Mycoplasma Detection for Cell and Gene Therapy

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In this episode, I meet with Mark White, Ph.D. Associate Director of Biopharma Product Marketing, Digital Biology Group at Bio-Rad. We speak about cell and gene therapy with a focus on mycoplasma detection.

I'm Dan Limbach, your host and producer of the PharmaVOICE Webcast Network.

Dan: Welcome to the podcast program, Mark.

Mark: Hey Dan, great to be with you.

Dan: So just as a baseline Mark, what is mycoplasma contamination and how does it affect the manufacturing of therapeutics?

Mark: Mycoplasma is actually a common bacteria that contaminates cell culture across many different modalities of scientific research as well as production. And in a lot of the therapies we make there are cells that are producing either the protein or the therapy, and so what ends up happening is that frequently these cell cultures are infected by this bacteria. A lot of times it's coming from the lab personnel themselves. It's a challenging bacteria because in many cases it's resistant to antibiotics and it can cause potential issues if it's present in a therapeutic dose for the patient that's receiving it. And so it's really important that we make sure that whatever we're producing or whatever our customers are producing is not contaminated by this mycoplasma bacteria.

Dan: Very good. So what are some of the methods used today to test for mycoplasma contamination?

Mark: Traditionally the method of choice was a very long what's called direct culture method. It took about 28 days or more where we would take a sample from whatever material that we were trying to test and put it into culture media and let it grow, and if there is mycoplasma bacteria it would grow out and you'd be able to see it that way. And then more recently a qPCR-based method has been developed that provides a quicker turnaround time, but there are some challenges with that method. That was one of the reasons we sought to create a droplet digital PCR-based version of a test that could be used to determine whether or not there was mycoplasma present in a solution.

Dan: Let's talk more about some of those challenges. What are the biggest challenges most cell and gene therapy companies face today?

Mark: If we're talking about kind of contamination testing, I would kind of narrow that question a little bit to contamination testing. Time to answer is a big challenge, so they're trying to create doses and sometimes even with cell therapies releasing a dose to a patient very rapidly is really critical for that

patient's therapeutic success. And so how long a test takes is a big challenge. So there's been a big push within the industry to minimize the time taken to get a test done.

And then reliability and reproducibility, ease of use, all of those things are really critical, like having an assay that performs well across sites, operators, instruments is really crucial. The best way to create an assay that performs well in those conditions is to make it as simple and robust as possible. So that's another big challenge.

And then a third one that's I think underappreciated maybe outside the field, but within the field is pretty well-known; is just getting people enough human power to run these assays. So if an assay is fairly manual and labor intensive, it requires a specialist to run it. And finding, hiring, training those types of people is harder and harder as these therapies become more mainstream and more popular, that supply of human capital, if you will, is constrained. And so it's really important that we have assays that are efficient in how much hands-on time a lab operator has to spend with that assay.

Those are, I think, kind of the three big ones that we try and address when we're developing assays.

Dan: All those are pretty significant. So let's move on to solutions. What solution does Bio-Rad have to address the challenges of mycoplasma detection and how is it beneficial specifically to scientists?

Mark: The direct culture method with a 28-day turnaround time just is really, really too long, and so moving to a qPCR-based method was a big benefit, I think, for people in the turnaround time. But there is in the field that's kind of well-known that these qPCR-based methods are a little bit less specific and have unfortunately a fairly high false-positive rate. The problem with a high false-positive is that you're testing for a potential contaminating bacteria on what could be a very, very expensive dose of a therapy; it's taken a lot of time and energy to create it, and so if you have a false-positive, you may be pulling that dose out and that's a big cost as far as time and energy that's taken to produce that and now it can't go to a patient. So that's a big challenge those false-positives.

So either you're scrapping a dose that was good if it was a false-positive or you're rerunning that test to make sure whether or not it was a false-positive. And then if you have to rerun a plate or an assay then you're effectively doubling the cost and doubling the time it takes. And so really a very highly specific test that has a low turnaround time and a low false-positive rate is really crucial in this case. And that's what the Vericheck ddPCR-based mycoplasma detection kit really addresses.

So I think that because we made the assay pro-based, so it's got basically three different pieces of DNA that target the mycoplasma, DNA targets that we're going after, makes them a much more specific test that is much less prone to false-positive rates. Very, very low. So that gives our customers the confidence that they're only going to have to run the test once. It's a simpler test as far as training goes.

