.

Now one caveat here is that the recovery rates that Rose has talked about are based on her system that is extremely sensitive. In order for me to generate these types of recovery rates, typically I have to spike in about 3000 copies of bovine Coronavirus per millilitre. Rose is working with smaller concentration, lower concentrations because her system is actually designed to be much more sensitive.

She uses a digital PCR system which is extremely well suited, and I'll let her explain the advantages of that. She has the advantage of being able to detect very, very small quantities of either spike or actually SARS-CoV-2 in wastewater.

Rose:

I just want to highlight that that difference probably comes from the fact that over those 7000 samples that we've tested, many of them have been grabbed samples, versus these samples from the primary influence.

As someone mentioned earlier on the podcast, those are much trickier samples. There's lower flow, there's more inhibitors present, and so that could be kind of driving our numbers down. But also, we have identified that we do lose virus in our filtration method if we actually add that bovine Coronavirus. After the filtration, we do see those viral recovery percentages up near 70%. But that just doesn't really work with the high throughput nature of our processing.

Host:

Okay.

Dave Goad:

In addition, Paul, that's her method of spiking the sample and then going through the entire process is more representative of what the sample looks like and takes into account. When you're looking at concentration of a sample, a lot of people get really concerned about percent recovery or percent recovery of your target. When in reality, there's other factors at play here, including sample processing steps and pre-processing steps, so forth, and all those need to be taken into account in a what we like to call a true concentration factor, or I guess some people would say a final concentration factor is calculated, taking into account the volumetric concentration that you've got, in other words, for example, going to say from 50 millilitres to 300 microliters, and the recovery percent of your target. But then also you need to take into account losses in your system, for example, from extraction steps, from other preparation steps to generate actual concentration

Host:

Okay. True concentration is just allowing those factors that you've just mentioned there and taking them into account. That's what basically true concentration is.

Dave Goad:

Yes. Dave, would you agree with that?

Dave Alburty:

Yeah, absolutely. Yeah. The concentration factor itself, with the actual recovery efficiency, combined in there is what really gets towards the limit of detection. The lower your limit of detection, of course, given the ability of your analytical technique, combined with that concentration step, and all the other sample prep, is what really enables you to achieve the limited detection that you want that's really meaningful for your project goals.

Host:

Most of the samples that you were receiving Rose, were they composite or grab samples?

Rose Nash:

When we monitor a community, it's always a composite sample that's been collected, not a grab sample, at the primary influence, but we monitored a lot of individual facilities, so dormitories, large employers, penitentiaries, and those were all grab samples. They were often monitored more frequently. We got a lot of our samples were these grab samples.

Our hypothesis for why they're just in general, harder to work with, is that they're often collected when there's very low flow through the system, and oftentimes, basically, the way they do this is they have a beaker or a bucket that's kind of tied onto the end of a stick, it's very high tech, and they dip it down into the manhole and bring the liquid up. But they often get into the sediment that's been settling over many years on the bottom of the sewer. Whereas a wastewater treatment facility sample mostly looks like water with a couple solid chunks floating around. But the samples that come out of a grab are usually almost black, they're really quite gross to work with.

We were so impressed that the InnovaPrep could actually work with those samples, that it could pull up that more viscous and more solids heavy samples, and then we just had to make sure that we were running the appropriate amount in our digital PCR to not see the inhibitors or the downstream ramifications for kind of what comes up with those grab samples.

Host:

One important thing to remember when doing wastewater epidemiology is to normalize your data. By using a control such as human faecal indicator like the pepper mild mottle virus that everyone sheds in feces, which you can use to correlate your viral loads with a probable number of people in the community at that time.

Which of these indicators, Rose, do you recommend, and why?

Rose Nash:

We actually prefer to normalize to an internal process control. Coming back to that bovine Coronavirus that we spike into every sample that we process. Rather than normalizing to a fecal indicator which has some pros and cons that I'll discuss in a minute, we actually normalize to a viral recovery to overcome any variation within sample processing.

