Coordinator: Welcome and thank you for standing by.
All lines are currently in a listen only mode.
Today's conference is being recorded. If you have any objections you may disconnect at this time.
Now I will turn the meeting over to Dr. Beard at CDC. Sir, you may begin.

Ben Beard: Good morning. This is Dr. Ben Beard in CDC's Division of Vector-Borne Diseases at Fort Collins, Colorado.

I want to welcome everyone here this morning to the HHS Federal Research Update on Lyme Disease Diagnostics.

I hope everyone can see the presentation. I'm trusting that you can. I'd like to begin by just saying a little bit about Lyme disease in the U.S. As most of you probably know it's the most common vector-borne illness in the U.S.

In 2007, there were over 33,000 cases that were reported to CDC, making it the sixth most common reportable disease in the U.S. And for those of you on the East Coast you probably realize that it's actually the second most common disease in the northeast behind chlamydia and the third most common, chlamydia then gonorrhea then Lyme disease so we recognize its importance and it's a high priority to all of us in Health and Human Services.

One thing that we all know and it's very important to us is that early and accurate diagnosis and treatment is the most effective way to avoid more serious illness and the potential for long term complications related to Lyme.

So the primary objective of our webinar this morning is to advance Lyme disease diagnostics by informing potential stakeholders of the resources that CDC, FDA and NIH are currently making available in an effort to promote development and clearance of new Lyme disease diagnostic tests.

And to this end we'll have three speakers this morning, a speaker from NIH, one from CDC, and one from FDA.

I just want to remind everyone that as we go through the presentations we have approximately an hour and a half for the whole webinar so there should be plenty of time for the presentations and also for questions.

So if you have a question you can submit it online through the webinar interface.

Just please if you don't mind, provide your identity so we'll know who the question's coming from and may enable us to answer it more effectively or and also indicate who the speaker is to whom you are addressing the question.

For clarifications we'll take a few questions after each of the presentations and at the end we'll have questions for any of the speakers for anything that comes up during the course of the webinar.

So I think with that I will turn it over now to Dr. Joseph Breen who is Bacteriology Program Officer in the Division of Microbiology and Infectious Diseases at NIH/NIAID. And Dr. Breen's presentation will be entitled Diagnostic Projects in the NIH/NIAID Lyme Disease Research Program.

So Joe, take it away.

Joseph Breen: Thank you Dr. Beard. Good morning. As Dr. Beard mentioned my name is Joe Breen and I'm the Program Officer for the Lyme Disease Research Program in the National Institutions of Allergy and Infectious Diseases here at NIH.

Today I want to tell you about the diagnostic projects that are in the research program to give you an idea of the scope and scale of what research projects are ongoing in this important area.

So here's an outline of my talk. I'm going to talk just briefly about the state of the science of Lyme disease diagnostic testing and then I'm going to talk about the research program because I think that'll help frame the kind of things that we do here in the Lyme Disease Research Program in NIAID.

That's really the first half of the talk. The second half is really I'm going to be speaking about the projects that are underway so these are all active projects and using a reporter-based tool that I'll show you a little bit about called NIH Reporter.

And I'll be talking about ongoing projects that come in from researchers all across the country and the world, in fact, and then some that we specifically requested.

And finally I'll summarize some of those approaches and talk a little bit about next steps.

So the current state of the science regarding Lyme disease diagnostic testing, so I'm sure many of you are aware like blood tests for many other infectious diseases in fact the test for Lyme disease measures antibodies made in response to an infection.
So that's been very informative however the pathogen itself is not measured using the blood test. It's the body's response.

And that's created a number of challenges. It's good that we can do it. But as I think I'll describe in the research projects we're trying to find ways to do that better and to even potentially directly detect the pathogen itself, the *Borrelia burgdorferi*.

Current diagnostics are less than optimal for early disease because it can take weeks for a detectable immune response to be sufficiently measured.

So the early stages of disease which when treatment is typically most effective unfortunately are when serum diagnostics are least effective.

In addition current diagnostic measurements don't reliably change with treatment. So there's no way to measure effective treatment other than how a patient is feeling and with all the different disease manifestations. It would be wonderful if we had a way to test for effective treatment with antibiotics or even stage disease, something that will be a little bit more helpful in terms of identifying and stratifying patients along the treatment pathway.

So what do we do in the NIH/NIAID Lyme Disease Research Program? The goals of the program are to develop better means of diagnosing, treating and preventing this disease. And really to accomplish this, the research portfolio has a number of activities—primarily research grants to cover a broad range of things to better understand the disease. That's really when you get down to it, the better we understand the disease, better we understand *Borrelia burgdorferi* and the better we understand a response of the host to *Borrelia burgdorferi* then we should be able to better design diagnostics, better treatments and potentially prevent the disease using vaccine.
The way that this is done is really through two different broad ways. We call it extramural and intramural. Extramural is really about 85% of the work that's done with NIH/NIAID support. That is research labs all across the country and in fact the world. There's been a smaller percentage of the work that's done intramurally. And we have two laboratories I'll tell you a little bit about, some here in Bethesda where I'm located, and some in the Rocky Mountain Labs.

The extramural work, which is most of the work that I'm going to be telling you about in the latter part of the presentation today, is really investigator initiated so experts, researchers, and clinicians have an idea that they think will advance the field and they submit that in the form of a grant application.

And that can happen anytime during the year and those are evaluated three times a year and the most successful ones are eventually awarded.

The other way that that happens is that we specifically ask for a targeted area of interest. And we did that recently for Lyme disease and I'm going to tell you about that.

With any of these mechanisms, support for a research project is based on the scientific quality as proposed in the application, as judged by independent peer review panels of people out in the research public, and experts who are the experts in the field. Not NIH people, actually, but the very people that we support out in the research field.

And so the idea is that really the very best science is evaluated on a regular basis, judged and awarded.

And I'm going to tell you about some of these projects ongoing.
So in the extramural program studies are focused on—a lot of those studies are focused on basic biology and pathogenesis because again, better understanding of *Borrelia burgdorferi* itself should help us achieve the ends I mentioned and really be part of the mission.

Some of the things that have been done in the last, say five years, are better development of *Borrelia* specific genetic tools so we can knock out genes in *Borrelia* and better understand their function. At the same time, identification of key factors involved in dissemination, as well as better understanding of individual genes, will allow us to perhaps use those as new protein targets for

diagnostics for example. And that's very much what is happening in some of the research projects that I'll mention later.

So we support a variety of diverse projects aimed toward development of new diagnostics and vaccine targets. And that really falls out of the study of the basic biology.

