IN THE UNITED STATES DISTRICT COURT FOR THE

NORTHERN DISTRICT OF OKLAHOMA

W. A. DREW EDMONDSON, in his capacity as ATTORNEY GENERAL OF THE STATE OF OKLAHOMA and OKLAHOMA SECRETARY OF THE ENVIRONMENT C. MILES TOLBERT, in his capacity as the TRUSTEE FOR NATURAL RESOURCES FOR THE STATE OF OKLAHOMA,	)	
Plaintiff,	)	
vs.	)	4:05-CV-00329-TCK-SAJ
TYSON FOODS, INC., et al,	)	
Defendants.	)	

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THE VIDEOTAPED DEPOSITION OF

VALERIE HARDWOOD, PhD, produced as a witness on behalf of the Defendants in the above styled and numbered cause, taken on the 18th day of July, 2008, in the City of Tulsa, County of Tulsa, State of Oklahoma, before me, Lisa A. Steinmeyer, a Certified Shorthand Reporter, duly certified under and by virtue of the laws of the State of Oklahoma.

Q Okay, and you testified previously that you are not providing expert geological, economic chemical signature, medical or hydrological testimony; is that correct?

A That's correct. 09:08AM

Q And you were retained as a consultant to the law firm of Motley Rice; is that right?

A That's correct.

Q Okay. Have you received any funding directly from the office of the Attorney General of Oklahoma? 09:08AM

A No, I have not.

Q Now, apart from your -- the prior deposition and -- well, apart from the hearing, have you spent any time in the Illinois River watershed since your last deposition? 09:08AM

A No, I have not.

Q In general terms, Professor, could you summarize the work you've done in this case since your last deposition?

A Yes. Since the last deposition we have -- 09:08AM Roger Olsen and the CDM team has collected some more water samples. The North Wind Laboratory has done some more analysis on water samples, and I think that's about all we've done.

Q Okay. 09:09AM

A Of course, I've done some additional data analysis for the report.
Q Right, and you submitted a report?
A Correct.
Q We talked at your last deposition -- you talked at your last deposition a bit about fate and transport, and let me just run through some characteristics here, and I hope we can take care of these pretty quickly. Since your prior deposition, have you conducted any study of the fate and transport characteristics of any bacterium in the Illinois River watershed? 09:09AM 09:09AM
A No, I have not.
Q So you have not studied how bacteria is affected by temperature? 09:09AM
A No.
Q Desiccation?
A No.
Q Predation?
A No. 09:09AM
Q Osmotic pressure?
A No.
Q UV exposure?
A No.
Q pH balance? 09:09AM

A No.
Q Nutrient availability?
A No.
Q Have you studied how the movement of any
particular bacterium in the IRW is affected by its 09:09AM
size?
A No, I have not.
Q Its shape?
A No.
Q It's surface charge? 09:10AM
A No.
Q Location in the water column?
A No.
Q Presence of vegetation?
A No. 09:10AM
Q The media it's moving through?
A No.
Q Have you cultured the Brevibacterium that you
identified through your PCR process?
A No. 09:10AM
Q Why not?
A There has been no need to culture the
Brevibacterium.
Q Have you identified it any more specifically
than to say it's 98 percent consistent with 09:10AM

Brevibacteria avium?
A No.
Q And if you haven't cultured, I assume you also have not studied its fate and transport characteristics? 09:10AM
A That's correct.
Q Now, what you refer to as the marker, the biomarker in your term, what you're actually referring to is actually the DNA sequence that's contained by the Brevibacterium; is that correct? 09:10AM
A That is correct. We're referring to the DNA sequence, yes.
Q Okay. For clarity, I'm going to attempt to be consistent referring to the Brevibacterium as the PCR Brevibacterium and the sequence as the PCR 09:10AM sequence. Will those terms make sense to you? I just want to distinguish the two.
A Well, it's really a DNA sequence, so I guess --
Q We can call it the DNA sequence. 09:11AM
A DNA sequence.
Q If I refer to that, then we're talking about what you would refer to as the biomarker?
A Yes.
Q Now, we previously discussed or at your last 09:11AM

