UNITED STATES OF AMERICA

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DEPARTMENT OF HEALTH AND HUMAN SERVICES

FOOD AND DRUG ADMINISTRATION

IMMUNOLOGY DEVICES PANEL

MEETING

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Thursday, November 16, 2006

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The meeting came to order at 8:00 a.m. in the Ballroom of the Gaithersburg Holiday Inn, 2 Montgomery Village Ave, Gaithersburg, MD. Clive R. Taylor, MD, Chairman, Presiding.

PRESENT:

CLIVE R. TAYLOR, MD, PHD, CHAIR
SUSANNE GOLLIN, PHD, VOTING MEMBER
JAMES L. GULLEY, MD, PHD, VOTING MEMBER
TERRY R. LICHTOR, MD, PHD, VOTING MEMBER
MARC S. ERNSTOFF, MD, VOTING MEMBER
PATRICIA A. THOMAS, MD, VOTING MEMBER
MARILYN LEITCH, MD, DEPUTIZED VOTING MEMBER
M. MARGARET KEMENY, MD, DEPUTIZED VOTING MEMBER
GEORGE J. NETTO, MD, DEPUTIZED VOTING MEMBER
GENE P. SIEGAL, MD, PHD, DEPUTIZED VOTING MEMBER
ELBERT B. WHORTON, JR. MS, PHD, DEPUTIZED VOTING MEMBER
COLIN B. BEGG, PHD, DEPUTIZED VOTING MEMBER
W. JEFFREY ALLARD, MD, PHD, INDUSTRY REPRESENTATIVE
JOAN LONDON, MA, CONSUMER REPRESENTATIVE
DON ST. PIERRE, BS, FDA
RUFINA CARLOS, BS, EXECUTIVE SECRETARY

A G E N D A

P R O C E E D I N G S

(8:03 a.m.)

DR. TAYLOR: Thank you. At this time, I'd like to call this meeting of the Immunology Devices Panel to order. I would note for the record that the voting members present constitute a quorum required by 21 CFR Part 14.

At this time, I would like to ask each panel member at the table to introduce him or herself, and state his or her area of expertise, position, and affiliation. I'll begin. My name is Clive Taylor. I'm Professor of Pathology and Chair of the Department at Tech School of Medicine, University of Southern California. And then perhaps we could go around from my left.

MS. CARLOS: I'm Rufina Carlos, and I'm the Executive Secretary of the Immunology Devices Panel.

DR. GULLEY: James Gulley. I'm a Medical Oncologist and work with immuno therapy at the NCI.

DR. THOMAS: Patricia Thomas, Professor and Chair of Pathology at the University of Kansas, Surgical Pathologist and Cytopathologist.

DR. LICHTOR: I'm Terry Lichtor. I'm a Neurosurgeon and Neuro Oncologist at Rice University

Medical Center in Chicago.

DR. ERNSTOFF: I'm Marc Ernstoff, Medical Oncologist, Professor of Medicine at Dartmouth, and Director of the Immunotherapy program there.

DR. WHORTON: I'm Elbert Whorton, Professor, Epidemiology and Biostatistics, University of Texas Medical Branch in Galveston, and Professor of Microbiology and Immunology, same institution.

DR. ALLARD: I'm Jeff Allard. I'm the Chief Scientific Officer at Fujirebio Diagnostics in Malvern, Pennsylvania.

DR. TAYLOR: Susanne.

DR. GOLLIN: My name is Susanne Gollin. I'm Professor of Human Genetics at the University of Pittsburgh Graduate School of Public Health. I'm Professor of Pathology and Otolaryngology at the University of Pittsburgh School of Medicine. I'm a Board Certified Clinical Cytogeneticist, and my research concerns genetic biomarkers in cancer cells.

DR. NETTO: I'm George Netto. I'm an Associate Professor of Pathology at Johns Hopkins. My interest is surgical pathology, urologic pathology, and molecular diagnostic. I'm Board Certified APCP, and also molecular diagnostics.

DR. SIEGEL: I'm Gene Siegel. I am

Professor of Pathology, Cell Biology, and Surgery at the University of Alabama at Birmingham. And I am Director of the Division of Anatomic Pathology at the University of Alabama Hospitals.

DR. KEMENY: I'm Margaret Kemeny. I'm Professor of Surgery, I'm a Surgical Oncologist at Mt. Sinai, and I'm the Director of the Queens Cancer Center in New York City.

DR. BEGG: I'm Colin Begg. I'm Chair of Epidemiology and Biostatistics at Memorial Sloan-Kettering Cancer Center in New York, and my expertise is in biostatistics.

DR. LEITCH: I'm Marilyn Leitch. I'm a Surgical Oncologist and Professor of Surgery at the University of Texas Southwestern Medical Center in Dallas, and I'm the Medical Director of the Center for Breast Care there.

MS. LONDON: Good morning. I'm Joan London. I'm a Mass Communication Specialist, and I'm here as the Consumer Advocate.

MR. ST. PIERRE: Good morning. Don St. Pierre. I'm the Deputy Officer Director in the Office of in vitro Diagnostics at FDA.

DR. TAYLOR: Thank you. At this point, we'll ask Ms. Rufina Carlos, who is the Executive

Secretary, to make some introductory remarks. Rufina.

MS. CARLOS: Good morning, and if you haven't already done so, please sign on the attendance sheets outside. Information for today's agenda is also at this table. And as a courtesy to others in the room, please turn off your cell phones during the meeting.

Before I turn the meeting over to Dr. Taylor, I will read onto the record the deputization of temporary voting members statement and the conflict of interest statement.

"Appointment to temporary voting status - pursuant to the authority granted under the Medical Devices Advisory Committee Charter dated October 27, 1990, and amended April 20, 1995, I appoint the following as voting members of the Immunology Panel for the duration of this meeting on November 16, 2006; Dr. Marilyn Leitch, Dr. Margaret Kemeny, Dr. George Netto, Dr. Gene Siegal, Dr. Elbert Whorton, Dr. Colin Begg.

For the record, these people are special government employees and consultants to this or another panel under the Medical Devices Advisory Committee. They have undergone the customary conflict of interest review, and they have reviewed the

material to be considered at this meeting. Signed, Dr. Daniel Schultz, Director, Center for Devices and Radiological Health, dated October 24, 2006."

I will now read the Conflict of Interest Statement. "The Food and Drug Administration is convening today's meeting of the Immunology Devices Panel of the Medical Devices Advisory Committee under the authority of the Federal Advisory Committee Act of 1972. With the exception of the industry representative, all the members and consultants of the panel are special government employees, or regular federal employees from other agencies, and are subject to federal conflict of interest laws and regulations.

The following information on the status of the panel's compliance with federal ethics and conflict of interest laws covered by, but not limited to, those found at 18 USC 208 are being provided to participants in today's meeting, and to the public. FDA has determined that members and consultants of this panel are in compliance with federal ethics and conflict of interest laws. Under 18 USC 208, Congress has authorized FDA to grant waivers to special government employees who have financial conflicts, when it is determined that the agency's need for a particular individual's services outweighs his or her

potential financial conflict of interest.

Members and consultants of this panel who are special government employees have been screened for potential financial conflicts of interest of their own, as well as those imputed to them, including those of their employer, spouse, or minor child related to the discussions of today's meeting. These interests may include investments, consulting, expert witness testimony, contracts, grants, gratis, teaching, speaking, writing, patents and royalties, and primary employment.

Today's agenda involves the review of a pre-market approval application for a laboratory assay designed for the rapid detection of clinically relevant greater than .2 millimeter metastasises in lymph node tissue removed from breast cancer patients. Results from the assay can be used to guide the decision to excise additional lymph nodes and aid in staging.

Based on the agenda for today's meeting and all financial interests reported by the panel members and consultants, no conflict of interest waivers have been issued in connection with this meeting. Dr. Jeffrey Allard is serving as the Industry Representative acting on behalf of all

related industry, and is employed by Fujirebio Diagnostics, Incorporated."

We would like to remind members and consultants if the discussions involve any other products or firms not already on the agenda, for which an FDA participant has a personal or imputed financial interest, the participants need to exclude themselves from such involvement, and their exclusion will be noted for the record. FDA encourages all other participants to advise the panel of any financial relationships that they may have with any firms at issue.

Thank you, and I would now like to turn the meeting over to our Chairperson, Dr. Taylor.

DR. TAYLOR: Thank you. The panel is here today to discuss, make recommendations, and vote on a Pre-Market Approval, PMA P060017 for the GeneSearch BLN Assay. This is a qualitative in vitro test for the rapid detection of clinically relevant, that is greater than 2 millimeter, metastasises in lymph node tissues removed from breast cancer patients. Results from the assay can be used to guide the decision to excise additional lymph nodes and aid in staging.

At this time, we're going to proceed to the first of two one-half hour open public hearing

sessions for this meeting. The second half-hour open public hearing session will follow the panel discussion this afternoon.

For the record, prior to the meeting, no one had asked to speak at this morning's open public hearing segment of the meeting. Is there anyone who does wish to speak at this time from the public? In that event, we will proceed to the next phase of the meeting.

I would like to remind public observers at the meeting that while this meeting is open to public observation, public attendees may not participate, except at the specific request of the Chair.

We're now going to proceed to Veridex' presentation for their device. The first speaker will be Lubna Syed, Manager of Regulatory Affairs for Veridex, and then she will introduce the other sponsor speakers.

MS. SYED: Okay. Good morning, everybody. My name is Lubna Syed, and I'm the Manager of Regulatory Affairs for Veridex. I want to thank you all for being here. It's really hard work that you do. We sent you a big, fat binder beforehand, and you're all very dedicated to go through all of the materials and come here. And, after all, we're all

consumers of the healthcare system, and I think that what we're doing is very worthwhile.

With that in mind, when we're designing products at Veridex, we try to keep that in mind in terms of, is this something that I would want to use for my mother? Is this something that I would want to use on one of my loved ones, and so we try and design a safe and effective product, and we're hoping that we've done that, and we'll present that information to you today. You're the experts, and you'll get to vote on that.

