Steve Lewis 00:09
Welcome to Speaking of Mol Bio, a podcast series about molecular biology and its trending applications in the life sciences. I'm Steve Lewis and my cohost for today is Melody Savea, general manager of the molecular diagnostics business within the biosciences division of Thermo Fisher Scientific. Our guest today is Beverly Wood, coming to us from Raleigh, North Carolina. Bev is the supervisor of the molecular diagnostics lab at the North Carolina Department of Agriculture and Consumer Services, where she's worked for over two decades. We loved hearing about all the ways she's used molecular biology to keep her state safe and healthy, and how technological advancements have changed her work over the years. We hope you enjoy the conversation.

Beverly Wood 01:03
The main zoonotic disease we deal with is avian influenza. We do probably, I don't know, 30,000 samples per year to look for avian influenza in turkeys and chickens. That's the main disease that would have zoonotic potential. Swine influenza, we do some of that. Most other tests that we do surveillance for are not really zoonotic, like foot and mouth disease, classical swine fever, African swine fever, all those are animal diseases, per se. The zoonotic potential of avian influenza, you know, becoming a human pathogen, that's of concern, and something we're always aware of.

Melody Savea 01:47
So, Bev, I have a question for you. Obviously, you've been with the department for quite some time. If you think about when avian flu, and then West Nile, were really starting to emerge early on. I'm just curious, from a technique perspective, the challenges that you were facing at that point in time to how it is evolved to your ability to even go—I'm assuming that you're in a surveillance mode right now versus active outbreak. So, I'm just curious how sort of the technology has evolved in the way that you actually are surveilling, you know, what really could be large geography and large population and flocks of birds, you know, from poultry producers and things like that?

Beverly Wood 02:37
Before 2002, we really didn't have any way of doing surveillance with molecular techniques. After 9/11, there was a Homeland Security directive that released a lot of money and we, our lab, was the recipient of some of those funds, and we were able to build infrastructure and buy equipment. Initially, the technology was, you know, more of the spin column extractions, we use the Cepheid Smart cyclers, you know, it was very low throughput and very time consuming. And over time, we started getting the 96-well platform machines, the Applied Biosystems™ 7500 machines that we still currently use, we have 10 of them. We were doing manual extractions, even up to like 10 years ago, then we purchased Thermo Scientific™ KingFisher™ Flex machines that use magnetic bead–based technology. So, between the five KingFisher instruments and the 10 9600/7500 machines, we can put out some samples. We are not currently in disease outbreak mode, but we have been as recently as 2022, and then there was another one in 2020. Right now, it's not in commercial poultry but you know, it could be this afternoon, you know, when we discover the next outbreak. So, we're, you know, we're always ready.

Melody Savea 04:09

And from a testing throughput perspective, as you said, you know, you've had challenges, you know, from a throughput when you're in active outbreak versus surveillance. How does that testing volume change?

Beverly Wood 04:24
I would say in an outbreak, you know, the number of samples that we get in total is not that huge, just probably like 5,000 samples, maybe, over a period of, that's extra, in addition to our regular load, over I would say, a period of maybe 12 weeks. It's usually about three months. And part of that's active surveillance around the site, the locations that the disease was detected, and then 10 kilometers around that and then any additional premises where we detected anything. And then after that, then you've got to do the follow-up testing to make sure you're free of disease. And that cycle continues as long as any more premises, you know, they can pop up along the way. So, in terms of numbers, basically, we have to just work really long hours. They might come at, you know, 11 at night, you know, when they, you know, or in the middle of the night or, but you still have got to be ready to test those samples.

Steve Lewis 05:28
Tell us a little bit, Melody, if you don't mind, in your experience, I think this idea of active surveillance across the US might be new for some of our listeners. Can you share your experience in working with some of the broader laboratories in the space?