1 deposition you discussed that when a bacteria dies,
2 its DNA remains in the environment for some period
3 of time after that. Do you recall that?
4 A Yes, it can remain for some period of time.
5 Q Do you know how long the DNA sequence at issue 09:11AM
6 in this case can remain in nature apart from the
7 Brevibacterium that carries it?
8 A Typically in nature, bacterial DNA is rapidly
9 degraded within -- and it depends on the
10 environment, but within a matter of hours to several 09:11AM
11 days.
12 Q Okay. You said it depends on the environment.
13 A Correct.
14 Q What kind of characteristics affect how
15 quickly the DNA degrades? 09:11AM
16 A Characteristics would include the amount of
17 ultraviolet radiation. It would include the amount
18 of pred -- or not predation but the amount of
19 organisms that would consume that DNA because
20 they'll use it as a food source. So it would depend 09:12AM
21 on the trophic level. So in a more eutrophic
22 nutrient dense environment, then that DNA would
23 probably be consumed more quickly than in a more
24 allegatory thick environment.
25 Q Can DNA move in the environment after the 09:12AM

bacteria that carried it had died, become inactive?
A DNA could be transported along with water, yes.
Q Could it move in any other way?
A It would not be able to be motile on its own. 09:12AM
So it would have to be transported by the movement of water or some other matrix.
Q Okay. Let's talk briefly about sources of bacteria in the IRW. Since your last deposition, have you studied sources in the IRW, apart from 09:13AM poultry, of any -- of fecal indicator bacteria?
A I have not.
Q Okay. Has anyone associated with the State's case?
A Roger Olsen of CDM has done some work with 09:13AM bacteria in cow manure.
Q Okay. Are you familiar with the nature of his work?
A I have read his report, yes.
Q Have you studied any sources in the IRW, apart 09:13AM from poultry, of E. coli?
A No, I have not.
Q Okay. Of Enterococci?
A No, I have not.
Q Campylobacter? 09:13AM

A No.
Q Salmonella?
A No.
Q Any other bacteria?
A No. 09:13AM
Q Have you undertaken yourself to quantify fecal production levels by any animal in the IRW?
A No, I have not.
Q Have you undertaken quantification of bacteria loading from any particular source in the IRW? 09:13AM
A I have not.
Q Now, you submitted a journal article to the Journal of Applied and Environmental Microbiology; correct?
A That's correct. 09:14AM
Q And we were provided a copy of that a couple of days ago. You're on the editorial board of that journal?
A That's correct.
Q Okay. Have you discussed your article with 09:14AM any of your colleagues on that board?
A No, I have not. That wouldn't be -- you don't do that.
Q Okay. You submitted it on June 11, at least according to the cover E-mail; is that correct? 09:14AM

regrowth, what are you referring to?
A E. coli and Enterococci have the ability in some environments to persist for months, and there are some -- there is some evidence that they may actually multiply in some environments, especially 09:17AM
in sediment, and the multiplication would be slow but it could have -- it could potentially occur.
Q Do you have any evidence that the Brevibacteria you identified through your PCR process might grow in the environment? 09:17AM
A No, I don't have any evidence of that.
Q Okay. If the Brevibacteria did grow in the environment, how would that impact its correlation with indicator bacteria?
A That's almost impossible to say because it 09:17AM
would really depend on how the Brevibacteria responded to nutrients and environmental stresses. So I mean it could respond very differently than E. coli or Enterococcus.
Q If they responded differently to the same 09:18AM
environment and they're in the same environment, how would that impact the correlation?
A Again, the factors are so complex that I'm having a hard time thinking about how they might respond, but certainly if one -- if one group was 09:18AM

next week actually, but I'm thinking that we would have results at least sometime in August.

Q Let's look to Exhibit 3, Subtask 3, which, as I understand it, appears to be testing for Salmonella and Campylobacter in the IRW using a PCR assay. 09:45AM

A Uh-huh.