So who are we? Veridex is a Johnson & Johnson Company. We're located in Warren, New Jersey. We're about 100 employees. We're dedicated to providing cancer diagnostic products to enable earlier disease detection, more accurate staging, monitoring, and therapeutic management. Our first line of products was a cell search product line which has been on the market since about 2004, and the GeneSearch BLN Assay is the first of the GeneSearch product lines, which is CE marked, and was recently launched at the International Sentinel Lymph Node Society meeting in Rome, November 1st through 3rd. So I'm up here and I will present the following speakers. I'll do the introduction. I'll be followed by Dr. Janet Vargo,

who is Director of Clinical Affairs, who will be giving the product description and performance. That will be followed by Dr. Don Berry, giving the statistical summary, Dr. Thomas Julian, who is Staff Surgeon Allegheny General, and who is also an Associate Director for the NSABP, will be presenting the surgical perspective. He was also Principal Investigator, and he'll be presenting Practice of Medicine Clinical Utility. That will be followed by Dr. Juan Palazzo, who is a Staff Pathologist at Thomas Jefferson University Hospital. He was also the central pathologist in the pivotal clinical trial, and he'll be presenting the current standard of care. Debra Rasmussen will be up last. She's our Worldwide Executive Director of Regulatory at Veridex, and she'll be presenting the conclusions.

I also wanted to let you know that two of the other participating Principal Investigators are in the audience with us today, but in the interest of time, they will not be speaking. It's Pat Whitworth, and Dr. Peter Blumencrantz. But if there are questions later on, they will be happy to be able to answer those.

So just to give a quick overview, the benefit of the BLN Assay is to be used

intraoperatively, so a surgeon would excise a lymph node from a patient, and that lymph node would be transited to the path lab. In the meantime, the surgeon would probably proceed to removing the primary tumor. The node would then be dissected by the pathology tech, and the RNA extracted using the sample preparation kit. There is some residual homogenate left over at this stage, and that could be used later, if necessary.

The RNA is then reverse transcribed and amplified using the GeneSearch BLN Assay, and this is loaded on to the Cepheid Smart Cycler II instrument, and a result is generated. It's a qualitative result of either positive or negative, and that is then communicated back to the surgeon. The surgeon could then proceed to remove the axillary lymph nodes within the same surgery, if the result is positive, or if the result is negative, they could proceed to closing up the patient. So we've run through this once before, so I'll go quickly.

The GeneSearch breast lymph node assay is a qualitative in vitro test for the rapid detection of clinically relevant that's greater than .2 millimeters metastasises in lymph node tissue removed from breast cancer patients. Results from the assay can be used

to guide the decision to excise additional lymph nodes, and to aid in patient staging. So there's multiple benefits for the product, and we'll go through these in great detail throughout the course of our presentation, but I just wanted to give a quick overview.

For the patient, there is reduced probability of a second surgery for nodal positive patients. A greater proportion of the node that's removed is being assayed with the assay than is currently being done by histology. Patient will not have to undergo the inconvenience, stress, and risks associated with additional surgery and more invasive lymphatic excision.

For the pathologist, there's improved support of surgeons implementing state-of-the-art commercial grade tests. The surgeon is providing improved patient care with reduction in the number of second surgeries potentially for breast cancer patients, and the oncologist is getting more thorough staging information of the lymph node itself.

So next up, I would like to introduce Dr. Janet Vargo, who will give you the product description and the performance.

DR. VARGO: Good morning. I'm Director of

Clinical Affairs at Veridex, and they're pulling up my slides. So just to give you a very brief background on the sentinel lymph node procedure itself, when the sentinel lymph nodes are diagnosed for metastasises, that presence or absence of metastasises in the sentinel nodes is about 95 percent accurate in predicting what is going on in the rest of the nodes remaining in the axillary basin.

If the sentinel nodes test negative, then that spares the patient from an unnecessary complete axillary lymph node dissection. If the sentinel nodes are found positive for metastasises, then typically, the surgeon would go on remove the rest of the nodes in the axillary basin, and it would aid the oncologist in their therapy decisions, as well as providing important prognostic information for the patient.

The current standard of care in diagnosing those sentinel lymph nodes is two-fold. Some labs, but not all, use some intraoperative histology methods. This has the benefit of giving a fast result, allowing the surgeon, if positive results are found with those intraoperative tests, to go on and do an immediate axillary lymph node dissection. That is an easier surgery to do, and it saves the patient coming back from a second anesthesia, a second surgery

at a later time point.

The gold standard, however, in terms of performance is permanent section histology, which takes one to two days as a turn-around time. However, it is more accurate, and will be detecting positives that are missed by today's current intraoperative procedures, meaning the patient has to come back for a second surgery. So the limitations of today's standard of care are that there is no combination, there's no rapid test that also provides high sensitivity.

Also, in all types of histology, for a number of cases, there is a subjective aspect. It requires expert pathologists with experience, and the methodologies are non-standardized. There is also a practical limitation on how much of that node can be sampled, so that you're looking at pieces of the node, and hoping they represent what's going on in the rest of the node.

The assay is designed to fill those unmet needs by providing a rapid result with high sensitivity, subjective, standardized, reproducible, and it can definitely test more of the lymph nodes. You're getting a better idea what's really going on in that particular lymph node. Remember that the goal of

the assay is to identify only clinically relevant metastasis, those greater than 0.2 millimeters.

For assay timing, which is important for an intraoperative test, first of all, one to six patients' samples can be run simultaneously, and technically you can actually run double that number with two different runs being run simultaneously in Cepheid. The turn-around time with an experienced operator is shown here, approximately an average 35 minutes for one to two nodes, and approximately below 40 minutes for up to three nodes. The results are reported as negative or positive, both on a per node basis, and on a patient basis.

The AnaLight markers used for the assay are two, Cytokeratin-19, which is expressed in epithelial cells, and Mammoglobin, which is expressed in breast cells. These markers have to have an appropriate cutoff to correlate with the 0.2 metastasises just talked about. That was done in a separate training set. We call it the Cutoff Study of 306 evaluable subjects. The goals of the company was to have specificity of the product be no less than 95 percent, approximately, and then to maximize sensitivity. And you can see here the cutoffs are 31 and 30 for the two markers. If either marker is

positive, or if both are positive, the node is considered positive.

The PCR system allows for sophisticated controls so that when you get a result on your node on whether the status is negative or positive in terms of the metastasises, you have a very high confidence in the fact that that is an accurate result, so there's an internal control, which is identification of a constitutively expressed gene, and that is there so that if you have negative cancer marker, negative CK-19 and mammoglobin, this should be positive so that you know everything went well in the amplification and all the technical processing.

Secondly, the external controls are there, and you can see that they're referred to as positive or negative external controls. Those names are referring to the status of their expectations for being positive for the cancer markers, but you can see that, in fact, the positive external control serves as a negative control for the internal control PBGD, and that the negative control serves as a positive control for PBGD, so all six results, all six marker results must be correct for the run to be considered valid.

Today, mostly I'll be talking about the results of the validation study in terms of

sensitivity and specificity of the assay, but a benefit of the assay is that it is highly reproducible. We did a separate reproducibility study at three sites, two operators per site with three lots, each lot tested over two days, and two replicates within each run of all panel members leaving 72 sample results per panel member. The panel itself was four different samples, some positive, some negative. And just as a very general result, conclusion here, there was 100 percent agreement with the qualitative result expectation of positive or negative, and the overall variability of the cycle time to positivities as measured by percent coefficient of variations were very low, ranging from 1.17 to 9.81 for all factors studied, and the median of 4.43 percent, so it was highly reproducible results.

So now I'll go into the validation study. This was an independent patient set from those that were used to determine the cutoffs, and we used, of course, the predetermined cutoffs. This is an overview of the trial design. The objective was to evaluate the sensitivity and specificity of the assay against permanent section histology. Remember, I mentioned that is the test that has the best accuracy,

although it takes time to get that accuracy. Positive by histology was considered positive if metastasis greater than 0.2 millimeters was identified and confirmed by two out of three pathologists. This was a prospective multi-centered study, results were blinded. The patient population was those with invasive breast cancer, and the testing with the assay was done on fresh tissue by site personnel, themselves.

This is a very important slide. In looking at any investigational test, you must compare it to something that people are pretty confident has a correct result to show how your performance is in your investigational test. In all cases, when you disagree with whatever the best gold standard is you can come up with to compare your assay to, the investigational test is always wrong. Whether it's really wrong or not wrong, you've got to take the hit as if it is wrong.

It's a particular problem for us in molecular field because we are testing fresh tissue to give an immediate result to the surgeon, and yet, the gold standard needs fixed tissue in order to give the best accuracy. This is a problem, means we cannot test the same tissue. It's not like blood where you

can different aliquots, pretty much the same thing. So we know going into the study that we are not going to have 100 percent agreement with the gold standard, and we can't, just as if you took sections, different levels of histology throughout the node, they are not going to be in 100 percent agreement with each other. So here, I just want to indicate that problem.

Here if you have micrometastasis that are distributed, which can happen, or may not happen, but if it does happen, then both test results would be positive. This is showing the cuts taken for histology, the red lines, and this is the assay piece which gets homogenized, and a true sample is taken from it to be tested, so here there's no problem, of course.

Here is the metastasis happens to be located only in the piece or pieces tested by histology, and the assay piece didn't have any metastasis, the assay could accurately give the answer of negative, but it will be considered false negative because the nodal status, as determined by histology, was positive; although, the assay gave the correct result for the piece it had.

The opposite can happen where the assay piece is either the only piece that had the

metastasis, and in a clinical trial against a comparator, the assay is going to be considered false positive, when, in fact, it could have been perfectly accurate. This is a case where the metastasis may be present. This is a macromet in both pieces of tissue, but if the sample didn't happen to be taken at the point of the macrometastasis in histology, again, the assay could be accurate, but would take a hit as being falsely positive inappropriately. Keep this in mind, because you are not going to see 100 percent agreement with the comparator result no matter what test this was.

This is sort of shown here, if you took histology against histology, and you did sampling of this piece of tissue with three other levels here, these levels results are not going to agree 100 percent with these levels results. It's a matter of sampling different tissue. So how did we share the tissue in the study? You can see that we took approximately 2 millimeter thick sections, divided the node in the way that you see here, alternating sections went to the assay, opposite sections went for histology.

The histology cutting that was done was two-fold. Here I'm showing you one piece of it.

There were three levels that were mandated by the study that had to be 150 microns apart. Those went to the study central pathologist to review. Also, the site pathology slides used for patient management were also evaluated for the study. If the site found positivity on their slides, confirmation had to be obtained for the study by the central pathologists, so you'll hear me talk about central slides and site slides throughout the presentation. These sections were taken very near to each other on the same face of tissue. And for performance evaluations, the cuttings that were done were all taken from one face of the tissue, for practical reasons. The sites were not willing to do more than this.

Here's the overall performance. There were 29 percent positivity rate from permanent section histology results. Sensitivity of the assay against the permanent section results was 87.6 percent, specificity 94.2. You can see the negative and positive predicted values with an overall agreement of 92.3 percent.

