Bin Guan, Ph.D., FACMG and Robert Hufnagel, M.D., Ph.D.

Behind the Mask

October 4, 2021

Barr: Good afternoon. Today is October 4, 2021. My name is Gabrielle Barr, and I'm the Archivist at the Office of NIH History and Stetten Museum. Today, I have the pleasure of speaking with Dr. Bin Guan, who is a Staff Scientist in the Ophthalmic Genomics Laboratory at the National Eye Institute (NEI), and Dr. Robert Hufnagel, the Chief of the Medical Genetics and Ophthalmic Genomics Unit as well as the Chief of the Ophthalmic Genomics Laboratory, also at the National Eye Institute. Today, they're going to be talking about some of their SARS-CoV-2 research and experiences. Thank you very much for being with me.

Guan: Thank you for having us.

Hufnagel: Thank you, Gabrielle. Thank you so much.

Barr: To begin, what were some of the complications of detecting SARS-CoV-2 in what is now considered to be the "conventional" way?

Guan: SARS-CoV-2 is an RNA virus. The conventional way was to purify or extract molecules from a specimen first before real time qualitative PCR [polymerase chain reaction]. The RNA extraction step is time consuming. Because of the pandemic, we have done a lot of research—and the whole world has done a lot of COVID-19 testing—so the RNA extraction reagents were in fairly short supply at some points. In addition, the use of plastics in testing, such as columns and tips, could also be an environmental waste, so we hope to be greener in our environment.

Barr: I would not have thought about that. That's really nice. What is chelating resin, and how does Chelex, a chelating resin, improve the process of testing for SARS-CoV-2?

Guan: Chelating resins are polymers that have chelating groups attached on them. Chelating groups on this resin have very high affinity for transition and heavy metals such as calcium, magnesium, and copper. Chelex stands for "chelating ion exchange resin" and can be used for removal of the transition and heavy metal cations from groundwater or other physiological fluids. In molecular biology, it is generally used for DNA extraction for use in PCR amplification because of its ability to chelate magnesium and calcium. These metal ions are essential factors for Dnase activity. It is also presumed to be able to absorb cell lysis products that interfere with PCR. In the COVID-19 testing, we hypothesize that the resin can prevent RNA from degradation by inhibiting RNase [ribonucleases]. The RNase enzyme is also dependent on magnesium for its optimum activity.

Barr: What made you think that could be the case—that the Chelex could do that and that the standard testing process could be simplified?

Hufnagel: The Chelex is protecting the RNA from degradation so that it can be detected. The conventional way of doing it is to extract the RNA quickly so that it can't be degraded and isolate it from everything else. In this way, we aren't pulling the RNA out of the sample, we're simply putting something into the sample to protect the RNA so that we can do the test on the sample itself. That's why we thought that would save time and energy—

and cost—because you don't have to buy an RNA extraction kit, you don't have to have somebody pulling the RNA out of the sample and isolating it via the extraction, and you also save time in not having to do those things. This permitted for us to collect the sample in a new type of media with this Chelex added, and then do the test directly from there.

Barr: When and how did you all begin showing the RNA extraction free method could match or exceed the sensitivity of standard test?

Hufnagel: We knew we had the ability to do this in collaboration with the Department of Laboratory Medicine at the Clinical Center. Dr. Karen Frank is the Director of all of the clinical testing that happens at the NIH Clinical Center in Building 10. We were able to synthesize or make synthetic samples in the lab. Bin was able to compare his method to the method used by the CDC [U.S. Centers for Disease Control and Prevention] to show that they acted similarly in a synthetic or an artificial situation where we knew exactly how much virus was going into all the samples. Then we were able to work with the Clinical Center where they were doing the live testing, and they were doing it at the standards that any clinical lab would be doing this as. We were able to work with them to test Dr. Guan's method with Dr. Frank's laboratory performing the CDC method, side by side, all on the same machines and by the same personnel. We were able to get the samples from the National Institute of Dental and Craniofacial Research Institute (NIDCR) from Dr. Blake Warner. He was one of the doctors in the car line collecting samples from volunteers who were coming in every day. It was a really fantastic collaboration between all these different Institutes and investigators and laboratories so that we could do this. That was really the only way we could have really compared these in what we would call "live testing."

Barr: How long did it take you all to set up your experiment and perform your analysis?

Guan: Initially, we saw very promising results in early June 2020. NEI submitted a patent based on our preliminary data in August 2020.

Barr: Congratulations.

Guan: Thank you. We don't know whether it will be approved at any time soon. This is the provisional stage. It took us much longer to collect all the patient samples. The last batch of patient samples was collected in December of 2020. Then we spent a few weeks writing the manuscript and put the data on medRXiv.