Melody Savea 05:46
Yeah, so I actually have pretty lengthy experience, and I actually worked with the California Department of Food and Health Safety. So, it's through the setting up and then sharing new protocols within NVSL (National Vet Services Lab) and the NAHLN Network (National Animal Health Laboratory Network) on new methodology for being able to test from poultry. Because the challenge, obviously, from field collection, is how clean the sample is. And then you know, what the detection limit is, depending on where you are in the amount of virus that actually could be present in any given sample. So, you can imagine, especially from what Bev does, is that when you're working with poultry farms, or wild bird populations, you know the samples aren't the cleanest. So, being able to take a cloacal sample and actually do real time, because it's an RNA virus, in this particular case, doing RT-qPCR. To do that, and make sure that you're getting true answers of is this, you know, positive or negative was really important. And I think that actually, then you take all of that information that was gathered at that time, from labs, like Bev has done, actually has helped with the way that we were able to then respond to COVID. You know, it's just getting a good quality, clean sample, being able to manipulate it as little as possible for risk of loss, and then being able to make sure that you have an assay that's sensitive enough and specific enough to truly say that, yes, this is a positive or negative. And then take the amount of sheer throughput that you have to do, you know, the size of a population that you're testing, are all critical things that were applied for what we just went through with the pandemic.

Steve Lewis 07:50
What are some of the challenges with those techniques and sample integrity?

Beverly Wood 07:55
Oh, well, the samples, they can be wildly different in how clean they are. For the most part, the Applied Biosystems™ MagMAX™ technology that we use, the bead-based technology for extractions, really is pretty good for cleaning up the samples and providing a clean sample at the end. For tissues, for cloacal swabs, tracheal swabs, those are our biggest samples. That's what we usually test. And it works, it works really well.

Steve Lewis 08:29
And then PCR after that, right, for testing the sensitivity?

Beverly Wood 08:34
Right. PCR, PCR with real-time Applied Biosystems 7500 machines. Yep.

Steve Lewis 08:41
How has RT-PCR over your time changed the pace? I know, we spoke a little bit about high throughput, but in terms of perhaps digital results even, has that made life a little bit faster and even more accurate?

Beverly Wood 08:59
Oh, sure. Yeah. So, we start with a matrix PCR doing the avian influenza screen, which will pick up all influenza A. And then, you know, to not have to wait all the way to the end after, you know, gel or something. You could see the positives coming up, you know, as you, after what, I don't know, 20 cycles, and then if you go ahead and start your next process of the subtyping for the H5 or the H7, hemagglutinin and so that yeah, that's immensely helpful. It really is. You know, there's no post-PCR manipulation. It's a closed system, you don't have to worry so much about PCR contamination. So, it's great. And it's, you could do 96 samples at a time. So, well, minus control samples plus controls. So, it's really a good system and we've got it down so that we can do it very efficiently, very quickly, and get results within two and a half, three hours.

Melody Savea 10:04
So how do you do your subtyping work?

Beverly Wood 10:06
Once we get a matrix positive, we can still use the same extract. There are two different H5 PCRs. One is, one detects the Eurasian and the North American strains high path and low path. If that one's positive, then we have a separate H5 PCR, real-time PCR that would detect the Goose Guangdong 2.3.4.4, the virus H5N1 that was, that's been the outbreak virus in the last few years. And then H7 has a separate PCR as well.

Steve Lewis 10:47
Fantastic. And I think that throughput definitely helps with some of the surveillance. Throughout this season, we are exploring new application areas of molecular biology and one of those specifically is in the biosecurity space. Many people don't know that in biosecurity, actually, animal health and food safety are a major part of that. And I know that we have somebody on our call today, in our cohost Melody, who's very experienced in that. Can you share a bit about it?

Melody Savea 11:18
Sure. So, from a biosecurity perspective, food supply is very important to us just as much as it is being exposed to someone who has the flu or has some other communicable disease. So, networks such as the Animal Lab Health Network, are responsible for and tasked by the government to test the various sources of animals, in this particular case, that may be entering our food supply chain. If you think about the things that people are eating, and we want to make sure that we are not exposing the population to potential, you know, infectious agents. So, some of the work that's being done, and Bev you could probably share a little bit more detail around that, is what your group is actually tasked with, you know, what species of population and the types of things that you are surveilling for, versus other labs doing other types of testing.