We did a study recently where we spiked our internal process control into a liter of waste water, and then we tested that sample from start to finish in replicate of 20. Without normalizing to the control, we saw a relative error across the replicate of about 40%. A 40% deviation from day to day, or sample to sample is enough for a community to raise the red flags that they're having an outbreak.

We really needed our process to be more precise than that. When we normalized those 20 replicates to the viral recovery, the relative error actually came down to just 4%, which is outstanding.

Our normalization technique is based on the percent viral recovery for the sample. We do however also test PMMOV, which is the pepper mild mottle virus, as well as an F-specific coliphage, these are two viruses that associate with the coli within our intestines. We use these to normalize for fecal material. What we found across testing in a hundred different sites is that each site really has a signature fecal content, so it varies very little from day to day.

Instead, we use those fecal measurements as more of a QC check to look for any large variation from day to day. The few times that we've seen that, we've been able to attribute the change to some dilution event that happened. For a small mountain town that we monitor here in the state of Colorado, they had a river flooding that infiltrated their wastewater treatment facility, and we saw a large dilution effect which we found through less of those of those fecal indicators, so it's really quite powerful that way.

I also like to point out the current recommendations by the CDC for wastewater monitoring actually calls for both of these, both the recovery and the fecal indicator normalization for these reasons.

Host:

Okay. For Dave Alburty. Wastewater epidemiology seems like a very reliable non-invasive methods for public health agencies to curb outbreaks and for the surveillance of pathogens be it clinical or field-borne. To what extent has this been done before the SARS-CoV-2 break? In your opinion, will wastewater epidemiology be given more attention post COVID?

Dave Alburty:

Well, yeah, we've been in a battle with these pathogens for millennia, whether we've known it or not, and we've only just now really developed a secret weapon that we can use to help find these pathogens in our environment, determine what they are, help us decide what to do about it, and then measure our effectiveness in our response. This started out probably recently with the dawn of rapid microbiological methods in the 80s looking for poliovirus in wastewater.

Wastewater epidemiology for viruses really started with the ability to use rapid microbiological methods that began in the 80s with PCR, for example, as Rose uses it. It was used to monitor for poliovirus in the environment to help monitor the effectiveness of the polio vaccine. That's led to some of the uses for monitoring for SARS-CoV-2 in our wastewater now. It's that same sort of approach. It evolved from looking at environmental waters, raw sewage waters for these viruses and bacteria and other things that are in an environment in a place where the concentrated samples can be found, which is the sewage.

They exist in classical methods and microbiology aren't sensitive enough to find these viruses really in the clean water without some amazing sample prep and sample enrichment or sample concentration steps. The rapid sample concentration that we've developed, aids the people like Rose

to really fully realize and use these rapid methods to find these viruses and find these bacteria or other pathogens in our environment, and let us know what those are.

There's a big future in this as this technology continues to evolve, and people want to look for additional pathogens or even future outbreak strains.

Host:

There's been a lot of research over the last 10 years into the microbiome It's logical to think that this is the new era that we're entering into wastewater epidemiology, and it will be given far more attention.

You see it continuing into the future. Definitely SARS-CoV-2 surveillance, but for other pathogens too?

Dave Alburty:

Yeah, I think so. You mentioned the non-invasive nature of it, and the fact that it's a population surveillance tool. Nobody needs to consent to be monitored, there's no trouble, you don't need to go into the lab, there's no painful swabbing. The cost per sample, for those implementing these techniques, is extremely cost effective.

Host:

I'm surprised that it hasn't been given greater attention than it's been given now. I mean, researchers are now saying the samples that we're getting, it's a treasure trove of information, that they're picking up DNA, RNA in terms of sequencing. Has it been done to a large extent, other than detecting polio?

Dave Alburty:

This is really the dawn of the widespread use of these techniques, I think, and many times there's a watershed event. We've had several other pandemics recently. These techniques like Rose are implementing just haven't been as widespread, accessible or cost effective as they are now. Now an instance like we have with a global pandemic of this magnitude, once a tipping point is reached, and a technology does become a little bit more in the popular press and everybody poops, so news stories are interested in that, and it's newsworthy when people like Rose stop a pandemic event in its tracks at a university dorm, for example, and allow the university dorm to stay open or the university itself to stay open.