Barr: In additional to looking at Chelex, you also looked at other buffers in your research. Can you talk a little bit about how you went about doing that and how it compared?

Guan: I guess you are talking about other RT-qPCR methods without conventional RNA extraction. For this purpose, we compared our method with SalivaDirect, a method developed by a group in Yale University. We found that our method is comparable to their method in terms of the simplicity. We think our sensitivity is a little bit better than theirs. There are a few other methods, like commercial products that are available on the market. But we did not compare those products with ours, because their published lower limit of detection looks to not be as good as ours.

Barr: That's interesting. Were you guys surprised at how well your research went? Were you surprised by your findings at all?

Hufnagel: You always set out with a hypothesis, but of course, a lot of experiments simply don't work. We do try a lot of experiments that don't work, and this is one of the ones that did. We're always ecstatic when something sticks. But Bin had a really, really great rationale. Bin actually has a great story he should tell, about his experience with Chelex when he was an instructor for college students.

Guan: Oh right, yeah. I got to know Chelex back in graduate school, around 2005 or so. I was teaching a molecular biology lab for undergraduates. At that time, the lab used Chelex to extract the DNA from mouthwash in a classroom. That product was commercialized by Bio-Rad and they called it InstaGene Matrix. It was likely named that because it's sort of instantaneously prepared the DNA for gene analysis.

Barr: How did you guys get into this research, considering some of the other research you do?

Hufnagel: We specialize in the extraction of DNA and RNA from patient samples. We extract DNA from patient blood, from saliva, and we have even extracted DNA from pathology samples that have been archived at the National Eye Institute. We have a lot of expertise in the detection of these nucleotides that are basically salts. We understand a lot of the things about handling these so that they don't degrade. That was the sticking point in Bin's mind—that we have these discussions about RNA extraction. We know we have all these kits in the laboratory, and we're always trying to reduce the costs and the workflow. RNA extraction kits are a cost, and they take up space in the lab. We were wondering about some of these direct detection things that might be more broadly applicable to other things. Chelex had been used for DNA previously, and so Bin thought to apply it to RNA, and now we're thinking about how to apply Chelex to other things. Can we detect other kinds of viruses? Can we detect other kinds of RNAs, like our own human RNAs? Can we use Chelex for that sort of benefit? One of the nice things that Bin's paper also shows is that Chelex permitted for shelf stability when in saliva. It also makes you wonder about being able to collect samples out in the field and then leaving them in your bag and shipping them. Maybe it takes a few days, but you know that the RNA is going to be stable. Whereas in a lot of other circumstances, we try to extract the RNA as quickly as possible so that the enzymes don't get at it and break it down. In this case, Chelex may allow for some extra time to get samples from remote locations into a laboratory, where they can then be safely extracted and detected. This, again, may be for viruses, or other organisms, or even our own DNA and RNA.

Barr: That's a really exciting possibility.

Hufnagel: Yes. We'll be testing out some of these, so we may have to meet with you again soon to talk more about it.

Barr: That would be wonderful. What were some of the obstacles and limitations you faced in your initial study?

Guan: Our supporting teams were very helpful, such as out Purchasing Agents and the committee in charge of research related to COVID-19. We got approval very quickly. Other than that, probably the biggest obstacle for us was to collect paired patient samples to compare our method to the gold standard CDC method.

Barr: You've already mentioned some people on your team, but can you talk about what each of your roles were and what you each did with this study?

Hufnagel: Bin did all of the actual experimentation. He designed the synthetic samples. He coordinated the transfer of samples and testing of samples between our labs and the clinical laboratory, and with getting samples from Dr. Warner at NIDCR. I would say my role was mostly as a sounding board for Bin's great ideas. I

felt like my job was to help remove some of the obstacles that Bin was talking about. Even though we're doing this in the time of COVID, we of course have to get exemptions to come in to do research related to COVID, emergency orders to get reagents quickly, and get special approvals for our laboratory to be able to use these viruses under safe conditions. And [my role was] also to put Bin together with the right partners to be able to execute his vision. We played very complementary roles. Fundamentally, it's Bin's idea and Bin's credit for putting everything together.

Guan: Yeah. Of course, we had a lot of great discussions along the way.

Barr: When do you think this method, that you all have shown to work, will be used in the clinic and labs around the world?

Guan: That's a really good question. We are in talks with a few diagnostic companies. We hope to be able to convince them that this is a good test and it's worth the investigation.

Hufnagel: The provisional patent is filed globally or in multiple countries. We're hoping to have the opportunity to talk to companies in multiple places that have a need for the advantages that this would provide for them. Hopefully, in the U.S. and in other countries as well.

Barr: That's exciting! How much time and resources does your process save in comparison to the conventional way of doing the COVID testing?