As well we are supporting some very important areas of research, studies of persistence of infection after antibody treatment using a variety of animal models—primarily mice, but also nonhuman primates.

We're also looking to improve Lyme diagnostics and I'll tell you about our recent solicitation about that.

We do intramural research as well, which is important to the overall mission. Out in the Rocky Mountain Labs there are three sections dedicated to aspects of *Borrelia* and tick biology with some expertise in medical entomology gene regulation. In fact some of the genetic tools that I mentioned earlier were developed there, as was the original discovery of *Borrelia burgdorferi* from a Rocky Mountain Lab researcher, and a team of people of course, and a section on molecular genetics.

In Bethesda we have a clinical studies group where there are more than 400 volunteers enrolled in ongoing studies. And these would include development of new tests and biomarkers, still trying to understand STARI, southern tick associated-rash illness, and the role that immune response in Lyme disease and post-Lyme disease syndrome, an area of intense interest of course.

So how am I going to show you this information?

All of the data that I'm going to show you and information from this point forward is available to the public at the search engine called the NIH Reporter, research portfolio online reporting tools.

So any project that is supported by NIH, actually HHS, can be found in report.nih.gov.

And there's a search engine in that. There's a pre-identified category for Lyme disease. But you can also go through and search using whatever search terms you're interested in to find actively supported projects.

So for the purposes of this presentation I did that using Lyme diagnostics and I made it centered on NIAID.

And that comes up with a long list here. And I don't intend to go through this in detail on this slide but actually this slide will be a useful reference in the future if people want to find out these projects because then with the principal investigator's name and the title you could find more information.

What's public about all these funded research projects is the abstract of the work, as proposed.

So as I mentioned the Extramural Research Program supports work throughout the year that comes in but we also at times recognize some specific (needs).

And in 2011 we issued what's called a Request for Applications for Lyme Diagnostics.

So the idea here is that we really understood it and we needed this initiative to encourage development of more sensitive and accurate methods of detection of *Borrelia burgdorferi* leading to improved diagnostics. We hoped that

projects that proposed would come in would provide benefits over what's conventionally available with serological-based diagnostics. And I mentioned some of the gaps in the first slide.

And we wished projects to consider host responses, perhaps differentiate different strains and focus on development of a way to identify stage of disease and discriminate between active infection and previous infection or what we know is often true if symptoms persist and we don't have a good way to test for cure as I mentioned in the beginning.

As a result of this initiative in June of 2012 we awarded four new awards to do some of the things that I mentioned here. And I should mention that three out of four awards will utilize the CDC NIH serum repository that Dr. Schriefer will tell you about more in the next presentation.

So those four awards are listed here. So these research projects, which are two year projects, were awarded in June and we're very excited because these are really - we're really asking investigators to try some new approaches here and to give them support to do that.

And the awards were made to Tulane University with Monica - Dr. Monica Embers as the PI.

Yale University was Dr. Linda Bockenstedt.

Colorado State University was John Belisle and University of California Dr. Alan Barbour.

And these all have different advantages and approaches. Dr. Embers is really trying to utilize some new protein antigens, new ways to measure the disease that will be responsive to treatment. And actually a more refined way to do that then what we currently do today.

Dr. Bockenstedt's program is looking to utilize a host response mechanism that would be faster presumably than the traditional antibody-based approach so ideally would be able to detect disease in that early window when treatment is more effective.

Again these are research projects but so these ideas are being tested right now.

Dr. Belisle's project is looking at metabolic biomarkers and biosignatures using very advanced chromatography methods and really some advanced chemistry to try and look for new ways to measure Lyme disease using some technologies that haven't really been brought to bear in a big way before.

And finally Dr. Barbour's approach is actually trying to develop a different type of immune response measurement that again might be a better early marker than what we have currently and his particular project is trying to take into account some of the different strains that may be responsible for different forms of the disease.

So that was an advance—that initiative that we asked for specifically. However as I mentioned we—NIAID does have a research program that accepts applications all through the year and evaluates them in the manner I mentioned to look at basic biology of *Borrelia* but also diagnostics.

And so again I'm pulling out the diagnostics projects here. There's a project that was awarded recently to Paul Arnaboldi from Biopeptides looking at development of a Cytokine Release Assay and again this is hoped to be a host response that is from the person, for example, getting the infection that would be measured sooner than what you can measure with a delayed antibody response.

Dr. Bockenstedt has a connection to a company here looking at a T-cell assay again, which would hopefully be an earlier response for disseminated Lyme borreliosis.

And we also have a project ongoing from Dr. Schuster at Bioscience Development where he's actually looking at a nucleic acid coupled to mass spectrometry in ticks and now in humans. And actually this award is unique in that it's looking for more than just *Borrelia burgdorferi*, looking at other co-infections that are known to exist in ticks.

And he's actually helping to characterize that and now trying to correspond that to what he finds in patient samples. So this as it's mentioned is broad detection system.

So I'm going to go through just a few more of these awards just to give you an idea of the scope of what we're supporting.

There's a rapid point of care test that's been actually under development from a company called Immunetics, which is not unknown in the field, wherefore a Lyme serodiagnosis based on a novel ultrasensitive detection technology so again this is the theme. Trying to get more sensitive, more early stage testing when we know treatment is very effective.

This is a development of some early Lyme *Borrelia* using novel antigen, some proteins which have never been used in traditional diagnostic assays before to try and detect earlier disease.

So these are research projects. We don't know which ones are going to be more fruitful.

But again the idea is to try better understanding and better support for a diversity of approaches should help us get to better candidates to move

forward, a lab - another point of care assay for rapid serum diagnosis of Lyme disease, an approach being done at Biopeptides with Maria Gomes-Solecki.

And this is a list of several others. There's investigators out in California using some microfluidic approaches, again for a point of care.

At Columbia University there's a very important study going on with Dr. Armin Alaedini where he's identified some protein changes that he's seen to occur in post-Lyme disease syndrome patients, in patient samples.

And he's trying to understand immunological responses of those proteins which is interesting of course for the biology of what's occurring but it's also interesting because those may be excellent targets for diagnostics once we understand what protein patterns are changing in the host after inspection.

And Maria Gomes-Solecki has another investigation here. We're looking at Multi-Peptides Antigen Assays for the serodiagnosis of Lyme disease.

So what I'm trying to emphasize is there are a number of diverse research projects underway in the area of Lyme disease diagnostics. This is actually only a portion of what is being supported in the research program overall and I pulled out the ones that are relevant for Lyme diagnostics although you could say that the basic research ones could easily lead into better identification of factors that would be potentially useful in a diagnostic test.

So in, you know, summary we're looking at combinations of more specific and sensitive peptide-based tests which tend to be cleaner results but you don't want to lose the sensitivity of what's currently available.