For the purposes of performance calculations, those results that were invalid leading to a no test result in the assay were considered as negative for performance calculations. Why? Because

in an intraoperative setting, if an invalid result is obtained, it is not providing evidence of metastasis to the surgeon, he's going to have to act as if he has a negative result at that moment in time.

Now in reality, it is reported to the pathology lab on the report as invalid. It is reported as invalid, but for performance calculations, we treated them as negative.

This is just for comparison purposes to show that the performance in the pivotal study was very similar to the performance obtained in the cutoff study that I mentioned previously. That was nice to see confirmation. And there was a smaller and equal `78 study done at the Institut Jules Bordet, and, again, you can see the performance is very similar.

Speaking of those invalids, overall on the validation or pivotal study, the invalid rate for the assay was 8.1 percent. Note, however, that over time, with experience, if you separate when operators didn't have as much experience with the assay, and when they did have experience with the assay, you can see that both IC failure rates drop, and there's a significant drop in the EC failure rates. It should be noted that some internal control failures are, in fact, appropriate. For instance, if only fat tissue is

tested, then it should be invalid. There's no nodal tissue there.

You can also see that during the trial we learned from how operators were doing after our training of them. We ramped up our training program. Site 14 came on late, and Bordet was started much later. We were able to use the new training program at those sites, and you can see that their overall failure rates are much better than the overall trial was, so we feel we've made great inroads in reducing the initial invalid rates.

Here's a breakdown of the performance of the assay against the permanent section results for sensitivity, as determined by macromets found by permanent section histology, or micromets. You can see that the assay's performance in the pivotal study was very high sensitivity for macromets, not surprising. They're going to be bigger, they're going to be distributed better, there's going to be less sampling issues with those, and that the sensitivity for micromets was moderate. Again, the distribution of the micromets is going to be a problem any time you're testing one test against another, and you're looking at different tissue.

Here we're looking at how did, or how does

the current intraoperative test do? In this study, it wasn't mandated that every site had to do frozen section or touch prep. If they weren't used to doing it, it wasn't fair to them or to the intraoperative methods currently used to make them do something they weren't comfortable with, so you can see that most sites, there were 319 cases where frozen section was done as standard of care, and only 29 cases where touch prep was done. I'll concentrate primarily on the frozen section, because the ends are much more reasonable to look at.

You can see the overall sensitivity that the assay had better performance than frozen section, and this is despite the fact that the frozen sections are taken very near to where the gold standard is taken, the same pieces of tissue, typically along the same nodal face; whereas, the assay is testing a completely different piece of tissue, and yet, it beats the performance and sensitivity of frozen section.

Here you're looking at the intraoperative results for pivotal patients only for micromets - I'm sorry. Okay. So here you're looking at it broken down again by macromets by permanent section histology, or micromets by permanent section

histology, how did frozen section do versus how the assay did. You can see that the assay detected all the macromets in this patient population, and the frozen section missed some, and that particularly for the micromets, the assay's benefit is quite apparent.

Here you're looking at the same data with the pivotal and cutoff patients combined, just to give you more confidence that this was reproducible, and very similar results were found in both studies.

One of the challenges with today's current standard of care histology is identification of lobular metastasis. Lobular metastasis have a different staining pattern with H&E that's difficult to recognize. And, in fact, IHC can be quite helpful in figuring out whether or not the H&E is actually detecting lobular metastasis that are difficult to see, and frozen section makes it even more difficult to see. So we wanted to see how the assay did on these difficult cases, and you can see there were 45 patients that had invasive lobular cancer, and that the assay detected all of them in the subset that were tested also by frozen section. Again, a significant betterment of sensitivity over frozen section, and for comparison sakes, just to get the in up a little bit, you can see very comparable results were found when

you combine the pivotal and cutoff datasets.

Another important factor for nodal status is how many nodes are positive. So I've shown you data to indicate that the assay correlates very well with permanent section histology on a patient basis. The patient does or does not have positive nodes. Here I'm showing you the data on how many nodes are positive by the assay, versus by permanent section histology. The green diagonal shows you where there's perfect concordance. Overall, it was very high agreement with a kapa value of .75, overall agreement of 88.5 percent.

We believe that the performance of 87.6 sensitivity and 94.2 percent specificity is, in fact, an under-estimation of the assay's true performance due to the limitations in the clinical study of having to test different pieces of nodes than what the comparator test is testing. Here's some data to support that.

First of all, there's another element where it's difficult for an investigational test coming in and comparing to histology, and that is that certain cases for histology are difficult to put into the correct category, so here's an example comparing on the exact same slides, the central pathologist one

looking at it, and what answers he obtained, and central pathologist two on the same slides, what answers he obtained. And, in general, it's where the micromets where things fall down a little bit. If one pathologist identifies something as a micromet on the same exact slide, the second pathologist has a 25 percent likelihood, based on the study data, of saying that there's nothing there, or at least there's nothing that reaches the .2 level. Fifty percent chance of agreeing it's a micromet, and 25 percent chance of saying it's a macromet, rather than a micromet.

This is just to illustrate the fact that that categorization, once you get down to that level of metastasis, is difficult, even for the current gold standard. We're comparing ourselves to an imperfect gold standard. Things are just difficult for some cases.

There is also, as I mentioned, the sampling differences. And here I'm showing you some evidence of how much effect sampling differences may call when you compare two tests. Here you have, again, what I call the central slides, versus levels tested by the site, and you're seeing them compared here. Here are the results found on the central

slides, here are the results found on the site slides. And, for example, you have macromets identified on the site slide in four cases that were found negative on the central slides. The likelihood is they are both accurate for the slides that they had to look at, because they are sampling different portions of the node; albeit, they're very close to each other on the same pieces of node. If you pretend for a moment that the central slides are the gold standard, and the site slides are an investigational test for a moment, and you did sensitivity and specificity calculations, the site pathology would have a "4.2 percent false positive rate". Are they really false? No. It's just not present in the gold standards piece in this analysis.

I bring this up because the false positive rate of the assay is putatively 5.8 percent, not that much different than when you compare slides to slides sectioning different parts of the node. And, in fact, remember that the node is testing more tissue, and it's testing it further away from the gold standard.

The last piece of evidence I'd like to give you is an informal evidence, but it's one thing we can do. Again, the assay piece was tested with different tissue than histology can test, but we can

take that residual RNA left over from the assay and run it on independent molecular markers, and see if they corroborate positivity or negativity found by the assay. And, in fact, when we tested true positives in the study that were both histologically and BLN assay positive in this independent molecular test that we designed to have 100 percent specificity, most of them confirmed as positive. This assay is not designed to have 100 percent sensitivity, it's designed to have 100 percent specificity, so that you can believe a positive.

Interestingly enough, the putative false positives that the assay found, 11 of them were tested, available to be tested by this independent molecular test, very comparable confirmation of positivity was found with the molecular test. Again, all of these data support the fact that the majority of the BLN putative false positives, the 5.8 percent, are likely true positives that were simply not present in histological pieces.

So let's look at the risk benefit analysis based on these data. So as a reminder for the false negative rates, we have three pieces of data in the study. One is, frozen section against permanent section. That false negative rate was 14.4 percent.

The assay in the exact same patient population was 4.4 percent. For macromets in this, as a sub-analysis, frozen section missed 9 percent, the assay missed none. Micromets, the assay missed twice as little as what frozen section missed, so you can see that the assay for current intraoperative methodology is, in fact, quite an improvement.

What are the risks for false negatives? Intraoperatively, the risk is that the patient will have to undergo a second surgery for ALND, assuming that the permanent section picks up the missed positive. If the permanent section does not pick up the false positive, and, in fact, it just remains a missed positive, then you have a possible lack of adequate treatment; and, therefore, an increased risk of recurrence of the patient. **SW STOP 08:42:48**

Let's talk about false positives, and I have them in quotes, because for each of these test methods, the likelihood is that in the majority of cases, they are not false positives, but simply sampling differential. So in the frozen section comparison to permanent section in our study, 2.2 percent of frozen sections were not backed up by the permanent section result. What is that taken to mean in today's standard of care? It means, typically, at

least it's assumed that it means that the metastasis were exhausted in the frozen section cutting. It does not mean frozen section was wrong.

Likewise, when I showed you the site H&E levels compared to the central H&E levels, there was a 4.2 percent false positive rate, if you, for a moment, assume that central was the comparator, and site was "investigational", for a moment. And, in fact, of course, everyone believes that the level that has the positivity is correct. A level that is negative is correct for that level. We believe the same is true, and I've shown enough evidence to support the fact that the majority of the 5.8 percent false positives that we have to take a hit for in the assay are probably not false positives, but are due to sampling discrepancies.

What are the risks of true false positives? And that is, a possible unnecessary ALND with its subsequent sequella, possible over treatment with its possible sequella. And it should be remembered that the cutoffs chosen for the assay are done to minimize false positives. In conclusion for the risk benefits analyses, the assay sensitivity is shown by our data to be an improvement over current intraoperative methods. We have fewer false

negatives. And the assay's specificity is comparable to current histological testing, equivalent false positives.

There are benefits, in general, to the assay. The assay's performance is likely better than, or certainly equal to the current standard of care. It must be remembered that the study comparator method of the central slides and site slides consensus pathology review is above the standard of care, despite making every effort that was reasonable in a clinical trial to get a perfect idea of what truth is in that node, what we use is a study comparator method. It's still not going to be truth. For one thing, they didn't get to test the pieces of tissue that we tested, so we believe that in general, the GeneSearch BLN assay can raise the standard of care. It's rapid with better sensitivity than the current intraoperative methods. It's subjective and reproducible, and most labs can adopt it. It reduces the overburden on expert pathologists. You're going to get an objective, qualitative result that would be signed off by a pathologist, but the pathologist's critical expertise that is needed for many different aspects of histology can be better used on other difficult cases. And, this is very important, that

the assay can sample much more of the node, and get a better idea of what's really going on, instead of assuming that a couple of sections is representative of the whole.

In conclusion, we feel that the assay trial data support the use of the assay as a stand-alone intraoperative decision maker to go on for a complete axillary in the same surgery, as an aid to patient staging by accurate detection of clinically relevant metastasis. Therefore, if the assay is positive, we believe that the node should be considered N1 status for a sentinel node, just as a frozen section result that is found positive and not backed up by permanent section histology later is not considered false positive, it's considered that the metastasis was exhausted, and it stands as a positive result for that node. We believe the same should be true for the assay. If the assay is positive, the node is positive, the patient is positive.

That concludes the performance from my viewpoint, and next, Don Berry is going to talk a little bit more about the statistics of the trial.

