NEB Podcast #25 - COVID-19 Researcher Spotlight Series: Interview with Brian Rabe
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Transcript

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Interviewee: Brian Rabe, *Ph.D. candidate, Harvard Medical School*

Lydia Morrison: Welcome to the Lessons from Lab and Life podcast. I'm your host, Lydia Morrison, and I hope that this podcast offers you some new perspective. This is the first episode in our COVID-19 Researcher Spotlight Series. And today I'm joined by Brian Rabe, who's currently a graduate student at Harvard Medical School where he normally studies retinal development. As the coronavirus spread, Brian decided to take some of the new technologies he'd been using to explore gene regulatory networks, studying tiny little embryonic mouse eyes and apply them to improving and advancing Coronavirus detection methods. Hi, Brian, thanks so much for joining me today.

Brian Rabe: Hi. Thanks for having me.

Lydia Morrison: So I was wondering if you could tell us a little bit about your recent medRxiv publication.

Brian Rabe: Yeah. So, it's kind of funny. I usually study actually retinal development, but given the current pandemic, and I'd heard about this RT-LAMP technology from NEB, I decided I would try my hand at it before we close everything down. And I got some promising results initially, so I actually got to stay on and pursue it.

Brian Rabe: It's nice because it's essentially an alternative diagnostic assay for the virus that's proven to be quite sensitive, and it's really, really fast. And what's nice is you don't need any specialized equipment to run it. So unlike a lot of the qRT-PCR tests that are available that need really expensive machines and a lot of personnel training to run, this is an assay that could be run, potentially, in a van with a heat block. So we really wanted to increase accessibility of testing, and I think that's what we've been able to achieve.

Lydia Morrison: Yeah. So how are you able to achieve that?

Brian Rabe: Yeah. So it began just, really just ordering these RT-LAMP reagents from you guys, from NEB, and then designing several different primer sets, so, designing them to hopefully work out. It can be a little hard to predict which assays will work and which ones won't. And we got lucky, and actually the first one I designed worked really robustly. It works very quickly. So the amplification happens very, very rapidly, and it was a clear readout. And importantly, it had a very low background. So a positive result with this set of primers does appear to be, pretty much 100% of the time, a real positive, which was definitely necessary if we want to move into the diagnostic field.

Lydia Morrison: Yeah, that's fantastic. So how quick is quick?

Brian Rabe: From actually adding your sample to the reaction, it only takes 30 minutes and then you get a very clear readout. I think sort of peeking at reactions as they run, a lot of them will turn even sooner,

but for maximum sensitivity, 30 minutes works really well.

Lydia Morrison: That's great. And what kind of samples are you working with?

Brian Rabe: Well, for my work, it was all reconstituted samples. So I work in a biology lab, in an academic lab. And especially as this whole pandemic was progressing, there was no way I was going to be getting direct access to patient samples. I'm not medically trained and not at a hospital. So what I was working with was an RNA control that came from actually Twist Bioscience, and then I reconstituted samples. It basically means I stuck a swab down my nose many, many times to make, essentially, samples, whether they were nasal pharyngeal or oral swabs, or just collecting saliva in a tube. And then I would spike in these control RNAs to sort of serve as a fake virus in these samples, to see would the reaction still run with things like mucus from a nasal swab or saliva, to see how well they would run and see how low I could push the amount of RNA that I was adding and still get a result.

Lydia Morrison: And you mention that ease of the readout and the color change. Could you tell me a little bit more about what the readout in this LAMP reaction is?

Brian Rabe: Of course. So one of the really nice things about LAMP is that it generates enormous quantities of DNA. And the quantity generated is so enormous that every single base being added kicks off a proton, and with so much DNA being added, or being polymerized, you end up actually resulting in a huge change as long as your solution is buffered.

Brian Rabe: So the way this whole system works, and you can read about this more on the NEB site, is it's a non-buffered reaction with a pH indicator dye that turns from red to yellow when the solution is acidified. And it makes the readout really, really clear. I mean, it goes from being a sort of purple-ish red to being a bright yellow. And the nice thing with this primer set is it's really been very binary. There've been some reaction or primer sets in the past that people have been trying, but they sometimes get these sort of orange reactions that are kind of right on the line. The nice thing about this primer set is that it's been really, really binary.

Lydia Morrison: Oh, that's excellent. And how did you get involved in this project?

Brian Rabe: Oh. Well, a lot of credit there goes to my principal investigator, Connie Cepko. Basically, I knew about this, this reaction mix that you guys have been making for awhile. I'm a molecular biology nerd, so I read through catalogs from all these companies and I like to keep up to date with what's going on. I remember seeing them and thinking, "Oh, that's really cool. I'd love to try that sometime," even though it has nothing to do with what I normally work with.

Brian Rabe: But then this whole pandemic hit, and I realized that maybe we were a little behind in developing new tests. So I just went to my boss and I said, "I have this idea. Do you think I could try it?" And being the supportive mentor that she is, she said, "Go for it."

Lydia Morrison: That's awesome. And how do you, addressing sensitivity with the assay?