Q Has that been done yet?

A No, and we actually decided not to do that.

Q Why not? 09:45AM

A Basically expense and then we felt like we established the connection with the indicator bacteria.

Q Okay, and Subtask 4 just refers to technical memoranda summarizing the results of Subtasks 1 through 3. Do you know if any of those have been prepared yet? 09:45AM

A Those would not have been prepared yet.

Q Let's go ahead and turn to your report now, which you have as Exhibit 1 right there, and we're going to march through this page by page and hopefully get us all out of here at a reasonable hour. Let me direct you first to Page 3. Section 2 of your report here that starts by discussing waterborne disease, and while your report seems to 09:45AM 09:46AM

Q What do you mean by common?
A Common meaning one of the ways that people most frequently get sick.
Q How -- put that in percentage term. What's common? 09:47AM
A I'm sorry, I don't have a percentage off the top of my head.
Q What other routes would you say are common?
A Can you clarify the question? So what other routes are common for -- 09:47AM
Q Disease transmission.
A For disease transmission, sexually transmitted, airborne routes of transmission, foodborne routes of transmission would be among the most common, zoonoses from animals. Those are among 09:47AM the most common.
Q Okay. If you wanted to go find out how common one route of transmission is versus another for a particular bacteria or for a particular pathogen rather, is there a particular source you go to look 09:47AM at?
A That's fairly difficult. It depends on whether you are asking a question across the world or within the United States.
Q Let's say within the U.S. 09:48AM

A Within the U.S. generally I would go to the literature and see what I could find in there, and typically I would also go to the CDC, Centers For Disease Control.

Q Okay. I take it that the frequency of 09:48AM water-based transmission varies by pathogen?

A That's correct.

Q What diseases are more frequently or most frequently water transmitted?

A Do you mean in the United States -- 09:48AM

Q Sure.

A -- or do you mean in the world? In the United States our most frequent transmission would be -- Campylobacter is one of the very most frequent. Salmonella is frequent. We have the protozoa, 09:48AM Cryptosporidium in particular. The enteropathogenic E. coli are among the more common. Shigella is relatively common, and then there are a lot of viral pathogens as well.

Q Okay. Is -- say out of a hundred cases of 09:49AM Campylobacteriosis -- I'm going to slaughter that pronunciation at various times. Out of 100 cases, how many would you say are water transmitted?

A That figure I don't have off the top of my head. 09:49AM

1 person-to-person transmission, but there are usually
2 less person to person than there is from the
3 waterborne or foodborne, so I would say
4 proportionally less but I can't give you a
5 percentage. 10:00AM
6 Q Okay. Would the same hold for Campylobacter?
7 A To the best of my knowledge, yes.
8 Q Now, going back to your report, on Page 3 you
9 refer to full body contact. What do you mean by
10 full body contact? 10:00AM
11 A Full body contact would be when the person has
12 their full body in the water and --
13 Q Including their head?
14 A Including their head, yes.
15 Q Okay. So head under water. You note the 10:00AM
16 hundred thousand people using the IRW for recreation
17 that Dr. Caneday calculated.
18 A Yes.
19 Q Do you have any idea how frequently full body
20 contact occurs within those hundred thousand? 10:01AM
21 A No, I don't.
22 Q You also note in Paragraph 7 that the most
23 frequent result of exposure is intestinal, such as
24 enteric disease or gastroenteritis; do you see that?
25 A Is that on --

Q It's the first sentence of Paragraph 7.
A Yes.
Q What are you considering as exposure in that sentence?
A Exposure has a pretty wide range. It can 10:01AM
range from ingesting the water by swallowing the water or by drinking it on purpose. It could be accidental ingestion by when you are playing in the water or get submerged suddenly, but exposure could
also be aerosolization as if you are in a canoe and 10:01AM
slapping water or playing, even play fighting in a canoe, something like that. So exposure has a pretty broad range.
Q So exposure really means any exposure?
A Yes. 10:02AM
Q Okay. Do most exposures result in illness?
A I would say no.
Q Okay. So when you say the most frequent result of exposure to waterborne pathogens is
intestinal illness, is what you really mean the most 10:02AM
frequent result of infection or ingestion of waterborne pathogens, not actually just exposure?
A Well, if there's an adverse -- what that means is if there's an adverse outcome, if there is an
illness, it would be an intestinal illness. 10:02AM