Point of care testing you saw in several of the titles. I mean of course you'd ideally like to have a test that's available at the doctor's office and that you don't have to send away and wait for a response.

And assays based on the T-cell response—that's the early host response that several investigators are working on to try and get at this window of opportunity in early disease before you develop enough to measure in the typical serum diagnostics which are used today.

And again I've mentioned it several times because it's a real gap, you know, develop an assay for treatment. And continue to look for novel biomarkers. While we're trying to develop these ideas here we need to continue to support basic research and look for new markers because we don't know which ones are in the end going to prove the best candidates for a commercial test.

And finally that broad detection of pathogens to account for the fact that there are other pathogenic agents in these ticks and to try and see if that complexity matches what you find in patients is really important and again, developing new methods which is being done in this particular award.

So where is this going? And what do we want to do?

We're going to continue to support high quality science in the form of research application. And as I mentioned we have an ongoing way for people to do this.

We're going to support further development of successful early phase projects. Those four projects that I mentioned earlier from our specific solicitation are ones that are designed to be two-year projects and then actually they're evaluated after those two years. And the most successful ones will then be given more support to really push those ideas further.

Facilitate—we're going to continue to facilitate collaboration with our CDC colleagues, particularly in the utilization of the serum repository because it's critical to have a place to compare sensitivity and specificity in the

development of these new tests for reference. And as I mentioned Dr. Schriefer will talk more about that.

And we're going to continue to encourage principal investigators to contact the FDA once they're really in a stage where they have a potential research project that's really now moved into a test that could be tested and patient sampled either archived or prospectively.

We support of course testing and development of these projects from archive samples and also from prospective samples. But as they really start to get to where they can refine these ideas there—it's ideal if they start to talk to the FDA particularly if they're using new technology. And I think you'll hear more about that from Dr. Gerald in the third presentation today.

We at NIH, as I think I've described, support research projects from really discovery and then pilot phase and then we really are involved in analysis of archive serum samples and then even prospective analysis.

But it's really a pathway of development from the basic to the applied where, you know, manufacturers at that point have to work with the FDA and actually do the development and get it cleared.

And I think you'll hear a little bit more how to do that from Dr. Gerald.

So this is all the work that I'm talking about is supported through NIAID. Here's the web site for NIAID and my contact information.

And thank you. And I'll be happy to take any web questions now.

Ben Beard: So we have one question that's coming in now Dr. Breen. And the question is what's the average amount of yearly grant for each researcher, how much is being spent by NIH on a yearly basis for all grants for Lyme diagnostics?

Joseph Breen: Well I haven't looked at that specifically. The typical - can you hear me? Okay.

The typical grant size for a research project is $250,000 a year, $250,000 a year for the life of the project.

The pilot projects that I mentioned are a little bit smaller than that. They're basically a total of $300,000 over two years so they're slightly smaller in the pilot phase and then it - and it goes up.

So if you go into the Reporter, which I mentioned, you could pull out the amount. What I can tell you is in Fiscal Year 2011 the Lyme disease of a category, there was about $26 to $28 million spent for everything that I mentioned, the basic biology, some of the clinical studies that I mentioned in Bethesda and including these diagnostics projects.

So the diagnostics projects that I've told you about—there were about a dozen. So if you go with the average then I think it will be a dozen by approximately $200,000 cost per year. That's a ballpark.

If you - I could get the actual amount or this person could actually go and get that because again all these awards and the award amount is public information.

Ben Beard: Okay. Thank you Dr. Breen and got a couple more questions that are coming in. Is the emphasis of NIH more on serology-based tests?

Joseph Breen: No. In fact we ask specifically for approaches in our RFA that were—that would not be the traditional serological-based approaches. It's, as I'm sure people are aware it's, you know, *Borrelia burgdorferi* only exists in serum for

a limited amount of time. So that's why the immune response is generally measured.

So we're actually trying to encourage people to do different ways of measuring *Borrelia burgdorferi*. It turns out that we did award some excellent projects where they - it looked like they could improve that process substantially.

So we'd like to have a different way to do it, but that's sort of the state of the science right now is, we're trying to improve what we can do and as well as try new approaches.

Ben Beard: Okay. Thank you Dr. Breen. The next question - there is one more question but it actually gets into the content of the next speaker, and it has to do with the availability of samples from the CDC repository.

So I think with that we will move onto the next presentation which will be Dr. Marty Schriefer.

Dr. Schriefer's Chief of the Diagnostic and Reference Laboratory in the Bacterial Diseases Branch in the Division of Vector-Borne Diseases at the National Center for Emerging and Zoonotic Infectious Diseases at CDC.

Dr. Schriefer will be talking specifically about the reference reagents for development and evaluation of Lyme disease diagnostic assay.

So Dr. Schriefer, we'll turn it over to you.

Marty Schriefer: All right, thank you Dr. Beard and good morning. As Dr. Beard mentioned I'm the Chief of the Diagnostic and Reference Laboratory here in Fort Collins for Bacterial Vector-Borne Diseases. We serve as a clinical laboratory, a CLIA-certified laboratory. Test anywhere from hundreds to a couple thousand

samples a year for Lyme disease as well as we do research on improving diagnostics.

What I will go over briefly today is just a little bit of context, the Lyme disease in the U.S. Many of these, the points that I'll make are applicable outside the U.S., but wherever possible I'll focus on U.S. specific data and needs.

I'll give a little bit of information on the state of laboratory diagnostics. As Dr. Breen mentioned, our standard diagnostics are approved. Our recommended diagnostics are serology-based but there have been others that are utilized which are not FDA-approved. Some of them are useful and may warrant review and clearance by FDA.

I will address a little bit on sensitivity and specificity of these different tests and some of the problems or gaps that we see, stage specific or sensitivity, specificity issues.

And then finish up with some of the resources that we and NIH are providing. We'll hear from Gerald at the end about the FDA review of diagnostics tests and where we might hope to go in the future.

Very briefly, most of this is common information to most of the people on the line. Lyme disease was first investigated in the U.S. in mid-70s among apparent cluster of juvenile rheumatoid arthritis cases in Lyme, Connecticut. About seven years later, the etiologic agent *Borrelia burgdorferi* and the vector tick, at least on the East Coast of the U.S., *Ixodes scapularis* was identified.

In the early 80s, we began informal surveillance reporting cases. In 1990, Lyme disease became a nationally reportable disease.

And I'll just - although I won't dwell on this, it was actually mentioned briefly by Dr. Breen as well. We currently do not have a vaccine available. There was one on the market for a few years but that was removed in 2002.