Brian Rabe: Yeah. What's nice is that the primer set that we developed appears to be really quite sensitive, at least when I'm using RNA controls. The trick when we went over to clinical samples with our clinical collaborators over at MGH is a protocol, sort of a sample preparation step that comes before the assay, a really rapid step based on a protocol that actually came out of the Broad, called HUDSON, that does a couple of really neat things all in one step.

Brian Rabe: It inactivates all of the virions in this sample. You're basically boiling your sample with a few chemical additives. And that's really nice because it makes the sample safe for the people who are actually running all the tests. At the same time, it also breaks open all of these viruses so that they actually release the genomic RNA, which is what the sample is or what the reaction is actually testing. So it makes the RNA accessible to the enzyme.

Brian Rabe: And then it also inactivates patient enzymes, samples things like RNases, that would immediately destroy that RNA under normal circumstances. This protocol also completely inactivates them, and we found that the sample is actually quite stable after that. And so the nice thing there is, that alone, combined with the primer set we've been working with, has given us a really impressive sensitivity down to, say, 40 virions per microliter.

Lydia Morrison: Wow.

Brian Rabe: Yeah, it's been really impressive. We've been really grateful for our clinical collaborators' help in determining that. And what's, I think, is sort of next in the research pipeline, although not so much what I'll be doing, but what a lot of people are looking at now, is what level of sensitivity do you actually need in order to identify people who are infectious? I think there's growing data that, of course out of an abundance of caution, we've been... Anyone who's had any sort of positive with the qRT-PCR tests, which are even more sensitive, that we're considering them to be infectious, but we don't really know.

Brian Rabe: On the other hand, we also developed a really crude purification and concentration protocol that uses some really widely available reagents, things like basically glass powder that's used in the ceramics industry, and nifty little protocol that's really, really cheap and really easy to run and, in most cases, doesn't even need a centrifuge, and can allow someone to further concentrate the RNA that's found in a sample to the point that you can increase that sensitivity down even one virion per microliter. At least, that's what it looks like in with the RNA controls.

Brian Rabe: And what's nice about this, and sort of why we were able to do it, is the LAMP reaction has proven itself to be very, very robust to a lot of things that would normally inhibit enzymatic reactions. And so we're able to do this really fast and cheap and thorough purification, and the reaction is still able to tolerate any small amounts of inhibitors that get carried through. And that's been really impressive.

Lydia Morrison: Yeah. I was going to ask about that. So the additions of chemical agents to help lyse the cells and prevent RNA degradation, those don't have any effect on the color change or the buffers or the other components of the reaction?

Brian Rabe: No. I had to fiddle around them to make sure that the reagents I was using were at the proper pH when they're added, but luckily they don't appear to have enough buffering capacity or anything like that, to throw off the reaction.

Lydia Morrison: Well, that's incredible. So what kind of samples have you tested so far? I know your paper was mostly focused on nasal pharyngeal swabs, either sort of with or without this RNase protection treatment, but what other... Have you tried other samples, or is that something that's coming in the future?

Brian Rabe: Yeah. So I've actually tried, and I think this is something as far as clinical samples, that's in the pipeline, but I've tried with reconstituted samples, straight saliva.

Lydia Morrison: Wow.

Brian Rabe: And that's been something elsewhere where LAMP has, I think, outperformed certain other tests, in that the saliva doesn't appear to be particularly inhibitory to this reaction, especially when you run this inactivation step first. I think a lot of other reaction types that are sort of assays that are being developed, tend to be much more sensitive to those, but the two enzymes in LAMP appear to be incredibly robust.

Lydia Morrison: Wow. That's amazing, and really promising to hear. So where do you see COVID-related research heading in the near future?

Brian Rabe: That's a good question. And I've been talking to a lot of people who are involved in this. Well, on the one hand we're sort of creating... There's a group led by Mike Springer in HMS that's going to be comparing a lot of these assays head-to-head. So there will be several different LAMP assays. So the primer set that I developed as well as some that have been developed at NEB and Cornell, and I believe at least one or two other places.

Brian Rabe: Then Mike Springer's lab has come up with an RT-RPA based assay, and then there's a CRISPR based assay coming out of the Broad, although those either have LAMP or RT-RPA backends. And so they're going to be testing those head-to-head with the exact same clinical samples in order to get a really good idea as to how they compare.

Brian Rabe: Outside of the actual diagnostics, There's also been a lot more work and more attention being paid to sort of upstream, everything from, "Can we get enough swabs? So do we need to go to saliva, or can we stick with swabs," to, "What sample types actually have enough virus in them to detect well, when you swab other places?" Again, can you just have someone spit in a tube? And also sort of perfecting a lot of the collection protocols.

Brian Rabe: We've been talking a lot about, "What can a patient do themselves so that they reduce the risk of infecting whoever is testing them?" You know, obviously we think the patient can spit in a tube on their own, and probably close it and spritz it with disinfectant. So we're looking at things like that. And this is sort of leaving my wheelhouse very, very quickly, but there are a lot of people who are focused on this.