Beverly Wood 12:32
You know, the impacts for the food supply for our testing, it would have little to do with, you know, infecting humans really. I think any infectious agent detected in an animal, if it was zoonotic, such as avian influenza, those birds are, they're depopulated; they don't really enter the food stream. When we're diagnosing domestic diseases, PRRS or PCV2/3, swine influenza, those diseases, again, are generally in populations that are not, that don't enter the food supply. You know, I guess we're not so much concerned about passing on human disease, except for avian influenza, if it were to mutate and become a human pathogen. But, you know, as far as people getting animal diseases from animals that we've tested this, that's probably not going to be the issue there. The biggest risk for human diseases for the avian, the H5N1, or any other low path avian influenza that might be circulating, could become high path and adapt to humans. But as far as you know, why should people care about what we're doing? The economic impacts of agriculture, specifically poultry, in this day are huge. I mean, you know, billions of dollars, I think is something like 150,000 jobs are provided by poultry alone. So, when stuff happens in agriculture, it does affect our pockets and the availability of, I mean, I think after the 2022 outbreak, you could tell some that there was less poultry, you know, chicken and turkey on the shelves, but you did notice a big price hike, that's the other side of the coin.

Steve Lewis 14:37

Steve Lewis 15:09
Focusing a bit more on techniques, what is the most difficult challenge that you experience in the laboratory?

Beverly Wood 15:21
From strictly a molecular technique standpoint, for us as we're trying to chug through so many samples, you know, per day in the year, making sure that we don't produce any false positive results. Generally, you know, since real-time PCR, the least that we're doing is a closed system, there's not a whole lot of amplicon problems. We have a nice brand-new facility that we just moved into in 2021, that has lots of separation of space. We have an entire room with two different biosafety cabinets for master mix. We have an entire room just to do sample handling. We have an entire room just for extractions. In that extraction room, we have four separate biosafety cabinets. So, we can keep clean from dirty really well. I guess our biggest place for introduction of cross-contamination would be in just the sample handling, every one of those samples has to be decontaminated and, on the outside, racked in a 96-well rack and then we go ahead and pre-plate our samples from those racks. So that we have a plate of 96 samples to use to go from there to the KingFisher extractions, I would say that's the biggest place that you can introduce cross-contamination.

Steve Lewis 16:50

Now I remember a time, and I think perhaps this might have been due to funding, doing RNA extractions by hand, and you discuss some wonderful instruments that not only save time, but also for some, the old reagents like Invitrogen™ TRIzol™ reagent, are probably safer to use the instrumentation. This is a question for Melody: How have you seen new advances in instrumentation speed up the field overall?

Melody Savea 17:25
There's two things that can impact speed to results. Obviously, you've seen some improvement in cycling times. The time that it takes to go up and down in temperature. You see a lot of improvements in that regard, with a lot of them having Peltier-type controls, but a lot of it is that ramp rate of the heating and cooling of the reaction. Because as Bev said, you know, sometimes, and especially in a real-time reaction, if you're doing 35–40 cycles that can actually, you know, you want to compress the amount of time that it takes to actually perform that because speed to response is really what's key in driving, you know, the success and the needs and demands within the diagnostic space. But now where you're actually seeing the evolving process to also get faster reagents to go with it because just through normal thermodynamics, it's only going to heat and cool so fast. But if you can actually put an enzyme in there, a polymerase or whatever in it that can actually complement what the instrument does, that just continues to compress the overall time. And that's where you've seen those advances, in a lot of the DNA polymerases for PCR than it used to be. I remember when I worked in the lab, and it was like one kb a minute was what you used to calculate. So, if your target was going to be, you know, a four or five kb, you know, target, that you actually had to do a four- or five-minute extension. You add, you know, your denaturation and your annealing times to that. And so, for a cycle it could have been, you know, five, six, seven minutes. So multiply that times trying to do a 40-cycle reaction, I mean it's it's a whole lot of time. And now if you have an instrument that can cycle, you know, really, really fast between annealing time and, you know, an actual extension time, and you've sped up the polymerase to be, say, 15 seconds per kb, now, in a real-time reaction, your amplicon targets are usually smaller, but you know, still you want to be able to, you know, get through all those and confidently be able to extend all of those amplicons in a timely manner to compress the overall time. The last phase that we're starting to see now, which is more applied to PCR than real time, is when you start to multiplex, and you want to be able to detect more targets in a sample. You know, it used to have to be a whole lot of math and doing T_m calculations and everything to be able to make sure that you had the right temperature for all of those primer sets that you might have in the mix to be able to anneal to the target in a timely manner. So, now you're starting to see improvements in composition of the reaction to do universal annealing. So you don't have to do all of that math to know what temperature do I need to put my reaction at to make sure that all those primers can find their target and then be able to extend so that sort of the next piece in advancements and improvements in it to sort of pair the instrument, the enzyme, and now that primer annealing step to be able to optimize as many different variables as there might be in the reaction.