DR. BERRY: Thank you. My name is Donald Berry. I'm a Statistician from the University of Texas, M.D. Anderson Cancer Center, and consultant to

the company. Along with Scott Berry, I designed the trial from a statistical perspective, and analyzed the results.

Definitions of specificity and sensitivity, the reference test, as Dr. Vargo indicated, is based on Site H&E, and IHC histology. The BLN Assay is positive or negative based on the predetermined assay cutoff from the cutoff study. That was conducted prior to the data that you're going to see here to set the cutoffs. None of those cases were included in the sensitivity and specificity estimates that we provide here. So specificity is the probability that the BLN Assay will be negative, given that the reference assay was negative. Sensitivity is positive positive.

We conducted a Bayesian analysis, and as you'll see, over time calculated the probability that specificity would be better than the predetermined cutoff for specificity, based on agreement with the agency. We assumed something called Non-Informative Prior Distributions for specificity and sensitivity. What that means is that the conclusions were based, essentially, entirely on the data from the study as it was occurring, and the thresholds established for these probabilities, sort of like confidence intervals

in the more familiar frequentist approach to statistics, were based on the fact that we planned numerous interim analyses, and so adjusted the cutoffs for these specificity and sensitivity criteria based on the fact that we wanted to preserve the overall Type 1 error rate. And those cutoffs were .985 for both sensitivity and specificity, being better than the respective values of .7 and .9.

As I said, we planned interim analyses. We said after we get 200 cases, we'll calculate the probabilities that sensitivity and specificity are better than the lower bounds. And if we achieve greater than that .985 value, then we'll stop and conclude success. There is a corresponding futility calculation. We did predictive - we planned to do predictive probability calculations at each of these points, and if the predictive probability of a success at 700 cases was sufficiently small, then we would stop the study.

Otherwise, we'd continue to the next interim analysis, and, of course, stop at the cap of 700 cases in any case. And the .985 values that I indicated to you controlled the Type 1 error rate to be less or equal to .05.

The first interim analysis did not occur

at 200 cases. The first interim analysis occurred at 412 cases, and the reasons for that were logistical. The study was accruing moderately rapidly at 50 patients per month, and the amendment with the agency, and with the IRBs, delayed the first interim analysis.

What that means statistically is that the .985 values that we assumed were actually conservative. Had we planned to have the first interim analysis after 400 cases, we could have been somewhat more liberal. And so, these are the data that were available after 412 cases, essentially the same as what Dr. Vargo showed, and I'll come to that.

As she indicated, for BLN assay, if it was no result, that was treated as negative, and for the reference tests, if there was no result, the cases were not considered. What no result means is, no definitive result. The standard was to have two pathologists read each case, and if they agreed, that was accepted; if they disagreed, then it went to a third pathologist. When it says no definitive result, what that means is that there was no third pathologist reading to discriminate between the two. And at the time of the interim analysis, there were nine such cases, and we did not consider those in calculating sensitivity and specificity.

These were the interim results. The observed sensitivity was 89 percent. Remember, the cutoff for sensitivity was 70 percent, and we calculated the probability that sensitivity based on these data was, in fact, bigger than .7, which turns out naturally to be quite large, and so with respect to sensitivity, there was no question as to whether the boundary had been achieved. With respect to specificity, the observed value was about 94 percent, and the probability of being greater than 90 percent was approximately .99, which, again, achieved the cutoff, And so the study was stopped, meeting the criteria for success. That was on the basis of, remember, 412 cases. The final analysis involved 416 cases, four of the previously unadjudicated reference tests had been adjudicated, and so the no definitive result for those cases, again not considered, and the comparison was five.

So these were the final results. The observed sensitivity was .876, the probability of sensitivity being bigger - I mean, this is essentially the same as what you saw before - was .9999. The probability that specificity is bigger than its target is .996.

Dr. Vargo indicated that the reference

histology test is not perfect, but it is our gold standard in calculating sensitivity and specificity. If the sensitivity is 100 percent, then the specificity as we've -- if the sensitivity of the reference is 100 percent, then sensitivity - the specificity that we calculated on the basis of the 416 cases was 94.2 percent. If, indeed, the sensitivity of the reference test with respect to the truth, whatever that is, is less than 100 percent, then based on the references you see at the bottom, we calculated what the estimated specificity would be for the BLN Assay, and you see that if the sensitivity of the reference test is not as good as we were assuming, then, indeed, the assay specificity for BLN is a good deal bigger. The false positive rate is a good deal smaller than the estimated from the study.

This is -- Dr. Vargo indicated that for a frozen section, there are only 319 cases. This is expanding a bit, and showing in picture form the tables that she showed. So in both of these cases, the BLN and the frozen section, this shows an estimated categorization into true positives, false positives, true negatives, false negatives. The dark blue are agreements with the reference test in the case that the reference test is negative. The green

is agreements with the reference test, when the reference test is positive, and the pink and the red are the false values for the BLN and frozen section. The total error rate for BLN is 5.4 percent, for frozen section essentially the same, 5.6 percent. There is a disagreement as to which ones are false. And as Dr. Vargo indicated, and Dr. Palazzo is going to further discuss, the false positive may not be false.

So now I'm going to turn it over to Dr. Julian.

DR. JULIAN: Good morning, Dr. Taylor, panel members. Thank you for the opportunity to speak before you today. I am a surgeon, who is the Associate Director at the Allegheny General Breast Cancer Center in Pittsburgh, which is the home office for the NSABP, and Associate Professor of Human Oncology in the Drexel University College of Medicine system.

I believe today that one of the most stressful periods in a woman's history is when she is told and confronted with the fact that she has breast cancer. The first questions that come from her mouth are has it spread, and am I going to die? Fortunately, today, we do have methods to detect

breast cancer at an earlier time with use of screening mammography, and also patient information and awareness. This leads us to detect cancers at a smaller size, and hopefully at an earlier stage. Today, most breast cancers are being detected roughly around 1.5 centimeters. With that small size, there are fewer lymph nodes that are involved, and the metastatic rate seems to be a little smaller in those deposits.

The sentinel node concept evolving in essentially 1991, used in a clinical pattern, is based on a belief that metastatic disease to the lymph nodes is not a random event. It applies some of the Halstedian principles that we are still faced with, but it, instead, is an orderly progression of tumor cells to the lymphatic system. These primary draining nodes or sentinel nodes are the first to filter or contain those metastatic deposits. And biopsies of these sentinel nodes show an extremely high accuracy in predicting what happens, and the axillaness is borne by a multitude of single institution, multi-center, and also clinical trial reports.

Prognosis, local control, and treatment planning are the basis of why surgeons, medical oncologists and pathologists look at sentinel lymph

nodes, and lymph nodes, in general. The most prognostic factor that we still have in early stage breast cancer is the status of the axillary lymph nodes. Other predictors of the node status still have not replaced a lymph node biopsy.

Molecular tumor array analysis that we are currently using have - for detection or prediction for recurrence - still require the lymph node status to be known before it can be used and its concept. Following a standard axillary dissection, recurrence rates in the axilla can range anywhere from 2, to 3, to 4 percent. The standard axillary dissection, unfortunately, is associated with risk factors for a patient with lymphedema, pain, parastesias, arm weakness, and this can linger in 10 to 15 percent of patients in a chronic fashion.

The medical oncologist uses the information from the lymph nodes to help determine the need for chemotherapy, hormonal therapy, and today, anti-biological agents, such as Herceptin, and that's based on node status, number of nodes, and some of the tumor factors, as well.

Radiation oncologists, additionally use node status to determine whether or not the patient may require regional node radiation. And, again, this

is based on numbers of nodes, one to three nodes probably not going to receive regional node radiation, four or more nodes that are involved, they will have a higher request for use of radiation therapy.

This is a description of how sentinel node procedure is performed. Typically, prior to coming into the operating room, the patient is injected with a small amount of radioactive tracer, either in the skin of the breast. In the operating room, a small amount of blue dye is then injected either near the tumor or under the nipple. In the operating room, the surgeon will use a gamma detector to focus on the hot spot where the sentinel nodes are located, and then use that to help dissect into the axilla to minimize the amount of destruction in the axilla. And, here, on this slide, a portion of the node is being dissected out in a very focused fashion. And I've placed this portion of the slide to show you the distinction and difference between a sentinel node biopsy, which is located here, and a standard axillary node dissection, which, obviously, houses a fair amount of tissue. And, hence, the rationale for trying to use this technology and technique to minimize destruction in the axilla.

Sensitivity of axillary node involvement

has been reported anywhere from 70 to 100 percent. False negative rates have also been reported anywhere from zero to 29 percent. In the NSABP B-32 Study, which is the largest randomized study looking at sentinel node biopsy, the identification rate was 97 percent. The accuracy was 98 percent, the positive node rate was 26 percent, and our false negative rate was just a little under 10 percent.

It would be interesting if you compare this to the Milan Randomized Sentinel Node Study, which looked at tumors, or patients who had tumors that were two centimeters or less in size. I.D. rate, accuracy rate, the false negative rates were very similar, but an interesting finding was noted. They found a higher rate of positive lymph nodes than we have seen in American trials, and this is roughly 32 percent, compared to the 26 percent. And if you look at how they process their nodes in the operating room, the surgeon sends that lymph node to the pathology department, and that lymph node is processed frozen section realtime at 50 microns per section. And they then use an enormous amount of time, up to about an hour, sometimes a little longer, three pathologists, 20 technicians to process that node ad infinitum, and totally process it to identify their metastatic rate

into the lymph node, so it's kind of akin to an anatomical processing of the entire node and homogenizing it.

The sentinel node biopsy is supported by current ASCO guidelines, and several consensus panels, and societies, but I want to digress here for a second. Even though these guidelines are there, they are guidelines, they are not mandates. And, therefore, when one looks at path reports, operative reports, and the use of chemotherapy that is instilled by medical oncologists, one finds that the guidelines may not be followed to the highest level, as one would suspect, or expect in an ideal world. We live in a real world, and not all the times are the numbers of nodes, the size of the metastasis in the lymph nodes, the amount of blue dye, the amount of isotope detection recorded. And so, again, these are at the behest of the individuals who are using the technology.

Sentinel biopsy is associated with fewer complications in axillary dissection, and this is reported. Now it actually has been reported at the International Sentinel Node Society meeting in Rome this past month by investigators in the UK with their ALMANAC trial, and also Australian investigators with

their randomized trials looking at sentinel node, called the SNAC trial. Unfortunately, there is a risk with using the sentinel node biopsy. You could miss a metastasis, and there is a rare event of a blue dye allergy.