These are more eye-catching kinds of things that some people kind of latch onto, and then they can really utilize and extend in their imagination to other ways they might use the tech.

Host: Rose, just on to the instrument, the technology that's available to you at the moment, you've chosen the digital PCR as your detection method for the GT Molecular surveillance service. Why have you chosen this over RT-PCR?

Rose Nash:

At GT-Molecular, we pride ourselves with using the most cutting-edge technology available, which is of course why we use the InnovaPrep CP, along with digital PCR for analysis. There are two major

benefits for digital PCR over RT-PCR. One is that it's less affected by inhibitors, which is, of course, what everyone talks about when talking about wastewater. But I want to break that down a little bit.

Host:

What's an inhibitor? What's an example of an inhibitor?

Rose Nash:

When you think of a clinical sample, first, let's talk about a sample that's pretty clean, a pretty simple sample like a nasal swab. In that sample, you've got virus and maybe some snort. That's pretty simple stuff.

Now if you think about wastewater, we have human stool, which is chock-full of all kinds of bodily by-products, full of hemes and all kinds of nasty stuff that can inhibit these really intricate enzymes that are involved in the PCR amplification and that quantification step.

In this aspect, digital PCR has outperformed RT-PCR over and over again, and that's because digital PCR is actually an endpoint measurement rather than being reliant on the actual enzymatic kinetics, which RT-PCR relies on. There's been several publications cited in the literature as well as our own evaluations that have shown that digital PCR either by QIAGEN, or by Bio-Rad, is just superior at performing with these inhibitors present.

Host:

For someone that perhaps can't remember molecular 101, can you just remind me the differences there, the endpoint for digital PCR and RT-PCR is based on kinetics, can you just explain that for me, please?

Rose Nash:

What we measure in digital PCR is the number of droplets or the number of partitions that are positive or negative. That's where you get that digital concept. Zero or one, positive or negative. Then you count how many positives you have ratio that to the negatives, and you actually get an absolute measurement of the amount of virus that was present.

Whereas with RT-PCR, you're actually monitoring the kinetics of the amplification of the nucleotides. You're looking at the cycle number in which amplification overcomes some threshold. That's a kinetic measurement. You're watching the PCR amplification.

The process of those kinetics can very easily be affected by the presence of inhibitors or different components within the reaction. With digital PCR, we get that yes or no, zero or one measurement, and it's after that PCR amplification has occurred.

Host:

Okay, and you provide digital PCR kits, and you also provide RT-PCR kits under the GT Molecular brand for labs that want to carry out their own testing, is that correct?

Rose Nash:

That's correct. In January, when we started monitoring the UK variant in wastewater, we were actually the first to detect the variant in wastewater in the United States in early January, we realized that even though we had this service, and no one else had this service, that communities were slow to switch testing labs, because there's been such an emphasis on monitoring trends over time within wastewater, and it's very disruptive to change labs. Even though we had this service that other people didn't have, people didn't want to change.

We realized we could get more variant data out there if we actually started selling our own tests and our own reagents to our competitors, really, but other laboratories that were doing this type of analysis. We started doing that. We started selling our reagent kits. We did that for both digital PCR, for both the Bio-Rad and the QIAGEN system as well as RT-PCR.

Host:

You would prefer digital PCR for wastewater samples?

Rose Nash:

Absolutely.

Of course, there's a drawback to digital PCR in that it's fairly expensive to onboard, the equipment cost is very high. However, there's several newcomers coming into the market right now that have digital platforms that are more affordable. I'm excited to see that barrier of entry come down for the community.

Host:

Okay. If you're providing digital PCR information from your wastewater samples, and the public health officials are using RT-PCR for the clinical results, is there any problems there? Is there any difficulty in correlating the results that public health authorities need to do?