Steve Lewis 21:18
Bev, are you adopting, as a laboratory, some of those techniques in multiplexing, and where are we headed?

Beverly Wood 21:26
We do. Some of the NAHLN assays have been multiplexed. Diseases like African swine fever and classical swine fever are pretty much clinically indistinguishable in pigs. So, it makes sense that you test them together in a multiplex rather than doing two single reactions. Same for foot and mouth disease, vesicular type lesions, we always test Seneca virus A, for the same, the

same symptoms, the same vesicular lesion. So again, that made sense to multiplex those two things together. So, yeah, the more we can multiplex the better.

Steve Lewis 22:06
When samples arrive, you're receiving them from multiple locations, perhaps not even in a nearby radius. When you're opening up boxes, are you ever uncertain what you're going to get? Or is everything pretty standardized in sample format?

Beverly Wood 22:26
There was a time when we would accept cow fecal samples in a glove, you know, for testing. So, we no longer do that anymore. So, things have changed a lot. But, the client, the submitters are pretty good about knowing what to send, and how to send it. And there's not a whole lot of surprise. I mean, there might be a few now and then, but no, I'd say it's pretty standardized and well worked out because they submit thousands and thousands of samples.

Melody Savea 22:56
How do you keep track of all of them once they come into the receiving department? What's your tracking system?

Beverly Wood 23:03
Yeah. Our LIMS, our lab information management system, assigns a unique case number to each case. And all the case information is what stays with that, that accession number, each sample in that accession number is entered into LIMS, we track the whole thing, the time it's received, everything about it, the species, the animal. It's all there. Any client communication, we have to make a record of that in LIMS.

Steve Lewis 23:34
What's the furthest away you've received a sample from?

Beverly Wood 23:38
We do get samples up from out of state. We might get wild bird samples from the West Coast. We might get samples from another state. Generally though, it's in the southeastern portion of the country. But occasionally, like I said, we may get samples from anywhere. We accept samples from anywhere.

Steve Lewis 24:00
Have there been any surprises with samples when you received them that you can disclose?

Beverly Wood 24:05
No, you know, not really. No. You know, just the, just the odd "What is that?" kind of thing. It's usually pretty easy to clear up by just calling the submitter. But, nothing too terrible really. The exception might be the individual farmer or, you know, the person individually has chickens or, you know, a few cows or something. Now, those might be a little more iffy. That's where your "Huh, what is that?" type samples might come from.

Melody Savea 24:41
Typically, how long would you retain a sample? So, you've done your purification step, you've done your, you know, your initial tests, maybe you had to go back and retest something later on. How long do you retain samples?

Beverly Wood 24:56
Our section has a one-month retention policy. Then we would save any, you know, sample that might be useful for, you know, validation studies later. In general, our retention policy is 30 days or as space allows.

Melody Savea 25:13
So, you said previously you moved into a new lab fairly recently. What was the biggest improvement that you experienced versus, you know, what you had before?