And just another point, looking at missing a false negative, or having a false negative for a sentinel node, that can occur for a multitude of reasons, and that may be the patient characteristics, tumor characteristics, could be related to also the surgeon's capability. But another issue could be that the metastatic focus in the node which was harvested was just not detected due to the limited pathology that may be undertaken.

If a positive sentinel node is not identified, a patient has the potential to be under-staged. This may mean that she may not receive adjuvant chemotherapy that she may need, and this could raise the risk of possible recurrence.

Current guidelines that a surgeon could use to guide them when they're in the operating room, if they have a positive sentinel node biopsy on H&E, the guidelines recommend strongly that an axillary dissection be performed. If micrometastasis were detected by H&E, which is anywhere from .2 to 2

millimeters, the guidelines still recommend that an axillary dissection be performed because the non-sentinel nodes may have a positive rate of anywhere from 10 to 35 percent.

The use of immuno histo chemistry analysis for positive sentinel nodes does upscale the positive rate. However, this area is fraught by a lot of conversation and discussion, and some of the management here is still problematic, because we don't know the real prognostic value of isolated tumor cells, or small deposits of only immuno histo chemistry detected nodes. And, therefore, the AJCC has classified these as pN0.

Using an intraoperative analysis, a surgeon can perform an axillary dissection at the same time. It avoids a second operation under general anesthesia for the patient. The associated risks that could be involved with that second operation are also deferred and removed from the patient.

If a positive node is identified intraoperatively, as I said, the surgeon can go on to perform an axillary dissection. If that node is deemed by pathologists to be suspicious or negative, the surgeon will wait and defer until the final H&E is derived. And if it is positive, the surgeon then has

to ask the patient to come back for a second operation.

In NSABP-32, the false negative rate of intraoperative analysis for sentinel nodes was as high as 40 percent, and this led to roughly 250 patients out of the subset that this was utilized on to be recalled for a second operation. Frozen section also has a high rate of false negative.

This does affect patient counseling in the fact that you've told a patient on her initial trip to the operating room, based on the intraoperative analysis that she has a negative lymph node. She's happy, she goes home, and is excited. She does not have metastatic disease. Three days later you're on the phone, or your nurse is on the phone with her telling her that the H&E analysis has now found that she does have a metastatic focus in the lymph nodes, and unfortunately, it's felt that she needs to return to the operating room for an axillary dissection. I can tell you from a personal effect that this is an extremely devastating period of time, again, for a patient to be faced with, and not only from the emotional standpoint, but also affects the family, husbands, significant others have to take time off. They have to bring the patient back to the operating

room, they have to spend time with them, and it just has an overall effect on not only the patient, but their family.

The GeneSearch Assay is a realtime rapid, reproducible, and robust analysis. It does permit an objective evaluation of a large amount of the sentinel lymph node, and hopefully will reduce the false negative rate, and aid in staging and treatment decisions. If the assay is positive, the surgeon can go on to perform a axillary dissection at the same time. And, again, that reduces the risks that we associated with that second operation that I've outlined previously. It also would help to reduce potential cost factors with that second operation, with hospital fees, professional fees, anesthesia costs, et cetera.

This assay does have detection limits which are appropriately matched to the histologic criteria; and, therefore, can be utilized for an intraoperative decision to be carried out, as per established guidelines.

All right. Thank you. I'm now going to introduce Dr. Juan Palazzo, who is a Staff Pathologist and Professor of Pathology at Thomas Jefferson University Hospital, and is the central pathologist

for this study. Thank you.

DR. PALAZZO: Good morning to the panel members and to the audience. I apologize for giving my back to some of my colleagues here. I'm a Professor of Pathology at Thomas Jefferson University, and I've been interested in breast diseases for a while. What I'm going to be showing you very briefly in the next few minutes is an outline of what are the predictors of axillary metastasis from the pathology point of view, which have the current guidelines and algorithm for node staging, which are some of the challenges that we face as surgical pathologists whenever evaluating these lymph nodes. And, finally, which I think are the benefits and the clinical utility of the BLN assay.

It is widely accepted by most people that the primary cancer is essentially in the evaluation of a sentinel lymph node, and in making a therapeutic decision. And I keep telling the fellows, we have to look at the sentinel lymph node, but we have to know about the primary cancer.

Most people would agree now that the larger the cancer, more than 2 centimeters in diameter, when it is truly identifiable vascular invasion in the primary cancer, the patient is more

likely to have axillary metastasis. If the metastasis in the sentinel lymph node is micrometastasis, meaning more than .2 millimeters in diameter, if there are more than one positive sentinel lymph node, and also, some people believe that if there is extra nodal, meaning extra capsular extension of the tumor, the patient is probably more likely to have no sentinel lymph node metastasis.

This has been analyzed by several studies that are listed here in the bottom, and this table shows the correlation between the size of the metastasis with the incidence of further known sentinel metastasis, so a micrometastasis is defined as being more than 2 millimeters in diameter, anywhere between 45 to 79 percent of those patients are likely to have metastasis. The micrometastasis defined from .2 to 2 millimeters, anywhere between 10 to 25 percent of those patients will have metastasis.

The submicroscopic metastasis, a somehow controversial field in the area of diagnostic sentinel lymph node, those patients are believed to have between 7 to 15 percent of metastasis. One group of sentinel lymph nodes interpretation that I, myself, find quite intriguing are those lymph nodes that the surgeon is convinced, has taking all the sentinel

lymph node, we've done our best with the current standard of therapy, but there is approximately 10 percent of patients that, indeed, have known sentinel lymph node metastasis. There are several theories, that the so-called skipped metastasis, the metastasis don't spread from the sentinel but go to the axillary lymph node, or also very likely there may be a component of lack of something of the metastasis in the axillary lymph nodes.

Which are the guidelines that are used at this moment to report metastatic or the status of sentinel lymph nodes? There are several guidelines, I think three of them, which are the most important ones, and they really are considered to overlap between them or the AJCC consultation manual. This is updated yearly or every six months, including the pathology literature.

The second one, which I find very useful because it's a really practical report, and I was one of the pathologist participated in this, was a proceeding of a guide - excuse me - the proceeding that took place in the City of Philadelphia every two years, and this was published in "Cancer 2000". And the third are the ones that were published in the "Journal of Clinical Oncology", and were supported by

ASCO in 2005.

Once the lymph node is received and the pathologist has the lymph node under his microscope, which are the methodologies to measure these metastasis? One is to use an ocular micrometer somehow in a way, very similar to how dermatopathologists measure the depth of invasion of malignant melanoma, insert it in the same microscope. Perhaps a more commonly used methodology to measure metastasis is to use either a ruler or a micrometer after identifying the focus or the foci of tumor in the sentinel lymph node. As you can imagine, this presents certain difficulties when you are dealing with something close to .2 or 2 millimeters categorical edges.

It is really difficult, I think, if you're looking at a sentinel lymph node to accurately measure in two dimensions what we're doing, likely complex three dimensional biological event. The AJCC's recommendations are the following ones. The lymph node should be regards as one when it's positive by H&E either during the frozen section or in permanent section. H&E means haematoxylon and eosin stain slice, and we report them as macrometastasis, or micrometastasis when they are .2 to 2 millimeters, as

an N0 when they are negative by H&e, as an N01+ in those cases that the pathologist decides to do immuno-cyto-chemistry as an N01+. The immuno-histo-chemistry is essentially the use, for those of you that are not familiar with it, of an antibody against a Cytokeratin that will identify these cells in the lymph nodes. And as N0, I0 as those that are negative by H&E, and also by ISC, if the lab decides to use that.

The use of ISC has been discussed in many conferences, and a fair amount of time was, indeed, devoted to it in the consensus conference. It is really not part of the guidelines; however, it is recommended by the AJCC, the ASCO, all the consensus conference, but is frequently performed.

I conducted an informal survey before the consensus conference, and I did find that probably 80 or 90 percent of the smaller hospitals that do sentinel lymph nodes, the pathologists are reluctant not to do immuno-cyto-chemistry for various reasons. One of them is that it does help in the interpretation of the H&E, whether you are suspicious that the focus may be cancer or not. The second one, that since you are cutting deeper in the lymph node, you're getting more tissue from the lymph node with the added plus that you are also doing a cytokeratin. And the third,

according to some papers, there is an increase in lobular cancers, and these more frequently identified makes tubular lobular cancer, is the identification of lobular metastasis, which they can be really challenging in frozen and in permanent sections.

To end with the AJCC's recommendations and coming to the molecular aspect, they recommend reporting as N0 molecular negative, those that by PCR are negative, and as N0 molecular positive - well, those cases are positive only by PCR. There's really at this time very little or no clinical data about this group of patients. I do think that as an additional tool that we could use as surgical pathologists, the GeneSearch Assay, provides sufficient data to support that when the assay is positive, the case could be considered SLN.

What are the three ways that surgical pathologists approach sentinel lymph node? One, which is here illustrated in the center, and there's a group of people that do this - they have done away, for several reasons, with frozen sections, and they do just as permanent. They fix the lymph node and the report comes out a day or two afterwards with or without IHC.

Another group decides not to do frozen,

not to use the frozen, and they only do touch prep. If you do the touch prep, you will have two results; one is negative, and one is positive. We cannot determine the size, but the patient is a candidate for the surgeon to undergo no sentinel lymph nodes. And the other, probably the most frequently used, is to do the frozen section again with the same results, as negative, as positive. In some sense, as we tried to do, we do the frozen section, and also the touch prep. I'm going to go through some details about some of the advantages and disadvantages of using one or the other method.

Regardless of the result, lymph node is embedded and we get H&E sections. And once again, we have a negative result, negative for tumor or positive, and we can give the size, and the patient then is a candidate for axillary lymph node dissection.

If we introduce the BLN Assay as a test along here, the priming of the lymph node is started with the BLN Assay, and the assay will pick up only micrometastasis being larger than .2 millimeters, and the patient is a candidate for ALND, or the assay is negative. The remaining of the lymph node can be studied either with H&E only, or with IHC. And, once

again, the result will be negative or positive with H&E results being able to provide the size of the tumor.

Some of the challenges that we see in surgical pathology practice in dealing with sentinel lymph nodes, frozen or not frozen, false negative. Perhaps if not one of the most important ones, is that we're just not sampling enough of the lymph node, and also, even though there is a classical distribution of the tumor in the lymph node, those of us that practice diagnostic surgical pathology, do know that cancer doesn't always follow a specific pattern of distribution in the lymph node.

The second potential negative possibility is lobular metastasis, which I've described, and also, the expertise of the pathologist. Interpretation of the sentinel lymph node in frozen section is something that residents and fellows tend to learn when they become attendings or faculties in different places. I don't think most places really teach, or do a very good job in teaching them interpretation of these, that can be quite difficult.