If you just look at the lower left corner of the slide here you can see the incidence of reported cases from 1982 up until early 2000s very sharp increase over those early years as you would expect for a newly reported disease.

And if we then look over the last 10 or 15 years we've had cases steadily increasing through this time period. I just show this lower left slide here to indicate that we've added a new category several years ago. In addition to confirmed cases we are now collecting data on probable cases as well.

So over the last 30 years we've had about 400,000 cases of Lyme disease reported to CDC.

So what is the state of laboratory diagnostics here in the U.S.?

I will just very briefly comment on culture and PCR, spend the bulk of our discussion on serology. And then go into some details on serology kind of the developmental years, standardization in '94, '95. We'll finish up the third part of this webinar with a presentation from FDA.

But I will finish up with some issues and where we hope to go in improving Lyme diagnostics here in the U.S.

Culture for spirochetes *Borrelia* has been available for about 40 years. There've been a number of modifications to the media that was produced at that time in the early 70s. And those—that media is still available.

The benefit of culture is you have the agent in the laboratory. It's really a gold standard. But the downside is that spirochetes are slow growing, take days to

weeks to detect. There has been some recent publication on how that might be spread up at least the detection of that growth. (Cultural) samples include ticks and reservoir animals and tissues from humans.

And I've just very briefly indicated some of those human sources, tissues that have been successfully cultured. The erythema migrans rash and blood have sensitivities in early disease of about 50% sometimes a little higher.

And then in much decreasing amount we've isolated *Borrelia burgdorferi* from synovial tissue of Lyme arthritis cases and CSF in neuroborreliosis cases.

So what really is the utility of culture?

It's been an invaluable research tool, particularly in the early years, to better understand and isolate the organism across the country. It continues to have great potential and I think we may see culture coming back, particularly in early disease, perhaps in Stage 2 disease as a diagnostic tool. But currently it's of limited diagnostic application.

PCR—and in general PCR has sensitivities similar to culture. And I will in the next slide show some of that comparative data.

Some notable exceptions for PCR are in Lyme arthritis and neuroborreliosis cases where the sensitivity of this approach, PCR, is much higher than culture. And I'll comment on it again on that in a few minutes.

On kind of the downside or the needs of PCR there are no standardized methods for PCR. A variety of approaches and targets and platforms are utilized. We do not currently have any FDA cleared PCR assays for Lyme disease and I think that's an area that we can discuss and look forward to some tests being submitted and cleared in the near future.

Again like culture, PCR is an invaluable research tool but has little current diagnostic application.

So in this slide we can look at a review of the literature. There are about well over 30 studies reviewed in this slide comparing culture and PCR.

So if we look at kind of the different stages of disease, first all looking at skin samples, erythema migrans culture, and PCR have sensitivities of just 50% or slightly better.

If we move to blood sample again in early disease erythema migrans patients, the sensitivity of culture is still about the same, a little bit lower than 50%.

We have seen though in the recent years that PCR or newer modifications of PCR have resulted in some increased sensitivity in this very early stage, first presentation of erythema migrans patients or in persons that early disease stage that may not present with erythema migrans. And that one particular study is referenced at the bottom of this slide from this current year.

So again I think we may hope to see PCR utilized at early - in early diagnosis, first presentation of early Lyme disease cases.

If we move then into cases that have neurologic involvement or arthritic involvement, culture is rarely successful in these cases and only anecdotally has been reported in that neurologic and joint involvement.

In contrast, PCR has had some realized sensitivity in both of these manifestations of Lyme disease. And again I'm looking forward to seeing a greater utilization of PCR as a diagnostic tool in the future.

So now moving onto serology, as most people know the early years in Lyme diagnostics from the mid-80s to the mid-90s where everyone was utilizing cultures in their laboratory to make antigen preparations and different diagnostic tests was very unstandardized.

And through a couple of meetings, often referred to as Dearborn Meetings in the late 80s and early 90s, an attempt was made to standardize diagnostics for Lyme disease. And that's shown in this slide here that this particular MMWR publication came out in '95 and described what is now known as two-tiered testing.

And I will just briefly, very briefly go over that. The first test is to be a very sensitive test, most commonly an enzyme immunoassay. In the older days IFAs were utilized but have fallen largely from use currently. If that test is reactive, either equivocal or positive, then we reflex to Western blots.

And this is where we run into some complexity and really some complications in how to utilize these tests and bring up a few needs that we need to address currently and in the future.

For the first 30 days of illness we expect an IgM response to be predominant and an IgM Western Blot is recommended. An IgG Blot can also be done and is sometimes reactive but typically the IgG response does not mature until 30 days or longer after onset of illness.

And as everyone knows, designating this time point cutoff is often not possible, not submitted with samples for testing. This 30 day cutoff has been an area of contention and need for improvement in the future.

I will go into a few more of the complexities and needs in this two-tiered testing format, particularly in Western blots.

Again just to review this slide, if a sample is not positive in the first test it is not recommended that it be further tested. And it is also recommended that a test— that a sample not be tested by the second-tiered test without having a reactive first-tiered test. So doing reduces the specificity of the sample resulting in false positive tests which I will address at least partially here in just a moment.

This slide shows again a review, a summary of dozens of published studies looking at the sensitivity of two-tiered testing as a function of disease stage. So we have stage one disease often presenting as erythema migrans with other constitutional, but not objective signs, typically. Stage 2 illness with neurologic or cardiac and/or cardiac involvement and late illness most often manifested as here in the U.S. as arthritis or chronic neurologic involvement.

I will just briefly go over the performance of the various components of two-tiered testing and a few modifications or newer tests that have been developed and looked at over the last few years.

The first-tiered test as I mentioned before is often an ELISA or an EIA in the early days and still now it is most commonly a whole cell preparation. These tests there are a variety of them out there and that have been approved by FDA, very significantly in specificity but most of them are in fact quite sensitive. If we look at performance of the whole cell ELISA in—as a function of disease stage—we can see early in disease at first presentation we're only detecting about 30% of patients.

As Dr. Breen mentioned, this is at a time point when the immune response, the humoral or antibody response has not matured and so it is not unexpected that at this first presentation that we would, in the absence of a full antibody response, that we would be able to detect a majority of the patients.

As we move into a convalescent time stage of Stage 1 illness, sensitivity of the ELISA increases about twofold so we're able to detect about two-thirds of patients.

As we move into later stage illness the ELISA actually does quite well. The point to remember here though about a whole cell sonicate or ELISA preparation of early *Borrelia burgdorferi* as a first-tier test is that the specificity although, it's variable, it's generally not acceptable. It's in the range of 85% slightly lower or higher. But it's not specific enough that we can feel comfortable in utilizing it as a single tiered test.