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1 epidemiological studies to elevated microbial
2 pollution levels, and I'm just wondering which
3 microbes.
4 A Well, so in this case what this statement was
5 about was about the linkage between high indicator 10:03AM
6 organism levels that indicate fecal pollution and
7 their connection. So not linked to specific
8 disease-causing organisms but to fecal pollution and
9 their indicator, the Enterococci.
10 Q Okay. Have you studied any incidents of AFRI 10:04AM
11 in the IRW?
12 A No.
13 Q Are you familiar with any incidents of it in
14 the IRW?
15 A No. 10:04AM
16 Q Are you familiar with any incidents resulting
17 from exposure to water in the IRW?
18 A No.
19 MR. TODD: We'll go ahead and stop and
20 change the tape. 10:04AM
21 VIDEOGRAPHER: We're now off the Record.
22 The time is 10:04 a.m.
23 (Following a short recess at 10:04
24 a.m., proceedings continued on the Record at 10:19
25 a.m.) 10:19AM

A No.
Q On Page 4 of your report, you quote the World Health Organization, this little block quote here, and you quote, characterization of illnesses -- infections and illnesses due to recreational water 10:20AM
contact as being generally mild; do you see that?
A Yes.
Q What do you take generally mild to mean?
A What I just described. So it's not mild to the person, but vomiting and diarrhea for two or 10:20AM
three days, again, missing work and school, but then recovering on their own.
Q Okay, but seeking medical treatment or not seeking medical treatment?
A Frequently not seeking medical treatment. 10:21AM
Q Okay. You testified previously that plaintiffs have not undertaken any epidemiological study to quantify disease in the watershed. Is that still the case?
A Can you say that again? Sorry. 10:21AM
Q You testified I think at your last deposition that -- you were asked whether plaintiffs have taken any study to document levels of disease in the watershed.
A Correct. 10:21AM

Q And that still has not been done?
A Correct, it has not been done.
Q So the plaintiffs haven't conducted any epidemiological study to assess levels of Campylobacteriosis or Salmonellosis? 10:21AM
A Correct.
Q Okay. Have you yourself ever designed an epidemiological study?
A I have written a grant for an epidemiological study with the aid of epidemiologists, but myself am 10:21AM not an epidemiologist. So I'm familiar with the methods used, but I would seek help from an epidemiologist when design and study --
Q You need to translate your field of jargon for me. You said you wrote a grant. Does that mean you 10:22AM got the grant and did it or proposed a project or --
A This particular grant is a proposed project for an Environmental Protection Agency and the Florida Department of Environmental Protection, and the first phase of it is funded but the second 10:22AM epidemiology phase is not yet funded.
Q Okay. Now, you note -- this is in Paragraph 9 on Page 4 still -- that infants, children, pregnant women, elderly and the immunocompromised are more susceptible to waterborne infections. 10:22AM

A Correct.
Q Do you see that? Do you have any notion of the hundred thousand individuals who Dr. or Professor Caneday identified, any idea how many of them are infants? 10:22AM
A No.
Q Do you suspect there are many infants going for floats in the Illinois River watershed?
MR. PAGE: Object to the form.
A I really don't know. 10:23AM
Q Do you have any idea how many of the hundred thousand are children?
A No, I don't.
Q Pregnant women?
A No, I don't. 10:23AM
Q Elderly?
A No, I do not know.
Q Immunocompromised?
A No, I don't know.
Q Let's turn to the notion of bacteria that are in a viable but not culturable state, and this is something you discussed and testified about previously. Viable but not culturable does not mean undetectable; right? 10:23AM
A Viable but not culturable means undetectable 10:23AM

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by conventional culture methods, but there are other methods that could potentially be adaptive for detecting them.