The potential false positive results are benign nevic cells, histiocytes, these two can be difficult in frozen sections more than in permanent.

But macrophages really in some of these lymph nodes can look at just everything, including *(9:22:00). And once again, the expertise of the pathologist. The results then are obtained one or two days after the lymph node is fixed and cut for permanent sections. Some of the limitations that we're faced with is that if you cut a lymph node for frozen section, you're essentially starting with a 2 or 3 percent of the entire lymph node, and this is a scheme of obtaining three sections, four sections of 5 microms every two or three millimeters, oftentimes the lymph node has been cut in half.

The touch prep is done very subtle over the surface of the lymph node, and placed on a slide to look at the cytology. There are guidelines, I do believe that how we process lymph node is not really standardized, and people tend to follow their guidelines, the guidelines according - among, other things, the resources that they have. And this is not used, as I said, in all pathology labs.

The performance of the current intraoperative histologic evaluation, I think has relatively low sensitivity, high specificity. Doing the frozen section, and this is something people that don't do frozen section believe strongly, is that the

sections can have more quality to interpret them, and they are more difficult to interpret. And then once you freeze the entire lymph node, some of that artifact is reflected in the permanent sections. The touch prep, really positive and negative, but lacks the context of cell for surgery, requires an experienced cytopathologist or surgical pathologist to evaluate. People that have experience, or have seen cases of touch prep to determine when they are positive or negative.

Using the different methods, what people have found, you can see here a few references to the left, this group using only frozen, touch prep, and combining both, pretty high specificity, most of them in 100 percent, slightly higher when you only use frozen section, lower when you use touch prep, and surprising, the paper from Turner in 1999, slightly lower when you're combining frozen section and touch preps.

What happens when we look at the lymph node in permanent sections, whether you've done or not a frozen section? Here we're looking at about 2 or 5 percent of the lymph node, because the permanent H&E gets about 5 micron sections for three levels for each 2 or 3 millimeters fragment of lymph node. If the IHC

is done, you add another 5 micron section in one to three levels, so you run the risk of missing anywhere between 10 to 15 percent of clinically relevant metastasis being more than .2 millimeters in diameter.

The limitations are people have decided to compare studies. What happens if we actually cut more of the lymph node, and the papers are listed here to your left. I think one of the important papers, is for Dr. Julian's recommended, is the papers that come out from the Institute of Tomaria in Milan. And this was also discussed. Dr. Veronsei was at the proceeding conference, and they, indeed, upgrade their cases about 15 percent when they cut the complete lymph node at intervals of 50 microns. The consensus in the conference was that we were all very happy not to be pathologists in Milan, because as you can imagine, anyone who has visited them, they have a great operation, but it's not really a realistic operation of having a large, large group of technicians, laboratory specimens, researchers and pathologists reading all these frozen sections.

This diagram is just to show you some of the pitfalls of how we see these in a sort of a tri-dimensional way when you are not really careful how you sample the lymph node, so this represents the

lymph node, and this is the metastasis. If one were to decide to take only sections and going both ways in here, you run the risk of calling this negative. If you move more to the center of the lymph node, we will call this on both sides micrometastasis, but then it could happen, and it does happen, we see it in deeper levels all the time, from negative from micro becomes a micrometastasis, and, indeed, cutting deeper, you may be surprised to find the presence of extra capsular tumoral involvement.

An example of a touch prep, I was trained not as a cytopathologist, but emphasis in cytopathology, so when I am on frozen, when we examine frozen section, you always emphasize this, that if we're going to do frozen, let's do also touch prep. Some of my colleagues and other people don't like it, but this is an example of a touch prep in the bottom of lymphocytes of an invasive carcinoma present in the lymph node, regarded as a clinically significant metastasis. We cannot give them the size, but we say that it's positive, and the patient becomes a candidate for axillary lymph node dissection.

I have to show you an example of a cytokeratin. As I said, it is not in the guidelines, but people still use it. I think you have to take it

in the right context, and I think that in this example shows right underneath the capsule, a cluster of five cells of cytokeratin positive cells. Yes, indeed, they look like tumor cells. We have no idea what those represent biologically. There are only four or five cells, and the other pitfall, which I think is important to consider is that many times these lymph nodes are done on a patient that have done other core or FNAs before the issue of misplaced cells. So as I said, you have to take this in the right context to make sure that you are not calling individual tumor cells or a small cluster of tumor cells, because they may be just misplaced cells. This is one of the reasons why those that do not recommend cytokeratin don't do it.

This is a case I had a few months ago. It was a lymph node. I knew the patient had lobular cancer. This is a permanent section. I was very careful that - I was a little bit suspicious about some of these cells that were present in this fibro septum, an otherwise benign lymph node. Not much happen in the lymph node, these are the lymphocytes. When I did a cytokeratin stain, I did find that a good number of these tumor cells were positive. There were some single lobular carcinoma cells, and then a big

cluster here that extended to the lymph node, so I ended up calling this a micrometastasis from a lobular cancer.

The next three examples are really examples from the trial that we conducted. On the left is a slide that I received as a central reviewer, and I call it negative. This is the *(9:29:03). When we reviewed the actual slide that had been interpreted in the site, we call it - it was positive. So, in essence, it was really a false negative case here, because the case had been interpreted as positive in a different level by the site pathologist. And the assay, in this case, was also positive.

This is an example of a lobular cancer. It was read by three different pathologists participating in the study. One person called it a macrometastasis, and two called it negative. The assay was positive, and there is a subtle micrometastasis that extended to the rest of the lymph node of lobular cancer right underneath the capsule, also with tumor cells spreading into the parenchyma of the node, so it was essentially regarded as a micrometastasis.

I apologize, this is a little bit dark, but this is different levels of another case from the

trial. This is level one of the H&E interpreted as a micrometastasis. This is the capsule of the lymph node, and the tumor goes from here to here, less than 2 millimeters in diameter. Deeper sections, level two, same case, the tumor becomes a micrometastasis, not only involves the subcapsular space, but also here spreads into the lymph node, and is a micrometastasis of an invasive ductile carcinoma. And the assay in this study was, in this case was also positive.

This is another example of a lobular carcinoma that I think is difficult to diagnose in an otherwise hyperpathic lymph node. There are some *(9:30:46) centers here. The capsule, there's nothing outside the capsule, but there is subtle metastasis of a lobular cancer underneath the capsule, single cells here, more cohesive tumor cells here, which really size-wise look very much like the lymphocytes that you would expect to find in the normal lymph node. And the assay in this case was also positive.

So in summary, what is the issues of the current histology and approach to sentinel lymph node? I do think that all the way from the time the lymph node is taken, to the time it is reported, for all the reasons we've communicated to you, should be done by an experienced person. That's not always the case,

but that's the ideal situation. That the node sampling, whether with frozen, or without frozen and doing only H&E, is limited. The nodal interpretation can be subjective, and is dependent on certain specific techniques used by the lab or sampling and staining.

The evaluation can be difficult, even for those pathologists that have experience specifically in breast pathology, and in interpreting sentinel lymph node, so it's not really a fast test with high sensitivity to guide same surgery, that would decide whether the patient is a candidate for axillary lymph node.

The benefits that I see as a surgical pathologist of this assay is that it's rapid *(9:32:14) and allows fewer second surgery for the patient. I think it's objective and standardized, and decreases the possible inter and intra pathology variability, that you are sampling more of the lymph node. I don't have lymph nodes left in the *(9:32:29) whatsoever after the assay only, or after the assay and the permanent H&E. So the results are really more representative of the entire lymph node. I do think that it helps avoid some of these false negatives, taking complete sampling of the lymph node, lobular

metastasis. Helps confirm whether I have a positive or negative H&E, and/or ISC examination.

I'm not introducing freezing artifact with the assay in the frozen section interpretation, or in the permanent section interpretation. And I think that as a surgical pathologist, it does reduce the workload, and also the better utilization of resources. So there are two things that I would like to finish with. One, is, and this has been discussed in many meetings *(9:33:16), and so far is that this assay is not intended to replace the surgical pathologist, number one. And number two, I think it's an additional tool, that as a diagnostic surgical pathologist, I can use or I can offer in certain cases to better stage these lymph nodes.

With that, I conclude my presentation. I would like to introduce Debra Rasmussen, who is the Worldwide Executive Director of Veridex. Thank you.

DR. TAYLOR: Ms. Rasmussen, we're running a little late for the sponsor, so how long do you expect you need at this point?

DR. RASMUSSEN: About five minutes.

DR. TAYLOR: That will be fine.

DR. RASMUSSEN: Thank you, Dr. Palazzo. Good morning, panel members, good morning, Bob, Pat,

Gene, Max, and colleagues that are here for this panel meeting. I will be giving the concluding presentation for this morning.

This is our GeneSearch Breast Lymph Node Assay. The intended use - the intended use, we've demonstrated with the results the clinical study results and the results that we presented today, that the GeneSearch Breast Lymph Node Assay is a qualitative in vitro test for the rapid detection of clinically relevant greater than 0.2 millimeters metastasis in lymph node tissues removed from breast cancer patients. We've also demonstrated that the results from the assay can be used to guide the decision to excise additional lymph nodes, and to aid in patient staging.

The benefits of the GeneSearch BLN Assay - benefits to patients, it's improved care, it's improved care that also is a benefit with surgeons, so there are not second axillary node dissection surgeries required for patients. It reduces the emotional stress, it reduces the inconvenience.

As Dr. Palazzo presented to you, it can be another tool that the pathologists can use in their analysis of sentinel lymph nodes. And for the oncologists, it provides a more thorough staging

information for patient care.

Clinical utility - we have two, two main points I want to bring home to you today. The GeneSearch BLN Assay is a stand-alone intraoperative sentinel node test. It enables same surgery axillary lymph node dissection. It has better sensitivity, as we've shown you, in comparison to permanent section H&E, than current intraoperative histology. The assay complements our comparator permanent section H&E results, and improves patient staging by being able to have greater portion of the node sampled.

This is a diagram, again. Frozen section, permanent section are not medical devices today. They are standard of care. They are what we're comparing to. BLN Assay is the assay that we're recommending, and will hope that you recommend also for the approval of this PMA, whether it's frozen section, whether it's BLN assay, or the permanent section, if you get a positive, the positive then recommends to the surgeon that they proceed with an axillary node dissection. For frozen section touch prep, just like BLN, it can be intraoperative. The additional information - positive, positive, positive, is provided for staging.

Here's our stand-alone. It can be the BLN Assay, and then you can still have material, as we've

shown in the slide that we presented today, to still use permanent section H&E, positive BLN, positive permanent section. Here's a recommendation for axillary node dissection, and provides additional staging information.