So we again in following with that thinking have recommended further testing to increase the specificity by utilizing IgM and IgG Western blots shown in the second and third rows here.

One can easily see that the IgM Western Blot has its greatest utility in Stage 1 and Stage 2 disease. In chronic or in late stage illness, IgM Western blots are of little utility.

The IgG Western Blot as we've mentioned takes a number of weeks before one detects a positive response there. So it has - is relatively insensitive in Stage 1 illness either acute or convalescent samples. By the time we reach Stage 3 illness the IgG Western Blot performs very well.

One test that we've all heard a lot about in recent years is VlsE or subcomponents of that, the C6 Assay and the real advantage of this particular testing format has been realized in early disease.

So as we'll see again in a few minutes a number of two-tiered modifications which utilize VlsE or C6 as part of that testing format have targeted early disease and realized increased sensitivity in that particular stage of illness.

At the bottom of this slide is again a summary of two-tiered testing performance. And as we've discussed performance at first presentation is quite low about 30% of patients. Increases at convalescence to about two-thirds of patients and continues to improve but still has needs in Stage 2 illness and actually does quite well in late stage disease.

So I'll now go into a few of the things that we've identified over these last 15 and or 20 years in the utilization of two-tiered testing as problems or gaps.

And we've discussed some of these very briefly. The IgM Western Blot cutoff of 30 days is a complication. Again most samples are not submitted with a well known definition or time point of the exposure to illness.

I will also talk about the false positivity in Western Blot, IgM Western Blot here in the next slide.

And we've already addressed insensitivity of two-tiered testing in early disease.

And I'll spend just a moment on the complexity of Western blots and where we might hope to go in the future for diagnostic serology.

This slide shows a figure from a recent publication, 2011, addressing issues with IgM Western Blot.

I really don't want to spend a lot of time on the actual strips shown here. But as most of you know, the issues in Western blotting, being IgM or IgG, are several-fold. One is the intensity of reaction to any antigen that it meet a cutoff—and so that under low reactivity or/and nonspecific reactivity not be scored. Secondly, alignment of blots is a major issue. And thirdly, just the complexity of Western blots, although one develops an expertise in actually

developing and reading these over years is still considered by CLIA to be a technically complex approach as a diagnostic.

So again this study found in a fairly well controlled clinical environment utilizing two-tiered testing that up to 27% of IgM blots were probably false positives.

And again just to summarize what the reasons for those were, many of the samples, 45 of 50, had symptoms of greater duration than four weeks so should not have been utilized for IgM Western blotting. About half of them did not have an antecedent positive or equivocal ELISA and about 10% of them actually scored incorrect bands.

So then again this just really summarizes what many of us have known for a number of years—that IgM Western blots can be useful in some situations where there's a lot of expertise but are problematic in the general diagnostic setting.

Another approach that's been utilized over the last five years or so to minimize the complexity in Western blots is to use striped blots in which purified or recombinant antigens are striped on blots and that's shown in these figures.

In the blue boxes we have regular Western immunoblots. In the red boxes we have striped blots. So looking at the blue boxes on the left you can see the three bands that are required or scored for reactivity in the positive control strip. Next to that is an actual test strip, fairly realistic looking test strip, and you can see reactivity to a number of bands, some of which are the diagnostic bands, others are very close to those but are not the diagnostic bands.

So if we move from that particular strip to a striped strip in which just the three diagnostic bands are striped we can see that we've removed a lot of that

complexity. We still have the issue of cutoffs and various manufacturers have attempted to address that issue of band intensity in cutoffs by utilizing internal standards and scanned blots.

Moving just briefly to the west—the right side of the Western blots again you can see in the blue boxes the native or the normal Western immunoblots, the complexity of all the bands in those blots. We're trying to look for ten specific bands and score them. Many bands are in the vicinity of the bands that we're interested in. That actually complicates the reading of those blots.

If we move to a stripe blot in the far right red box, we can see that a lot of this background and complexity has been minimized and greatly facilitating the reading of these blots and simplifying the process itself.
So this is one of the approaches that's been utilized, again in the last five to ten years, to minimize the complexity of Western blots.

There are a variety of approaches that have been utilized in the last ten years to improve two-tiered testing. Some of these have been single tiered tests; others have replaced parts of the first or second-tiered testing.

And shown in this slide is just one example, one report of probably several dozen that have been issued, so I don't want to focus on this one. But it is a good example of what has been done to try to improve sensitivity and maintain specificity through a single or a two-tiered testing approach.

If we look at the data here in the first column, we have standard two-tiered testing. The second column is a two-tiered ELISA algorithm which was a whole cell ELISA followed by a C6 ELISA. And in the third column we have the C6 testing by itself.

And very quickly you can see that if we look by different stages of disease that two-tiered testing again as we've already discussed— is insensitive in

early disease, increases to about two-thirds of the patients detected in Stage 2 illness, and actually does quite well in Stage 3 illness.

If we utilize, as this study did, a two-tiered ELISA format which is much simpler, removes the complexity of the Western Blot, we can see a substantial increase in sensitivity in early disease getting us up to a little bit over 50% of the samples detected, very good performance in Stage 2 and in Stage 3 illness detecting 100% of the samples.

And then one important point to notice here is that the specificity in large number of controls was maintained by this simplified two-tiered testing approach and found values over 99%.

Utilizing in the third column a C6 approach by itself increased the sensitivity in both Stage 1 and Stage 2 illness, maintained it in Stage 3 illness but realized a slight decrease in specificity of a little bit over 1% which may not seem like a large amount but when you consider the several millions of tests that are performed in the U.S. each year, a 1% increase or decrease in specificity results in many thousands of cases being falsely reported as positive.

This slide I just included—and this came out in 2005—and again a caution to users of tests and developers of tests. This is in no way meant to discourage new test development but I'll let you read that on your own if you haven't already.

Just reminding us that newly developed tests need to be evaluated, preferably in a blinded fashion in multiple laboratory settings and publication, is crucial to the review and acceptance and in particularly in any change in our testing format.

So we've talked about a few of the needs, PCR standardization, multicenter blinded evaluations. Some other needs as Dr. Breen mentioned in the prior

talk are markers of infection, markers of cure and would be very good to have markers of prognosis. What do we expect in a in a particular Lyme patient?

What I will address in the remaining slides are some of the ways that we at CDC and, in some cases in combination with efforts from NIH, and efforts and funding from NIH, have attempted to address some of these needs.

Over the last 20 years, CDC has provided, as I've mentioned, clinical diagnostic service. Anyone can make use of this, submit samples here to us. We've also served as a clinical trial site for comparative studies, multicenter studies, as well as applications to FDA as part of the 510(k) review process.