Q They could be detected, for instance, for DNA-based methods, such as PCR; is that correct? 10:23AM

A That's correct.

Q What are the -- what are the relative advantages of doing culturing instead of -- over PCR?

A The biggest advantage of -- well, I guess if you can clarify that a little bit, so you asked me what are the biggest advantages of doing culturing over PCR show. In what context are you referring to? 10:23AM

Q That's a good question. Which one is faster? 10:24AM

A PCR was faster.

Q Which one is cheaper?

A Oh, that depends on the method. So some kinds of culture method are cheap and some are not.

Q If the PCR assay is already developed, so science has been done and it's been verified and it's known to identify, say, Campylobacter, so that's all in the box and you pull it off the shelf and you are going to use it, is it cheaper to do that or culture? 10:24AM

have you been familiar with the concept?
A I've been familiar with the concept since graduate school, so 1990.
Q Have you ever yourself studied it?
A Yes, yeah. We're doing some work right now in 10:27AM
my lab on viable but not culturable E. coli and Enterococci, for example.
Q What are you doing?
A We are assessing the extent to which the bacteria may persist in sediment samples in a viable 10:27AM
but non-culturable state.
Q Are you doing that for this case?
A No.
Q Apart from the work you're doing in your lab right now, have you ever written about any 10:27AM
bacteria's ability to enter that state?
A No.
Q When did you first consider the VBNC state in connection with this case?
A I would -- I would think it would be -- I 10:28AM
would think it would be from when I started working on it, which I think was 2005.
Q Okay. Did you at any point suggest that in order to generate a more accurate count of pathogens
in the IRW, it would be appropriate to use a test 10:28AM

other than just a culture-based test to identify it?
A We had some conversations about using PCR, and knowing the results that we were getting with the indicator bacteria and then moving toward the development of the biomarker, we just never went any 10:29AM
further with the PCR tests.
Q Let's talk a little bit about Campylobacter. I take it, based on what you told me earlier, that the State hasn't done any additional testing for Campylobacter since your last deposition? 10:29AM
A Correct.
Q You note on Page 6 now of your report that Campylobacteriosis is usually limited to mild to severe gastroenteritis but that it can also result in Guillain-BarrT Syndrome and Reiter's -- is it 10:29AM
Reiter's or Reider's?
A I think it's Reiter's.
Q Reiter's Syndrome. You say usually. Can you translate that into an incidence rate of one versus the other? 10:29AM
A I believe that Guillain-Barre Syndrome occurs in less than 5 percent of people that are diagnosed with Campylobacteriosis.
Q How about Reiter's Syndrome?
A Reiter's Syndrome, I'm not sure, but it's less 10:30AM

1 common that Guillain-Barre.
2 Q Since your last deposition has anyone
3 associated with the State's case studied
4 Guillain-Barre Syndrome in the IRW?
5 A Not to the best of my knowledge. 10:30AM
6 Q Are you familiar -- are you aware of any case
7 of Guillain-Barre Syndrome in the IRW?
8 A No.
9 Q What is Reiter's Syndrome?
10 A It is -- you know, I can't say for sure. I'm 10:30AM
11 sorry.
12 Q So you've never studied it?
13 A No.
14 Q Okay. Have you ever studied Guillain-Barre
15 Syndrome? 10:30AM
16 A Not beyond reading articles, not specifically
17 in my lab.
18 Q What you include in your report about the two
19 syndromes, I take it, is just based on your
20 literature review? 10:30AM
21 A Correct.
22 Q I take it -- are you aware of any case of
23 Reiter's Syndrome in the IRW?
24 A No.
25 Q Are you aware of any case of Reiter's Syndrome 10:30AM