Assay safety - the FDA is going to bring up assay safety, and they'll bring up assay effectiveness, and the consequences of those. Yes, there are going to be false negatives. Yes, there's going to be false positives, and the false negatives and the false positives will have possible lack of adequate treatment, or possible unnecessary axillary node dissection. This is the same as current standard of care. It's the same thing that you'd have with current intraoperative. It's the same thing you have with permanent section today, except for we believe that it's at least equivalent, if not better, because you do have more sampling.

Here's the assay performance. On the 416 patients that we used in the pivotal trial, 87.6 percent sensitivity, 94.2 percent specificity. And we think this is an underestimation, but the best we can do in terms of not being able to compare the same pieces of the lymph node as a direct comparison. We have shown you that there's an improved sensitivity in

comparison to current intraoperative methods. We have an objective assay, reproducible, and we do believe that the benefits outweigh the risks in terms of safety.

Effectiveness - GeneSearch BLN Assay, we can provide an intraoperative result, compared to permanent section histology, but not having to wait one to two days. Greater accuracy, more of the nodes being used. And, again, objective, reliable, and consistent results.

Conclusion - as I said, we believe that the GeneSearch BLN Assay is a stand-alone intraoperative sentinel lymph node assay, that we do believe that it's going to aid patients in their staging. As Janet had represented, and you know, assay positive equals node positive, equals patient positive.

So this concludes our presentation for today. I want to thank all the presenters, and I want to open up to the panel, and thank you very much. Dr. Taylor.

DR. TAYLOR: Thank you. So we have an opportunity for the panel to ask questions at this point. There is another opportunity this afternoon. We've scheduled about 15 minutes of this, so if any

panel members have questions, would they signify by raising a hand, and then we can proceed from there. Yes.

DR. SIEGEL: Thank you. On page 57 of the information provided to me, you criticize intraoperative step frozen section, and rapid immunohistic chemistry with being "labor-intensive", and "leading to a significant increase in operating room time". With that in mind, I'd like to ask you about your claim of being able to provide rapid results.

DR. RASMUSSEN: Okay.

DR. TAYLOR: Let me expand on that, if I could, for a moment, to maybe help. Right now there are two pathways, one is from the operating room to the gross room, pathology, and the second is from the operating room to the frozen section room, to the gross room. Once the specimen is received in pathology, be it the frozen room or the gross room, the specimen has to be examined, dissected from the surrounding fat and breast parenchyma. As your own information states, breast parenchyma would contaminate the results. And then it would have to be split to hold it for permanent section, and perhaps frozen and/or touch preps. From there, it would have

to be carried to the molecular lab. According to the information you provided, you recommend that the molecular lab actually be multiple rooms for pre and post analytical work. And that, therefore, assumes that a technician is standing by, would drop everything else and perform this assay. The assay, you say, would be 30 to 40 minutes for two or so nodes.

In practice, many times a surgeon is operating in multiple rooms simultaneously, and may have multiple nodes from multiple patients that need examination.

Lastly, that would have to -- the data from the assay would have to be returned for a pathologist for his analysis and sign-off, and that information transferred back to the operating surgeon. So can you talk to me about rapid turnaround time?

DR. VARGO: Okay. I can't promise that I'm going to be able to remember everything there. I'll start with the rapid. I will try to remember some of the other aspects. Before I forget one of them, though, you had mentioned that we recommend, and it's true - for any type of amplification testing, it is always ideal to have the prep room separated either by distance or actual wall from the amplification

room. I can tell you that I believe we had one of the 14 labs set up for the clinical study who could do that. It's ideal. It's not mandatory, and the data that you see today were actually done in real world environments, and most of the labs, most of the assay procedures were set up near or in the pathology department, not a molecular department. So we had to fit in during the trial at 14 labs, where to do the assay.

Was it something they had to think about and figure out? Yes. As we all know, pathology space is extremely limited in most hospitals. They found a space in every case, and it was hodge-podge where they put it. Regardless of where it was put, the robustness of the assay and the closed tube system is developed by the Cepheid platform, was really beautiful in the fact that contamination just wasn't an issue. So, although, ideally PCR would be nice to have it done in separate rooms, the real cases, they probably aren't going to have that ideal situation in many laboratories, and our own data showed that it isn't mandatory, by any means. Those days of PCR are just about passed, really.

In regards to timing, I can let, perhaps, some of the surgeons speak to how long they wait for

frozen sections. I can tell you that hearing from different ones, it's 10 minutes to 40 minutes, depending upon whether they can find somebody immediately to read it, because they need the expert pathologist, et cetera. But in terms of the timing that we have down on the slide - and I have some extra information here on this slide for the assay - this timing covers the different things that you talked about, so the first thing is, obviously, removing it from the patient. And then transporting it to usually the gross lab where you're going to trim it down, take any pieces, separation, sharing, however you want to do it, take touch preps, et cetera. And it includes weighing of that tissue, which is part of the assay procedures, the hands-on part of the procedure, which is shown here. I believe it's the four to six minutes, plus the two to three minutes prep time hands-on with the assay, two to three more minutes for the PCR pipetting into things, and then the automated amplification is 19 to 20 minutes.

Most labs have figured out who do frozen section, how to get that answer back to the surgeon quickly. Some labs fax it in, some labs call it in, so that's already been worked out because of the frozen sectioning being done. This timing that you

see of the 30 to 40 minutes average ranges for one to two nodes, and 37 to 46 is real time data gathered in the clinical trial.

I can tell you that we did some video analyses after the clinical trial, not using human nodes, but having them go through the entire procedure, and it was interesting that just having the person being videoed was able to cut out a few minutes. And the video camera, in a sense, was acting like a surgeon strumming his fingers saying get me that result. Remember during the trial, the results were blinded. The surgeon was not using the results, or even getting the results of the assay. So we feel that the performance, the timing, and the setup, and everything is certainly doable, shown by having it been done at 14 different laboratories, not to mention the Institut Jules Bordet, et cetera.

Do we agree that it would be lovely to have it all done in 10 minutes? Yes, that would be perfect. It's just not technically feasible at this time.

DR. TAYLOR: Okay. Any other questions? Yes?

DR. VARGO: I'm sorry. Could Dr. Palazzo comment from his --

DR. TAYLOR: Well, we have about 15 minutes. If you've finished with this issue, we can always go on to another issue, or if you've got anything you really need to add. Dr. Palazzo.

DR. PALAZZO: Two minutes. It is my experience, Dr. Siegel, that, indeed, the test would add a few minutes. A good number of these patients are undergoing a simultaneous excisional biopsy of the breast, and the surgeon, that would take them more than 20 or 30 minutes, and they can wait with this. The majority of the lymph nodes that we, at least, process are done during the day, meaning 7:30-8:00 to 5:00, so the people doing the molecular analysis know a day in advance because of the schedule, when the lymph node is going to come. Indeed, I agree 100 percent that if I were to take two lymph nodes, process them as I do now, and not doing that and only the assay, it would take a few more minutes, but I think it can be done in an effective way and transmitted to the surgeon.

DR. TAYLOR: Okay. Dr. Kemeny, question?

DR. KEMENY: I'm wondering, because the node is homogenized, how do you actually know that it's greater than .2 millimeters? Why isn't it just 2.1 millimeter nodes?

DR. VARGO: Two ways. One is the data I've shown you today with the correlation with histology, albeit in a different piece of the node, having to be .2 or greater. One could even argue that perhaps the assay -- in the trial really what has to happen is the assay is detecting something that's equivalent to about .2, when another .2 is in a different part of the node, if you think about it that way. But the second piece we have is analytical data, which I think Elsa is pulling up right now, on the number of cells that it takes to be positive with CK19 expression or mammoglobin expression, to kick the assay over to the positive zone passed its cutoff, or before its cutoff, I should say. And then relate those number of cells to the number of cells that are likely to be in a theoretical spherical .2 millimeter metastasis.

The theoretical number of cells in a .2 perfectly spherical, which, of course, doesn't happen. Metastasis is about a thousand cells. The data that we have here, you can actually - I'm going to skip down to it - this was a culture study done with cells. The source was human epithelial cells from mammollary gland, and what we did was evaluate the number of cells it takes to kick the assay into positivity, and

I'll just show you the final results. For CK-19, that number was 2,000 cells, confidence interval of 1 to 4,000 cells, and for mammoglobin 25,000 cells, 18 to 35 CK-19 is a bit more sensitive. Therefore, it's analytical evidence supporting that the level of positivity correlates with the number of cells that would theoretically make up a .2 metastasis.

DR. TAYLOR: Okay.

DR. KEMENY: But I still don't understand why can't it be 2.1 metastasis?

DR. VARGO: I see your point. The question is whether -- does the assay add up whatever is there, and the answer is yes.

DR. TAYLOR: Okay. Thank you. Dr. Thomas has a question.

DR. THOMAS: I do. It's about reproducibility. Did you say you did a reproducibility study, and maybe I missed it, with four specimens?

DR. VARGO: Reproducibility studies are typically done with a panel of samples that are a transcript, and those were four different samples tested 72 times each. Do you want to know what the panel was, or what is the question?

DR. THOMAS: Right. Was the number of

samples, and considering that the invalid results are so operator-dependent, wouldn't you think you might need to do more samples?

DR. VARGO: The purpose of the reproducibility study that I showed you were two-fold. One is, basically, you do get another idea of the invalid rates, which were extremely small in the reproducibility study. I think it was two results, or something like that, for the whole panel, for the entire panel. It was a very small invalid run rate, partially, probably because reproducibility study was done late. The operators have had a lot of experience already with the assay. But mostly what you're trying to see is, can you repeatedly get the same result in the same sample, so we made a huge vat of homogen, same thing, frozen it down in different aliquots, looked at it across operator sites, lots, test multiple days, et cetera.

DR. THOMAS: Were there four sites only?

DR. VARGO: There were three sites, which is the standard expectations for a reproducibility study. Three sites, two operators at each site.

DR. THOMAS: I have another question. What about, we know that you can have benign epithelial inclusions in lymph nodes, and you can have

them in axillary lymph nodes. What about false positives related to that?

DR. VARGO: Yes. Maybe Dr. Palazzo can back me up on this in terms of the number of cells.

DR. PALAZZO: That is, indeed, a problem. The only thing is, in my experience, and at least in some of the published literature, they would probably, most of the time, be considered a submicroscopic metastasis, and would be falling more into the ITC group, more than a submicroscopic metastasis. I think it's pretty unusual to find just a misplaced gland, ectopic breast, or nevic cells, but nevic cells would be negative, that they're more than .2 millimeters or more than 2 millimeters.