We also have a panel of monoclonal antibodies that is available, no charge, and has been distributed over the last 15 years.

Stock cultures—there are a variety of sources of these but we have quite a few too. If there are cultures that you're interested in, contact us and see if we have something that can help you out.

The last category which I'll spend the remainder of my time on is serum samples. We had a manufacturer's panel of about 45 sera that was developed in 1994. And it has been distributed in the last 17 years or so to hundreds of laboratories in the U.S. and abroad.

This panel as is indicated here was about 45 serum samples, 40 of them were from Lyme patients, just 5 controls. Some of the shortcomings of this panel, although it was very well described and documented, is that a number, a goodly number, of the samples were collected months, in some cases even years after the patient had been treated and cured and it lacked samples from look-alike diseases or patients that had cross-reactive antibody responses.

So I'll move there from what we've been doing over the last four or five years. We've initiated a new serum repository to address some of the shortcomings of our prior panel. There are over 400 patients that have been included in this repository.

The goals were to increase the number of Lyme disease patients from all three stages of illness. And we collected over 100 patients, Lyme disease patients. We wanted to include a large number of look-alike diseases or persons with potentially overlapping antibody responses, and those are listed here in this particular section from infectious mononucleosis to multiple sclerosis.

We also wanted to include a large number of healthy controls, both endemic and non-endemic.

What would we do with this panel? We, as I'll describe in the next slide how different panels are made up, we want to make them available for test validation. A lot of laboratories don't have positive or negative controls so to meet that need, for research development test, comparison and FDA application for 510(k) clearance.

Again this effort over the last four years was funded both by NIH and CDC through 11 contracts.

In the next slide—I will not go into detail on this at all. But just shows you the three groups of patients that are included in this panel; all three stages of Lyme disease in the upper section in green, the basis of inclusion or exclusion and additional information that we've collected on each of these patients.

The middle section here shows our cross-reactive challenge samples, if you will, and from mononucleosis to fibromyalgia, syphilis, periodontitis, looking for possible oral treponemes and cross-reactivity in assays with spirochetes,

rheumatoid arthritis and multiple sclerosis, and then our healthy endemic and non-endemic patients.

The lower two sections, of course, exclusion criteria with a history of Lyme disease. And as you can see in the last column in addition to inclusion and exclusion requirements we've collected a lot of information on each of these patient types.

When will this panel be available?

We've been working on it for the last—a little over three years and it is currently ready for release, at least in part.

We have decided to make this panel available or parts of it available in different categories. To meet the needs of validation we'll have ten samples available that will include both positive and negative controls. These will be provided in an un-coded fashion and they will be available here next month.

A research panel—a little bit larger number of samples, a little over 30 samples—will be available next month as well. This is basically moving to a larger sample set including more samples from Lyme patients as well as the different categories of controls.

And then finally a large panel that we will require persons to at least have some experience with one of our smaller panels and provide results of their testing in a confidential fashion here, not to be released to anyone unless they ask us to. But this coded panel will be available here in a couple of months. Right now we're targeting 1 to 200 samples, a very robust panel again including large numbers of Lyme disease patients in the various controls.

And then we've also given consideration to custom sets of panels if a person is or a laboratory is developing a specific diagnostic looking at a particular

stage of disease or a patient type. We will try to identify samples meeting those criteria in our repository and make those available.

So just in summary, what have we discussed?

There are a number of needs in laboratory diagnostics for Lyme disease. Simplification of the two-tiered testing approach, particularly the Western immunoblotting, is a much recognized need. We have still need for increased sensitivity in early diagnostics. We've talked briefly about perhaps some non-antibody responses that can be measured.

And Dr. Breen mentioned some current research looking at non-humoral markers of early infection as well. Just to underscore again there is a great need for markers of infection as control—as compared to pure.

And as we move more into the molecular diagnostics and are able to identify particular strains, it's possible that we may be able to provide prognostic information and diagnostics as well and then just finally, wrapped up with some of the things that we as federal agencies are doing and are available are to do in making reagents available to investigators, to people that are performing tests routinely and the general scientific community.

With that I will wrap up and take questions. Thanks.

Ben Beard: We are running just a few minutes late. So there are a couple of questions, and I think Dr. Schriefer can answer them real quickly. Then we're going to go to the next presentation.

Then I think we'll have a few minutes at the very end to come back to these.

But the first question for Dr. Schriefer is a clarification question, and I think you actually addressed this in the talk but it came in before your presentation.

Are samples available from the CDC Serum Repository to generate preliminary results for grants?

The second question maybe you can take before we move on is what is the gold standard of diagnosis against which new tests are benchmarked and how good do you think that is?

So we'll take those two questions then we'll move on and then we will take the rest of the questions at the end if that's okay.

Marty Schriefer: Okay, sure. Yes, as you mentioned samples are available. We very much encourage people to utilize these whether they're just in the process of test development, grant application, or FDA review. I know Dr. Gerald will address the FDA review in the next talk so I won't get into that too much other than to say to address briefly the question about gold standard.

As far as serology is concerned, the gold standard is two-tiered testing so equivalent or an improved performance is against two-tiered testing is kind of the benchmark by which new tests are reviewed.

Did that address at least briefly both those questions?

Ben Beard: I think so. There are a number of other questions that have come in, but we're going to move onto the last talk, and we'll come back to those questions at the end.

And so for now I think we'll move onto our third and final speaker in the series. This is Dr. Noel Gerald who is a Scientific Reviewer in the Division of Microbiology Devices in the Office of In-Vitro Diagnostic Device Evaluation and Safety, CDRH FDA.

So Dr. Gerald will be speaking on FDA review of IVDs that aid in the diagnosis of Lyme disease.

Noel Gerald: Okay. Thanks for having me. So hopefully my talk will now ducktail nicely with what Dr. Schriefer and Dr. Breen have presented in terms of when you have your concepts and you're ready to go to market, important things that it'd be good for you to know.

So my presentation outline, I'll very briefly talk a little bit about the overall regulation of In Vitro Diagnostic Devices and then the bulk of my talk is just going to really focus on the data that we look at when we review clearance of Lyme disease IVDs.

And in the last few minutes I'll briefly introduce a Pre-submission Program and review some information resources.

So we have this definition from the regulations for In Vitro Diagnostic Devices. And the highlights are that they're reagents, instruments, and systems intended for use in the diagnosis of disease or other conditions for use in the collection, preparation, and examination of specimens from the human body.

So FDA's mission for IVDs is to ensure that devices and systems that are on the market are safe and effective, while getting safe and effective devices to market as quickly as possible.