And then we've also implemented automatic gating that is used based on a positive control that's run with the assay to tell the operator where the positives and the negatives are. And so that's really

important in a qPCR-based method. A lot of times it's up to the operator to set the cut-offs of what's truly a positive and what's a negative. With our test, you run a positive control on the well plate and it automatically sets the positives and the negatives and again, it's reducing hands-on time and energy, reducing training, allowing the operator to get that yes or no question faster and easier.

So it kind of all ties together with those key challenges that these cell and gene therapy companies are facing today in trying to minimize that time and energy to get those tests to run.

Dan: Well, that sounds like that it could be a game changer. So let's talk about adoption. Why do you think QC labs are switching to digital PCR when testing for mycoplasma contamination?

Mark: I think a couple of reasons. As I just mentioned, this high false-positive rate is really disconcerting, especially when you're talking about a contamination in a production environment where you're manufacturing a therapeutic that's going to go directly into a patient. You want to be really confident in that test, and so you're going to make sure to move to the best test available that can be validated. And so I think that's one of the big reasons that people are considering in moving to this assay is just, if you have an assay that sometimes gives you a false-positive that doesn't make you feel good, you're not going to necessarily trust it and then you're going to repeat it a bunch. So getting away from that mentality is something that's trusted and has a lower false-positive rate is really, I think, valuable.

I think another key part of why people are moving is that we have strong adoption of droplet digital PCR within manufacturing for measuring viral titer in A, B or other gene therapies. So having other tests to run on a single instrument has value for our customers and kind of consolidating into one type of platform that has inherent positives about it in that it's reliable and robust, you get an absolute quantification of these molecules that they're trying to count, whether it be the number of genome copies from the viral vector or whether or not there's a contaminating piece of DNA that's coming from a mycoplasma, for example. So being able to kind of bring similar assays on to a trusted platform, I think, is also a big reason that our customers are kind of moving across to our test.

Probably a third one is that our sensitivity is higher, so we can detect one colony forming unit per mil, whereas kind of the leading test that is in the qPCR space is a 10 colony forming units per mil, so about 10 times more sensitive. So if you're trying to make sure that you have the best, safest therapeutic for your patients having the most sensitive test for contaminating bacteria is valuable. So that's another, I think, big reason for the move to the ddPCR-based mycoplasma test.

Dan: Excellent. And finally Mark, Bio-Rad has a white paper related to this topic called Transitioning from Quantitative PCR to Droplet Digital PCR for Mycoplasma Detection. What can you tell us about this paper?

Mark: We're excited about this paper. It's something that we're doing kind of for most of our assays and kits that we're developing in the biopharma kind of manufacturing space is a guide for how to transition from the quantitative PCR-based method to a droplet digital PCR-based method. I think in some areas droplet digital is still new to some of these operators, and so having a very detailed kind of explanation of how to transition from one type of assay to what we believe is a better assay and walking people through how that's done and how you do those comparison studies to gain confidence in the increased sensitivity, the increased robustness, lower false-positive rate with data and kind of explanations, material setup and things like that will really help our customers make that transition and gain confidence.

I think it goes together with the larger package that Bio-Rad is offering with our kits and our platforms is it's not just the kit and the platform that's needed to get up and running; there's a lot of other things that need to happen. These bridging studies like we're detailing in the white paper support and making sure that these things work having experienced field application scientists that can help our customers. All that goes together into making it as seamless as possible to move to droplet digital. And not to mention the fact that Bio-Rad has now been working with our customers in the cell and gene therapy space for over five years, and so we have the experienced team to help people bring up these, I think, gold standard assays that will kind of be able to give them the best results possible.

Dan: Well, I would certainly recommend that anyone that's listening to this podcast should also download this paper and get some more valuable information. Mark, I want to thank you for sharing your thought leadership and expertise with us today.

Mark: Happy to do it with you, Dan, to share what we're doing and excited for everybody to check out the white paper and what else is going on with droplet digital PCR at Bio-Rad.

That will do it for this episode. Learn more about Bio-Rad at bio-rad.com. To download the white paper discussed in this podcast, visit pharmavoice.com/whitepapers or download it from this podcast page. And don't forget to check out our other podcasts, white papers, webinars, virtual panels, videos and more at pharmavoice.com.

Until next time, I'm Dan Limbach.