1 caused by exposure to bacteria derived from poultry
2 litter?
3 A No.
4 Q Have you ever studied Campylobacteriosis
5 itself as a disease? 10:31AM
6 A No.
7 Q Have you ever studied Campylobacter as an
8 organism?
9 A No, not beyond literature review.
10 Q You mention, and this is Page 6, carryover to 10:31AM
11 Page 7, you note antibiotic resistance in
12 Campylobacter and Salmonella. Does antibiotic
13 resistance vary geographically?
14 A That's such a broad question. I really would
15 have a hard time answering it. Can you narrow the 10:31AM
16 question down?
17 Q Sure. Would -- let's say that Campylobacter
18 becomes 50 percent resistant to a certain antibiotic
19 in a study in say, I don't know, Oklahoma. If I
20 went and looked at Campylobacter in England, would I 10:31AM
21 expect to find the -- could I expect to find the
22 same resistance or could I draw no conclusion on the
23 Oklahoma study as to what I would find in England?
24 A There are regional differences in antibiotic
25 resistance patterns in both the pathogens and the 10:32AM

assay to detect fecal pollution from any animal other than -- or any creatures other than poultry in the watershed?

A No, no.

Q Okay. At your last deposition we talked about the report that North Wind had sent you which set out the process that North Wind had created to set out the process you used to develop the assay, and that was dated December, and the considered materials that were produced this time around had that December report in them. Has there been -- is there a more recent version of that report? 11:35AM 11:35AM

A That report was the report of the procedure used to develop the qPCR, and there has not been a more recent version of that particular report. 11:36AM

Q There have been more recent data reports; right?

A Yes, that's correct.

Q Okay. Did you ever test -- have you ever tested poultry feces to determine whether they contain the PCR Brevibacterium? 11:36AM

MR. PAGE: Object to the form.

A We have tested contaminated litter to determine that it can contain --

Q Did you ever test poultry feces? 11:36AM

poultry litter would outnumber the indicator bacteria by many orders of magnitude?

A So are you talking about Brevibacterium avium there?

Q Well, the Brevibacterium that you identified in the litter. 11:46AM

A Brevibacterium avium has been cultured from poultry.

Q Are you now saying that Brevibacteria that you identified in the litter is Brevibacterium avium? 11:46AM

A It's in distinguishable from Brevibacterium avium based on the DNA sequence.

Q I thought you testified it was 98 percent consistent?

A That's right, and that's indistinguishable. 11:46AM The general rule in molecular biology is 95 to 97 percent identity. Greater than that is the same species.

Q Brevibacterium avium has been isolated in bubble foot lesions on poultry feet; correct? 11:46AM

A Correct.

Q It's not been identified in poultry feces?

A Correct. There's very little out on the organism.

Q Is there any possibility that Brevibacteria is 11:47AM

growing in the litter?
A Is there any -- yes, there's a possibility, but that wouldn't matter for its purpose as a marker.
Q Are indicator bacteria growing in the litter? 11:47AM
A They could be.
Q They could be?
A Uh-huh.
Q What would you look at to determine whether they're growing in the litter? 11:47AM
A You have to do studies. I mean you look at pH; you look at water content. Salmonella, for example, have been demonstrated to increase up to two logs, and litter when the pH and the water content are right, so you could have some growth of 11:47AM pathogens and of indicators.
Q If Brevibacterium were growing in the litter but indicator bacteria are dying in the litter, what would that do to your correlation?
A Well, you could go every single way with that 11:47AM comparison, and you could say this goes up and that goes down, and that goes down and that goes up, and they both go up, they both go down. So it's pretty obvious that if they go different ways, then they're going to be less correlated. If they go the same 11:48AM

way, they stay correlated, but we just don't know. We do know, however, that the numbers are correlated, especially the numbers in the Enterococci, compared to the concentrations of the poultry litter biomarker. 11:48AM

Q We'll talk about the correlations later.

A Okay.

Q You've validated -- you validated the specificity of your assay with non-target fecal samples. Who determined what animals would be used? 11:48AM

A What species of animals?

Q Right.

A That was done in -- that was a collaboration between myself and CDM. I had the most input into it certainly. 11:49AM

Q Okay. Who determined how many samples to collect from each animal?

A Again, that was a collaboration between Roger Olsen and I and -- Roger Olsen and I really.