And we do this considering the benefits and the risks of each device.

So the regulatory classifications for IVDs are based on the estimation of the risk to the patient from the likelihood of false results from that IVD.

So it's a premarket review at FDA and then also post-market reporting and compliance from manufacturers that ensures that the tests on the market produce accurate and reliable results so that ultimately physicians can make informed decisions to best treat their patients.

So as Dr. Breen and Dr. Schriefer already mentioned, currently all FDA cleared tests for Lyme disease are serodiagnostics, which means that they detect antibodies to *B. burgdorferi* in blood serum and/or plasma.

So these are Class II devices, which means that they require a premarket notification before they can be marketed in the U.S. and that's application to our office in the form of a 510(k).

Since they are serodiagnostics, we do evaluate them in the context of the consensus 1995 two-tiered interpretation criteria that Dr. Schriefer described so well.

So since the late 1980s when manufacturers first began submitting applications to FDA for Lyme disease there have been more than 40 tests—more than 70 tests cleared along the 510(k) pathway.

Since 1996 and the adoption of the two-tiered interpretation approach there have been 39 tests. And they've been about evenly split between first-tiered tests and second-tiered tests.

The majority of the first-tiered tests that have been cleared have been ELISAs or other enzyme immunoassays, some with fluorescent-based, and the second-tiered tests are mainly split between Western blots and the Lyme blot assays that we've already heard about.

So these are the major components of review that we look at to assess safety and effectiveness. And this is just in the broadest strokes. We look at

analytical performance data to see how reliably and correctly the test measures its analyte or intended target. We look at clinical performance data to see how reliably the test measures the clinical condition. And we do look at the labeling, which should include the intended use, any directions, warnings or limitations, interpretations or results as well as a summary of the device's performance.

So I'll start off spending a little time on the intended use which is really the driving force of our scientific review and how we begin to evaluate devices for clearance.

So you should make it as precise and clear as possible and include details such as who will be tested, where they will be tested, and when and what the appropriate sample types are, and how the results may be used in patient management.

So to give you an idea of how all these elements will fit together in an intended use statement, here's a mock example we have for a fictional first-tier test. This is the (Zippy) Test, *B. burgdorferi* ELISA which is a qualitative ELISA for the detection of IgG and/or IgM antibodies to *B. burgdorferi* in human serum and plasma. And it's intended for use in testing samples from patients with signs and symptoms that are consistent with Lyme disease.

And the statement goes on to mention that all positive or equivocal results should be further tested by a second-tier Western blot assay.

So when you submit your application to us you should obviously give us a clear description of your device, you know, you should have the principle of the assay, a list of its components or reagents, any platforms or instruments that it uses as well as controls or calibrators that you recommend.

You should say how the sample should be prepared, how the signal is being generated and how the end user should interpret the results.

So as I mentioned, performance data is very important in what we review and we do look at analytical studies to help demonstrate the evidence for safety.

So you should be investigating the likelihood of your test for producing false positives with studies that look at cross-reactivity and other interferences as well as carryover and contamination issues. You should also look for a likelihood of false negatives with studies for limits of detection and matrix effects and interference.

And we do look for clinical data to help demonstrate evidence for effectiveness.

So we are looking for well-controlled clinical evaluations, which means among other things, a clinical plan and protocol. And you should have predefined objectives and methods so these studies should be performed with a test device that has a standardized design and performance and not just a prototype which is likely to change before you bring your device to market.

Other evidence that we do look for is a reproduceability study in multiple labs using a well-developed sample panel.

So the clinical performance data is required for all tests whether they're first-tier or second-tier.

And importantly for Lyme disease serodiagnostics, performance data generated at non-U.S. clinical sites is not acceptable. And this is mainly because the U.S. strains of *B. burgdorferi* differ markedly from the European and other non-U.S. strains.

So along those same lines, any antigenic reagents that are part of your test kit should be manufactured from the U.S. *B. burgdorferi* sensu stricto strains.

Now we do have a draft guidance that outlines some of the specific clinical studies we would look for for Lyme disease serodiagnostic clinical studies.

And I'm going to go over four of these here. One is Sensitivity Study. And in this you'll test a minimum of 100 well-characterized pedigreed archive samples. So these samples should be from clinically or culture-confirmed Lyme disease cases and they should span different stages from early, early disseminated, and late disease stages with symptom onset times noted, if they're available.

We also do ask for prospective studies. So these are fresh samples from symptomatic patients that meet inclusion and exclusion criteria—so these are not selectively tested, which means that you test all comers that come prospectively between your defined start and end dates of testing. You should conduct this study at three geographically distinct U.S. locations.

So we've also asked for an Analytical Specificity Study which testing a minimum of 100 samples each from endemic and non-endemic regions. And these should be obtained from asymptomatic populations and they should not include prescreened blood donors.

And lastly Marty talked about his reference panel that the CDC has which previously was 45 samples. We've also required a study with those 45 samples with the results stratified by disease stage. We're looking forward to the release of the new expanded panel.

So just to sum that part of the talk, the overview of the principles for successful premarket submission are to have a clear and precise intended use statement, a complete description of your device, to provide scientific

evidence supporting your intended use along with data demonstrating your device's safety and effectiveness with analytical and clinical data.

I haven't mentioned it in this talk but you should have good manufacturing quality systems in place and you should ensure that your labeling fulfills of the requirements that are outlined in the regulations.

And so in the last few minutes I just want to turn to our Pre-submission Review Program to introduce it to those who may not know about it and to encourage everyone to use it. This is basically a free protocol review from FDA. This was formerly called a Pre-IDE. And this is really the sponsor's opportunity to submit specific questions to FDA for feedback.

So this is an informal discussion of potentially complicated questions which could involve regulatory pathway or study design.

And this should be particularly helpful if you are new to the medical device arena or if you have a novel technology or a novel regulatory issue that you would like us to look at.

So the results of the Pre-submission Review, the comments are not binding on the FDA or the sponsor. It has a 75 to 90 day review timeline. And it's meant to be interactive and flexible and, of course, it is confidential.

So if you think about the typical IVD device development pathway that Dr. Breen was discussing where you begin with a basic research idea and move through analytical validation and through feasibility analysis and then go onto clinical validation and data analysis and then at the end compile everything into a submission that you're going to send to us for review.

And I guess the take home message for here is to not only restrict FDA to interaction just at the end. And that you can take advantage of the Pre-

submission Program to really interact with us at the early stages before you have invested significant time, money and resources in the conduct of your analytical and clinical studies.

And it's your opportunity to really discuss any of these issues with the group that will likely be looking at your submission in the end.