Rose Nash:

I don't think so, because most clinical tests are positive or negative readouts. Most of them are not quantitative. I don't think that's very much of an issue. Secondly one benefit, the secondary benefit I wanted to bring up is that digital PCR provides an absolute measurement. What that means is it doesn't rely on reference material or a standard curve to convert a CT value to a concentration. With a relative concentration method like RT-PCR, you're really only as good as your standard material. We've seen some dramatic deviations between the concentration that some manufacturers report for their reference material, and the actual quantification that we perform on our absolute measurement system.

There are a lot of areas in which error can creep into measurements when using a relative concentration method.

Host:

Okay. From a public health perspective, knowing which SARS-CoV-2 variant is the dominant strain in a community has become an important tool to raise public awareness. How does the GT molecular service enable the surveillance of variants in the community?

Rose Nash:

In two ways. First, we offer a variant monitoring service to communities around the United States. In this program, a community will send us a wastewater sample from their wastewater treatment facility. We analyze it for the key mutations that define the current CDC and WHO variants of concern, and we provide it that way. Then secondly, as I mentioned before, we identified early in the pandemic that communities didn't want to switch labs. We also provide our kits to other testing labs, so they can provide this data as well.

We've seen some really interesting examples of viral evolution through this process, we saw the UK variant, which is now referred to as the Alpha variant, first arrive in the United States, in Florida, in the very beginning of 2021, and then we saw it become the dominant strain over just two months, which is a really nice example of a virus having a fitness advantage through increased transmissibility and to see it sweep the population.

Then later in the year, I believe it was May that we saw the Delta variant start showing up in communities, and it outpaced the UK variant, or that Alpha variant very quickly. That is likely also due to even further increased transmissibility of that variant, we can actually watch these variants arrive in communities, and then become dominant. We often detect these variants for several weeks before public health gets their first positive result for these variants from swab testing, which really highlights the benefit of this type of surveillance.

Host:

You can see the benefits. I know the Delta variant really did take over many countries in the EU so fast. I mean, you'd hear it on the news, and with a matter of days it was 90% of the strains being detected. But you can see, especially during a pandemic, if you're able to detect a variant early, you're able to develop a clinical-based test to detect that variant in a patient when they arrive at hospital. You can see definite benefits to waste water epidemiology.

For Dave Alburty, with the start of the century, you saw a need for faster, more efficient concentration methods to suit the molecular detection methods, which were obviously rising in popularity, and you launched the InnovaPrep concentrating pipette to meet those industry needs. Can you see ahead now and tell me what the future holds for InnovaPrep?

Dave Alburty:

Sure. Well, really the concentration factor that's provided by the CP, and the fact that the technology that underlies that is easy to automate and integrate with other technologies fits well within the workflow and can eliminate a lot of steps. I see us as kind of like Spotify as to the music industry. They bring all these ears to the music companies and they can sell lots more music.

We bring the ability for people in many different fields to actually utilize lots more kinds of samples that couldn't feasibly be analyzed before, because they were too dilute. They took too long to enrich, or for whatever reason. That concentration factor coupled with the incredible sensitivity of new, modern rapid microbiological techniques, is a powerful thing.

We're addressing several additional markets growing out of wastewater-based epidemiology. Some of them are a lot like wastewater-based epidemiology, aquaculture. The water that fish swim around in and breathe and poop in and everything is a lot like wastewater in lots of ways or a lot like environmental water.

This is true for environmental monitoring, air and water samples, also several kinds of food and food products, beverage products, and in product safety. Then there is a world of fields where people really have that need for speed foremost. The concentration factor enables that but the speed for bio-surveillance and bio-defense, for example, in human health or in animal health, that's where we can really enable people in the laboratory to not only do better work, but do it a lot faster.

Host:

Okay. Thank you, Dave Alburty, Rose Nash, and Dave Goad for joining us today.

Dave Goad:

Thank you.

Dave Alburty:

Absolutely. Thank you, Paul. It's wonderful talking with you.

Rose Nash:

Thanks so much.

Host:

We are definitely entering what you might say is the golden era of wastewater epidemiology. As long as our sentinels are being wise with the information provided by surveillance, we should be able to avoid the suffering that happens when virus gets out of control. Wastewater epidemiology is a dirty job, but someone has to do it now and for a long time to come. Thank you, the listener for joining us today. Until next time.