Q Okay. What factors did you depend on in your recommendation as to collect -- as to how many samples to collect for each animal? 11:49AM

A Really I depended on my knowledge, expert knowledge of being involved in many source tracking studies, and in testing and validating these, these 11:49AM

assays, I really relied on my experience there.
Q Okay. Did you perform any calculation to ensure that the sample size of feces, fecal samples collected for each animal was representative of the population of the animal in the watershed? 11:49AM
A There are no calculations to do that as far as you know.
Q Who determines the location from which samples would be collected?
A That was -- so the general sampling strategy 11:50AM of collecting some samples in the watershed and outside the watershed was agreed upon by -- between Roger Olsen and I and also talking to North Wind Lab, but the exact venues where the samples were collected was by CDM. 11:50AM
Q Did you take any steps to ensure that the sampling locations were representative of the entire watershed?
A I had assurance that they were collected from throughout the watershed, and then having -- and 11:50AM from separate farms which we agreed upon and then knowing that somewhere inside and outside the watershed there was also an assurance of having distribution of samples.
Q Okay, and that was the extent of the steps to 11:50AM

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inefficiency associated with it.
Q Okay.
A Really for an environmental sample being able to concentrate or to detect 2,000 copies per liter is good. 01:53PM
Q Your testimony, as I understand it, is that the PCR sequence, the actual DNA, correlates with indicator bacteria?
A In the litter.
Q In the litter. In the litter, and it 01:53PM correlates with more strongly with Enterococci than E. coli; is that correct?
A Correct.
Q I want to walk you through the process of developing the correlation just to make sure I 01:53PM understand it. So you calculated the correlation between gene copies of the PCR sequence and number of Enterococci?
A Can you repeat that to make sure?
Q Sure. It's the same question I just asked 01:54PM you, which is you developed a correlation between the PCR sequence and the Enterococci?
A In poultry litter samples, contaminated poultry litter samples.
Q Right. How many samples did you use to base 01:54PM

your correlation on?
A All 10 of the litter samples that we had at the time I did the correlations.
Q Okay, and do you recall the R squared value?
A It's in my report. 01:54PM
Q Okay.
A It would be .74.
Q Did you calculate a P value?
A Yeah. .0013.
Q Okay, and what was the nature of the 01:55PM relationship?
A Positive linear.
Q Okay, and now the same questions for E. coli. How many samples did you use?
A The same, the 10 samples. 01:55PM
Q Okay, and what was the R squared value?
A Let me look in my report.
Q Sure.
A It was about .35, but I want to make sure that I'm accurate. For E. coli, R squared equals .395 01:55PM and P equals 0.052.
Q Thank you, and what was the relationship there?
A That was also positive.
Q Did you calculate a correlation between the 01:55PM

PCR sequence and indicator bacteria in field soil where litter was land applied?

A No, I did not do that.

Q Okay. Did you calculate the correlation in edge of field samples? 01:56PM

A Between edge of field samples and what?

Q I'm sorry. Between -- in edge of field samples did you calculate a correlation between the PCR sequence and indicator bacteria?

A No, I did not. 01:56PM

Q Okay. Did you do it in surface water?

A No, I did not.

Q Okay. Did you do it in groundwater?

A No, I did not.

Q Did you do it for springs? 01:56PM

A Nope.

Q For wells?

A No.

Q Okay. Go back, if you would, to the few pages I gave you from your journal article you submitted. 01:56PM I forget what exhibit number it was. It was pretty early on.

MS. SOUTHERLAND: Exhibit 2.

Q Exhibit 2.