Beverly Wood 25:24
Oh, wow, our old building, the Rollins lab. Wow, it was known as “the dark hall of despair” pretty much. It was just a very, very old building and had a whole lot of mechanical problems. And it was a good building, but it served its purpose. And we had added on, you know, the BSL-3 facility. Over the time, you know, we had renovated spaces, but it just got to the point where it was was just too old to really function very well. And there were funds to combine all the labs into one big facility. And it's beautiful. It's just, we've got windows, we could see outside, there's light. And like I said, we get we have a lot more space, we're able to separate our spaces for sample processing and our clean areas. It's been a really nice. It's been truly, truly helpful in the whole workflow, how it goes, we're able to have a BSL-3 facility in this facility. It's got better security, it's just generally been, it's been a great thing.

Steve Lewis 26:44
Can I tug on that thread a little bit? Can you share more about the BSL-3 facility and what differentiates it from a two?

Beverly Wood 26:51
Well, the BSL-3 facility that we have now is a shared BSL-3 space. And it would be available to anybody in the complex. Mainly for us it would be for, it's really allocated for African swine fever, classical swine fever, FMD, because those are, if we wanted to store any avian influenza. Anything that's a select agent or considered a select agent would have to be tested and stored and used in that BSL-3 facility, and it’s got a HEPA filter. The exhaust is HEPA filtered, the biosafety cabinets are vented, the rooms are negative pressure, there are autoclaves in the facility, shower facilities, two-sided autoclaves on the open, inside and outside the facility. It's not a big space, but it would allow for us to have select agent registry. We're not there, we're not there yet, but the plan is to be select-agent registered.

Steve Lewis 27:58
That's often a multiyear process. Can you share just a little bit of insight about, I guess, select agent pathogens and why they would matter to a broader population?

Beverly Wood 28:11
In our case, you don't want to release a pathogen that's inside the lab to outside the lab, and then bring that pathogen into contact with animals that, you know, could be infected by those agents. The main thing is containment; you want to contain what you’ve got.

Steve Lewis 28:31
This has been a great conversation. And as we're moving toward the last pieces of it, one of the questions that we always like to ask our guests are, what are your keys to your success?

Beverly Wood 28:44
Oh, wow, um, well, it's just a lot of work. It's just a lot of attention and work. Like I say, I've been with the department over 25 years. And this, it's really been, it's an everyday type of commitment. Because things change quickly, and you really have to be of the mindset of I want to be a public servant. And I want to be committed to that. That's not really for everybody. But you know, it is, it works for me, and I like that.

Melody Savea 29:14
I truly appreciate that you are that committed to doing that. Because I agree that, yeah, that you're doing, while it can be repetitive work it's very meaningful and has purpose. So, I appreciate your commitment to that.

Beverly Wood 29:29
Right. Thank you. I appreciate, I appreciate hearing that. The average person doesn't really, you know, think about "Oh, what's happening at the animal diagnostic lab?" I mean, you just don't, you just don't have that, you're not exposed to that. And, you know, I think we fly under the radar a lot. But, you know, I feel like it's important, and that's partly why I wanted to be on this podcast is, you know, I think it's important for people to know what we do and that we're doing a real service. Lots and lots of committed people. They're doing a great job here in North Carolina. And I want to tell people about that.

Steve Lewis 30:05
I know there are many who are going to come out of listening to our podcast and have a new appreciation for everything that you and your team do. Final question from me: For anybody who might be interested in getting into the field or following in your footsteps, what would be some advice that you could share?

Beverly Wood 30:29
Well, be flexible, be willing to learn and change quickly and be willing to be committed. Because, you know, it's going to take, it's going to take some commitment. You can't be sort of halfway.

Steve Lewis 30:43
Passion and commitment with real material impact. We really appreciate you today, Bev. Thank you so much for coming on the podcast with us.

Beverly Wood 30:54
Thank you. I appreciate it. I really do appreciate it.

Steve Lewis 31:00
That was Beverly Wood, supervisor of the molecular diagnostics lab at the North Carolina Department of Agriculture and Consumer Services. Special thanks as well to Thermo Fisher's own Melody Savea for guest hosting this episode with me. Speaking of Mol Bio is produced by Matt Ferris, Sarah Briganti, and Matthew Stock. Join us next time for more fascinating discussion about the wide world of molecular biology. Until then, cheers and good science.