Brian Rabe: And then downstream of this, people are looking at ways of sort of connecting these results to contact tracers and things like that, so that we can hopefully contain any potential sort of little outbreaks that may occur in the future. And then there's just, how do we deploy these things? Do we collect at a lot of different places and send samples to one centralized location for testing? Do we send sort of little pop up labs to a bunch of different places? Things like, if you were worried about a school, can you send a team in a van with heat blocks to a school and have them run tests? How do you do surveillance? Things like that. So there's a lot of thought being put into that, as well.

Lydia Morrison: Well, that's really interesting. Yeah. I often wonder when we're going to be at the point where there's sort of a home test that someone could do. Someone could spit in a tube at home, and could that tube potentially contain the right reagents? Or, maybe you need to aliquot some specific volume. Do you envision this being something that eventually could move into home use, or maybe you spit in a tube and put it in the mail?

Brian Rabe: Yeah, I think any of those are good options. If you have a sous vide at home, it's very easy. We've actually tried that, just an immersion heater in water, and you set it to 65 and that's really all you

need. So yeah, I think a lot of people have been looking at how you would sort of formulate a kit or maybe a device, to make it easy to run. But yeah, I think a lot of people are looking at at-home diagnostics.

Lydia Morrison: Yeah. And I'll be really interested to see that study from the Springer Lab of HMS come out because, certainly, there's been a lot of fast and furious movement in terms of developing diagnostics to detect COVID. So it'd be really great to sort of see those compared in terms of specificity and sensitivity.

Brian Rabe: Oh, absolutely. And I have to give a lot of credit to Mike. He's put together a great group. And he's really, really built a sense of camaraderie. The way he sees it, his tests may work in a different way than ours, but he likes to think of us not in competition, but as sort of supplemental to one another. We have assays that work in different ways. They may have different pros and cons, and really importantly, different supply lines. So potentially, if we need to be producing millions of tests, tens of millions, even hundreds of millions a week, if we're thinking worldwide, having a number of different tests that are being made in different ways could be really helpful.

Lydia Morrison: Yeah, certainly. And I think that that's a great example of the global camaraderie that's really come out of developing diagnostic tests and the free sharing of scientific knowledge that's really surrounded the crisis.

Brian Rabe: Definitely.

Lydia Morrison: So, Brian, did you find that compared to your normal research, there was a particular sense of urgency with this project?

Brian Rabe: Oh, absolutely. I mean, definitely, we are always thinking that this pandemic is progressing. Every time you check the news, we see that it's spreading and yet our testing capacity is not growing enough to meet the needs. And then of course, states are beginning their reopening and we're worried that without enough testing capabilities, we could just be looking at another outbreak if we don't catch things quickly. So there is definitely a lot of urgency. What also, I think, helped was working with other groups developing these assays. We were all motivating one another, we were sharing results. It became a really fluid and very rapid process.

Lydia Morrison: So tell us something good. Do you have any good stories or reflections about your time in the Massachusetts stay at home recommendation that you could share with our audience?

Brian Rabe: Well, I can say that it's been nice to see it, in a lot of places. Like, I'm out here in Waltham and people are definitely taking things very seriously. I do see people are really practicing social distancing. I know you can hear on the news about things happening at various grocery stores and all that, but here it's been very... I think everyone's been sort of, "We're in this together," kind of spirit.

Brian Rabe: Those sort of drive-by graduation or birthday party celebrations have really, really taken off here. I think every evening we see three or four of them go by, so it's nice to see people supporting one another in a safe way. And beyond that, I mean, it's been nice to keep all the plants in my lab alive.

Lydia Morrison: Lots of time to sort of focus on some of the more simple things in life.

Brian Rabe: Oh, absolutely.

Lydia Morrison: Well, thank you so much for joining us today. And thank you so much for your work. I think it's been really important that you picked up this project. You were obviously able to identify some primers that made a huge difference in the sensitivity and specificity of the test. And I think that it'll go a long way towards really bringing a COVID diagnostic to more places, to more rural places that might not be able to afford the instrumentation to carry out some of the RT-qPCR tests that are more prevalent now.

Brian Rabe: Oh, definitely. And having a lot of family back in rural Kentucky, that's definitely been something I've been thinking about.

Lydia Morrison: Yeah. It's nice to know where people's motivations lie. And I think a lot of our motivations lie in protecting our friends and family and loved ones from this pandemic. So thank you so much for your work toward that.

Brian Rabe: Oh, well, thanks for having me.

Lydia Morrison: Thanks for tuning in for this episode. As always, check out the transcript of this podcast for helpful links to further resources. And please join us for next episode when I'll be interviewing Feng Zhang of the Broad Institute along with Jonathan Gootenberg and Omar Abudayyeh of the McGovern Institute of MIT about their recent publication describing STOPCovid, an assay which combines isothermal amplification with CRISPR-based detection methods. So be sure to catch the next episode and hear from the scientists currently making major breakthroughs in Coronavirus detection and diagnostics.