A All right. 01:57PM

contamination.
Q Okay, but in order for it to be an indicator of poultry fecal contamination, is it necessary that the PCR sequence share the same fate and transport as pathogens from poultry litter? 02:00PM
A Can you say that again? I just got to get the first part.
Q Sure. In order for it to be an indicator -- you've just said it is an --
A Indicator of poultry fecal contamination. 02:00PM
Q Right, and that fecal contamination you are talking about here is bacteria; correct?
A Correct.
Q Okay. So in order for the presence of the indicator -- 02:00PM
A I'm sorry. Let me go back there because we're not only concerned about bacterial fecal contamination from poultry, we're also concerned about nutrient contamination. So we can add nutrients and metals to that list. 02:00PM
Q We'll talk about -- let's table the nutrients and the metals for just a second and let's talk about bacteria. In order for it to indicate the presence of bacteria derived from poultry, is it necessary that the PCR -- that the Brevibacterium 02:00PM

that you identified share the fate and transport characteristics of other bacteria from poultry litter?

A It would have to have certain fate and transport characteristics in common. 02:01PM

Q Okay. If we compare the correlations that we discussed here, so the correlation, let's say, taking Enterococcus, for instance, the relationship between Enterococcus and the sequence in litter as .75 and the relationship between Enterococcus and 02:01PM the biomarker -- the sequence in water is .89, which is different; correct?

A It's different, but it's certainly within the bounds of what you would expect from regular sampling error. 02:01PM

Q Okay. How big a difference can you have within the bounds of regular sampling error?

A In environmental microbiology we're very happy to get correlations of .3 as long as they're statistically significant, even .2 sometimes. So 02:01PM there's a really wide range of what you can get from correlations and still be biologically meaningful.

Q Okay. So does it surprise you at all then that the correlation that you got between E. coli and the PCR sequence in litter was .39 you told me 02:02PM

at all is very encouraging and would not be likely at all to be the result of a chance event.

Q Okay. You mentioned statistical significance. What is the relevance of statistical significance to relying on the correlation here? 02:03PM

A So when you look at a correlation, you take several parameters into account, but the first one that you would look at is the P value and that would be the statistical significance of the result and if P is less than 0.05, then by most general 02:04PM statistical cut-offs, then that's a statistically significant correlation. It means that if you repeated that experiment 100 times, 95 percent of the time you would still get some sort of a correlation between the variables. That's what that 02:04PM 0.05 means.

Then you have the R squared. The R squared value actually tells you to what extent the variables co-vary. So if R squared is close to 1, then they co-vary tightly. If R squared is lower, 02:04PM then there's more variability in their relationship to each other.

Q Okay. Taking the litter samples, is it your testimony that based on the 10 samples here and the correlation that you developed, that if you took any 02:05PM

10 samples from anywhere in the watershed, you would expect to find these same relationships?

A I would expect to find similar relationships, not necessarily the same R squared, but I would expect to find a relationship between indicator bacteria concentrations and the biomarker. 02:05PM

Q Okay. Did you perform any calculations as to how many litter samples you should take to accurately characterize the watershed?

A No. 02:05PM

Q In the water samples -- background question. Poultry is not the only source of indicator bacteria in surface water in the IRW; correct?

A Poultry is a dominant source of indicator bacteria in the watershed. 02:05PM

Q I knew you believed that, but there are other sources of indicator bacteria?

A There can be.

Q There can be?

A Yes. 02:05PM

Q Okay. Are there?

A Okay.

Q Do you think it's possible that poultry is the only source of indicator bacteria in the IRW?

A Again, poultry are a dominant source but it is 02:06PM

possible that there are other sources.
Q Well, if they're a dominant source, then there must be other sources. Can we agree there are other sources?
A I can agree that there are other sources, yes. 02:06PM
Q Thank you. What -- when you did the correlation here for your paper between PCR sequence and indicator bacteria in the water, did you perform any -- did you do anything to control for ultimate sources of the indicator bacteria? 02:06PM
A We measured the poultry litter biomarker, but we did not have specific microbial source tracking tests for any other species.
Q Okay, and so the Enterococcus and the E. coli that are included in this calculation, the 02:06PM correlation in the water, those include all indicator bacteria or all E. coli and all Enterococcus regardless of source?
A That would include all E. coli and all Enterococci that were culturable. 02:07PM
Q Okay. Did you find the PCR sequence in all of your edge of field samples?
A No. I don't think --
Q You can probably look on Exhibit 12 and it will tell you. 02:07PM