Guan: For each sample tested, the RNA extraction takes about 30 minutes or more. Using this method, it can be done in less than 10 minutes. We're talking about saving at least 20 minutes of time. In terms of cost, the cost depends on how and where you get the RNA extraction reagents, so we're thinking around $5.00 saving for each test.

Barr: That adds up, though. That's very interesting.

Guan: It seems like $5.00 is not much for a single test, but when you think about millions of tests were done around the world, it could be significant.

Barr: You said that it's more stable than some of the conventional testing. How much more stable?

Guan: Well, unfortunately, I probably can't tell you a good answer for that. Being in research labs and also in a clinic, when people collect the sample, they try to put it the refrigerator first or they will freeze the sample at minus 80 degrees. After the RNA is extracted, the RNA samples are also typically stored at minus 80 degrees. With our method, we think it can potentially help store [the sample], for some of these steps, at room temperature. That may help expand the testing to more laboratories, so a lab doesn't have to find very expensive minus 80-degree freezers for these samples.

Barr: What are your next steps for this research? Scientifically, what do you hope to do next with it?

Guan: One area we would like to try is to expand the downstream application for this RNA preparation method. For example, at this point, we have not tried whether it will work with RNAseq, which is a whole genome scale transcriptome analysis method. We hope to test that soon.

Barr: That's exciting. Have either of you been engaged in other COVID-19 research projects or initiatives, either at NIH or outside of NIH?

Hufnagel: Not directly. Being at the Eye Institute, there are plenty of other labs that are studying COVID in various ways. Sometimes people ask us advice about how to detect the RNA or advice around other things that we might study in the lab, like single cell sequencing or other types of RNA sequencing. I don't have any formal involvement with any of the current studies. Actually, I take that back. The other area that I was involved in was in a questionnaire. It was a large survey of patient groups with rare diseases. This was working with Dr. Beth Kozel and Dr. Tiffany Powell-Wiley from NHLBI, the National Heart, Lung, and Blood Institute. What we wanted to do, and did, was to send a questionnaire out to many family groups of patients who have rare diseases. We wanted to understand if patients with rare diseases had different experiences with COVID infections or the severity of infections compared to their family members. We wanted to understand if there were things about that that we were potentially missing in the care of our patients with rare diseases. I'm a pediatrician and geneticist by training so I wanted to work with them. This is led by Dr. Beth Kozel, and I wanted to work with her and Dr. Tiffany Powell-Wiley to really try and understand if we were missing anything in terms of taking care of patients with rare diseases in the time of COVID.

Barr: Very interesting. In addition to being scientists during the time of COVID, you're also human beings that are experiencing both the good and the bad. What have been some personal opportunities and challenges for you both that COVID-19 has presented?

Guan: This is an interesting question. There are a number of personal challenges. The kids cannot go to school, so they are taking Zoom classes at home. It was very challenging to manage that. In terms of opportunities, I guess it also made me think more about [old friends], who you are not interacting with in normal times. We are spending more time on the social networks, and that probably made me connect with my old friends more often.

Hufnagel: I say for me, as the group leader for the labs, I was very much wanting to make sure to keep everybody safe. We immediately changed the way that we did our work. People were granted the maximal flexibility to come in when they were able to safely, so we were setting up schedules where we only have one or two people in our lab space, given the space constraints—and then working around these schedules to also make sure that we were maintaining some productivity virtually. As Bin mentioned, a lot of the folks in the lab have families, so their whole work-life combination switched, because their children are also in the next room on Zoom meetings all day while we're on [Microsoft] Teams meetings. We really have to work around that so that we can take care of ourselves, take care of the lab, and take care of our families—with the families being the most important, of course. We really adapted quickly to the virtual experience, as Bin said, and then we were able to focus on things that maybe we hadn't been able to focus on before that. We wrote a lot of the manuscripts that we had been meaning to write and it helped us to focus and think about the final experiments, or which papers were actually done and ready to be published. We were able to publish a lot of our papers. Then we were also able, as Bin mentioned, to think about what the experiments are that are truly important—and important to us. This was Bin’s passion project, and so he really took off with this and was able to get a really amazing project completed in a pretty short period of time despite everything.

Barr: It is really impressive that you designed it and executed it in just a few months.

Guan: Thank you.

Barr: Well, is there anything else that either of you would like to share about your research or about your COVID experience?

Hufnagel: I think you've covered everything really, really well. I don't have anything personally. Bin, do you have anything else?

Guan: No, I don't.

Barr: Well, thank you very much. I wish you guys the best with this research. It's very interesting, and it'll be really great to see what comes of it.

Guan: Thanks.

Hufnagel: Thanks, Gabrielle.

Barr: I also hope that you all stay safe with your families as well.

Hufnagel: You too.

Guan: You too.

https://pubmed.ncbi.nlm.nih.gov/34396082/