So these are just links for two of the guidance documents that I referred to most. The first is our draft guidance for Lyme disease serodiagnostics which really goes into all of the detail of the studies that we will ask for for those tests.

And then the second link here is our recent draft guidance for the Pre-submission Program which will give you all the information that you need to participate with that.

And of course all of our guidance documents can be accessed at our medical device's home page which is listed here which will also have access to our medical devices database and our device industry resources and much more.

And with that I'd like to thank you for your attention. And I'll be happy to answer any questions.

Ben Beard: Okay, thank you Dr. Gerald. That was a really interesting presentation, and we have a number of questions that are coming in.

And I think one of these questions is "What would be ideal in terms of size of a biorepository, and how could we move to a next stage of the current one?"

Noel Gerald: Was that for me or for Dr. Schriefer?

Ben Beard: Well it came in for you and it could be that maybe it's better to direct it to Dr. Schriefer but if any of you has ideas on that. Why don't we go first to Dr. Schriefer then others can comment if they want to, the other speakers.

Marty Schriefer: Okay. I'd be interested to hear Dr. Gerald's thoughts on that.

But the panel that we've put together right now has been somewhat limited in costs and just numbers of manageable samples that we can hope to get. It's taken us almost four years to collect these 400, a little over 400, well-documented samples.

But I think I'll stop there. See if there are more questions or comments on that.

Ben Beard: Any of the other, either of the other speakers want to comment on that?

Noel Gerald: No. I did have actually—I wondered about the volumes of the panel that the CDC will have because I guess that's one concern.

So normally these samples and these testings that we have this is part of the cost of developing the assay that manufacturers automatically assume, so it's difficult to gauge what the appropriate size of the number of samples would be. You also have to take into account the volume and how long this panel will be available.

Marty Schriefer: Yes. That's a good question. And right now we plan to make these available in 100 microliter aliquots. Occasionally someone will ask for a larger volume but we do not make those available in larger volumes unless there are extenuating circumstances and then that's just for a very small number of samples.

With that kind of a distribution we hope that this panel will last us for a couple of years.

Ben Beard: Okay, thanks Dr. Schriefer. And we have a couple more questions that really go back more to the repository as well. The first question is to see if you would make some comments on how this repository was put together in terms of how it was characterized, how the samples were characterized, what sort of testing criteria were done to call them what they are right now.

And then also the question about do you plan to collect and make available other types of samples in addition to serum? So this would be blood, biopsy, DNA, et cetera.

And so maybe Dr. Schriefer, if you can say something about how these samples were characterized, and then maybe you or Dr. Breen might talk a little bit about the plans for other types of specimens.

Marty Schriefer: Okay. So they were collected or acquired here at CDC through contract. So we had 11 different contracts. It was an open competitive process for people to submit proposals on how they would meet and characterize samples.

We have one of the criteria for sample validation or sample documentation was that we not get into a circular type of proof so that samples were not included based on previously tested and generated data indicating that they were Lyme patients.

So wherever possible we encouraged and required other non-serologic-based tests in addition to clinical presentation so that might have included PCR or culture or both.

We have characterized them ourselves by standard two-tiered testing and a number of other standalone tests and we hope to publish that data here in the next few months.

Other types of samples, good question, right now we only have (sera) and I think this is an increasing need for any disease and development of diagnostics. What other kinds of samples could we collect? It's possible that we could provide DNA from cultures but that's a limited utility. We don't have any provision or plans right now to collect other samples such as CSF or synovial fluid or skin biopsies. Obviously some of those would be in vanishingly small amounts to begin with.

But I think it's worth discussing the possibility of collecting a few other sample types.

Ben Beard: Dr. Breen or Dr. Gerald, do you have anything to add to that from your agency's perspectives?

Joseph Breen: This is Dr. Breen. I do. I mean we - and NIAID supports research studies who are looking to develop, novel detection strategies.

And so in that context we do have folks looking at PCR assays from other bodily fluids.

And I think the idea there is once there's a repeatable pattern then they would either request, to and for future funding to collect more to develop, more. Something along the lines of a repository but and then of course we'd want to get that broadly available to everybody.

But it's more (in the) discovery stage right now and trying to understand which of these might be the best way to do that. Is it going to be something

that's present in serum or will it be another easily accessible bodily fluid for example, we just don't know.

So we're supporting work that's looking in each of those areas. And I mentioned one looking at PCR specifically. And if, again, if that looks fruitful and, as Dr. Schriefer indicated, there's acceptance in the scientific community and that looks like they could even start to do a prospective trial which is what Dr. Gerald said is needed then I think we'd start to see the emergence of these alternatives.

But I think we're just - the state of things is that right now serum is still—dominates basically. And I think I am aware of studies where people are collecting skin biopsies in the context of ongoing prospective work.

And, you know, hopefully that will be better sorted out than what's available by culture because that's so difficult. Using molecular methods but it's still kind of early to determine which of those will be the most benefit.

Ben Beard: Taking my moderator's hat off and speaking here as Branch Chief at CDC in the Lyme program, I think I can say that we would be very eager to collect these other types of samples once tests that are being developed look promising.

I think that for example, there's a lot of talk about direct detection tests and I think we all realize that if these tests begin to develop and look real promising that they'll have to be validated and so clearly there'll be a need for the types of samples that would be used for those validations.

And so those types of efforts are quite expensive though, and so I think that probably it would be—our investment in that would hinge on seeing some of these tests begin to come into promise.

So with that I think that most of the questions that were asked have been addressed.

But you can see the slide here with the contact information. So if you have additional questions, feel free to send them our way.
And I noticed that my email is up here - not up here so I should put that as well. It's very simple. It's cbeard@cdc.gov.

I'll close just by saying that the session today was recorded. And that the presentations will all be posted probably hopefully in the next week or two and we'll have them posted at our web site, CDC, www.cdc.gov/lyme. And so you'll be able to find the entire presentations, both the recorded audio and the presentations themselves.

And I think probably the other agencies will provide the same links at their web sites.

So I want to thank you for your participation today. And I guess with that if there are any other comments, actually I'll let the other speakers have a chance to say anything that they might not have said.

Do any of you want to add anything?

Noel Gerald: This is Noel at FDA. And no, I just want to thank you for allowing me to speak.

Marty Schriefer: The same. Thank you Dr. Beard.

Ben Beard: Well thank you. And I think that this is the first time we've done so I apologize if it's not flowed quite as well as it might have.

But we're going to want to do more of these types of meetings. I think it's a way of the future.

So I thank all of you for taking time from your busy schedules to join us today. And I hope you have a great rest of the day. And with that we will sign off.

END