DR. TAYLOR: Okay. Is there any other member of the panel with a question at this point in time? Yes, Dr. Leitch.

DR. LEITCH: I am pretty certain this is the case, but I just wanted to verify. For people who were positive on the assay, but negative on any other examination, I assume none of those patients have had axillary dissection. Correct? So we would not know what the status was of other nodes. Correct?

DR. VARGO: Correct.

DR. TAYLOR: Thank you. Anyone else?

Yes, Dr. Netto. This is Dr. Netto.

DR. NETTO: You eloquently presented how where exactly the micromet or macromet, be it in the deep, which piece, and how deep it is may affect the issue of false positivity being really not false, but maybe a true positive that just compared to the standard. This same premise, though, is present, supposedly, in your prior study where you set up your cutoffs.

DR. VARGO: That's right.

DR. NETTO: So here we are, we establish the cutoffs based on one study. When we move to the application of these cutoffs, suddenly we're going to highlight that this can occur here, but it was occurring there, too. So that I have a problem - how can you elaborate on this? The same issue, the likely that these micromets could be in the piece that you sampled for PCR, versus standard sections, is also occurring in the cutoff study, and so you would think that would affect where your cutoff is, and once you set up that cutoff, now you're using it again in the pivotal part. And here you're saying probably, or you're not saying - you're saying probably a lot of these are true positive that are not caught.

DR. VARGO: Right. What you want to do,

ideally, is, of course, have a perfect idea of what truth is in order to set your cutoff. There are limitations to being able to do that, especially in the case of molecular being compared to fixed tissue.

The second, though, tantamount thing that you want to do is make your cutoff study and the data you collect as like as possible, as to how you expect the assay to be used, and certainly how you're going to validate it, so you want those two studies to be identical.

The limitations on being able to do more thorough sectioning aren't due to the assay. They're due to handling of tissue for mounting it in a cryostat and cutting it. If you get less than about a 1.5 millimeter piece of tissue, if you try to parse it more between the assay and histology, the assay can take it, it's going to get all mushed up anyway, it doesn't matter. But histology cannot handle it, cannot manipulate it, so if we had done more thorough sectioning, which we would have loved to have done, or more thorough sharing, which would have loved to have done, we would have effective patient management, because there would be much increased chance that when they put that tissue for permanent section studying on the microtome to cut, instead of getting a good nice

cut to be able to read, they could smoosh it, and you get nothing, so patient management itself made the limitations to that.

We do feel that the analytical studies done on the number of cells, et cetera, et cetera, backs up that it is certainly a cutoff that's in the ballpark of .2, and that the clinical study backs that up. Is it a difficult thing? Can I tell you that it's exactly .2? Absolutely not. Can histology tell you it's exactly .2? Absolutely not.

DR. TAYLOR: Thank you.

DR. NETTO: I'm sorry. Can I follow-up on that?

DR. TAYLOR: Surely.

DR. NETTO: Yes. My problem is not the .2, is it really accurately reflecting the .2. It is the justification of these false positives suddenly being applied to the pivotal part, but not to the cutoff part. You would think that that would have played a factor in your setup of your cutoff.

DR. VARGO: Yes.

DR. NETTO: It was accounted for, and then when you reapply it on your pivotal part, you should not show that. So for it to show, it could have not shown on the cutoff, because you play with your

cutoff. Correct? You keep raising it, your CT. You say I'm not going to take any CT less than 19, or something, for the possibilities. So it's affecting your CT there, and then you adopted the CT, and now you're applying it again in the pivotal part, and suddenly you're highlighting this different and trying to justify it. I do have a problem. I don't think you answered the question.

DR. VARGO: Okay. Song Bai would like to address that.

DR. SONG BAI WANG: I'm Song Bai Wang, a biostatistician from Veridex. I think because we knew there are some problem in the cutoff, as well, our cutoff actually is set at specificity, it's 95. If there's a perfect result, then we would have set up as 100 percent specificity. Because we knew when we set up of 95 percent *(9:58:35), that probably is very close to, it would compare to the truth, which nobody knows, that's probably close to 100 percent, so that's why. As you can see that our pivotal study and the cutoff study specificity is very, very consistent. It is at 95 versus 94.2.

DR. TAYLOR: Thank you. Yes, we have a question from Dr. Begg.

DR. BEGG: I have a question. Although it

wasn't mentioned in the presentations, it was my understanding from the written material that the design was changed during the course of the study to lower the lower bound for the sensitivity estimate from 80 percent to 70 percent. And my question is that given your concern about false negatives, what was the rationale for making that change? And is 70 really a reasonable lower bound for a study of this nature?

DR. VARGO: Well, I might be able to sort of cut to the chase on that one about the false negatives, in the sense that despite the fact that we did change the amendment, and I can address why after I give you the answer, and you could tell me if you still want to go into why - that we still met the criteria for .8 in the final analysis.

DR. BEGG: Right, but I would like to hear your --

DR. VARGO: To know, okay. So the reason why the -- there were a number of changes. When I showed you in the slides what sections were taken for the comparator assay, they involved the central slides and the site slides. In the original protocol, we only had the central slides, and what we found when we looked at the cutoff data set is that the assay had

more false positives than we had expected, putative false positives. We checked into that, and though well, maybe histology is just not deep enough cutting. We had gotten what we were told and advised was the most cutting that we would get sites to be willing to do. We wanted ideally to have them cut all the way through the permanent section pieces, and we're told they won't do it.

Next best thing was well, they had to do some cutting for patient management, let's go get that data. It's what we can go get. When we did that, we found, lo and behold, that quite a few of the assay putative false positives weren't. They were backed up by site pathology slides, so the major change was we said we need a better comparator. We need the central slides, and the site slides, all of which have to be confirmed by a two out of three rule.

When we did that, we also came to grips with our naivety about how good the gold standard was. In doing that, we had originally had ourselves to have a lower confidence bound for sensitivity of 80 percent. We knew we would have to bias the study for patient safety reasons toward specificity, keeping it as high as possible, to not cause unnecessary axillary lymph node dissections. For that reason, we were

concerned that because we're testing different samples of tissue, and because we were seeing, to us, quite significant differences between adjacent histological sections, that our estimate of how much the assay was going to take a hit, because of the imperfect comparator, partially due to limitations in cutting that piece, and partially due to the fact that the comparator doesn't get to cut our piece, we lowered the expectations to be something in the realism that any assay could achieve. It ended up that the sensitivity did, in fact, meet the original criteria, however.

DR. TAYLOR: Thank you. Okay. We'll take one more question now, and then we'll defer other questions until this afternoon.

DR. SIEGEL: I just want to ask a point of information. Wasn't the final number .79, when the set that were thrown out for - I don't remember the exact term - inadequate, included back in?

DR. VARGO: Can you define .79, for what? I'm sorry. I wasn't following you.

DR. SIEGEL: You said you met the 80 percent rule. I thought the final number was .79. It didn't make the 80 rule.

DR. VARGO: Here it is right here. Thank

you, Elsa, for magically having it appear. Here you're seeing the changes that I mentioned. We added the site slides. I didn't mention, but we also added IHC, which ended up only having a difference in one case, where IHC was positive, and H&E was negative. We did a lot of work for not much gain. And you can see that the lower bounds were changed, as mentioned, from 80 to 70. However, what was achieved was 80.4.

DR. TAYLOR: Okay. There will be further opportunity for questions later. We are running slightly ahead of schedule, which is good, because usually things deteriorate later in the day. It's 10:02. We are scheduled for a 15-minute break, so we will come back at 10:20. Thank you.

(Whereupon, the proceedings went off the record at 10:02 a.m., and went back on the record at 10:21 a.m.)

DR. TAYLOR: Thank you. I would like to thank the sponsor and their team for their presentation earlier this morning, and now the schedule is for us to proceed to the FDA presentation. The first speaker for the FDA will be Dr. James Reeves, who is the lead reviewer for Division of Immunology and Hematology Devices, and then Dr. Reeves will introduce the other FDA speakers. Dr. Reeves.

DR. REEVES: Thank you, Dr. Taylor. Good morning, panel members, representatives from Veridex, FDA colleagues, and other attendees. I am James P. Reeves, or informally, Pat Reeves. I was the lead reviewer for this FDA submission. My introductory comments are intended to set the stage for FDA presentation of our review. It's my hope that our comments are accurate, precise, and sufficiently thoughtful so as to assist the panel in reaching a decision in this important submission.

We hope to briefly highlight the importance of various surgical, histopathological, and clinical items in node staging, and its consequences for early stage breast cancer patients. The GeneSearch BLN Assay is an in vitro diagnostic device submitted for approval for the intended use projected here. The assay's development and evaluation have been oriented toward intraoperative use after sentinel lymph node dissection, although the intended use does not limit its use to that setting. Please note that the intended use does not address the coordinated use of the BLN assay with other diagnostic methods, whether in an intraoperative setting, or not. The concept of clinically relevant metastasis greater than 0.2 millimeters will be discussed later in our

presentation.

The FDA's pre-market review focused on several aspects of the device for which we seek advice from the panel. There is experience with axillary lymph node dissection and evolving experience with sentinel lymph node dissection for the management of low stage breast cancer. We especially ask for the panel's advice concerning the clinical validity and clinical utility of the GeneSearch BLN Assay. Each of the FDA speakers who follow me will address one or more of the review areas noted here; assay design, intended use, population and setting, analytical issues, clinical validity, and clinical utility.

Dr. Roxolana Horbowyj is a Board Certified General and Critical Care Surgeon specializing in breast surgery, and she is a Medical Officer with the Office of Device Evaluation of the FDA. She will describe the highlights of sentinel lymph node dissection in breast care.

Dr. Max Robinowitz is a Board Certified Anatomic Pathologist, and he, too, is a Medical Officer at FDA in the Office of In Vitro Diagnostic Device Evaluation and Safety. He will address the surgical pathology of sentinel lymph node biopsy in breast cancer.

I'm a lead reviewer in OIVD, and will speak next to address the analytical performance of the device, the clinical study design, and some results from the clinical study.

Dr. Gene Pennello is a Mathematical Statistician with the Office of Surveillance on Biometrics at FDA. He will present the FDA's statistical analysis of the clinical results. After these presentations, I will return again to summarize and pose questions to the panel.

From our pre-market review to-date, the FDA believes that several characteristics of the device will require special attention. Our presentations will address these characteristics in detail, and I will briefly describe them here to you. These characteristics, combined with analysis of the performance data from the analytical and clinical studies are the motivation for posing the specific questions that we will ask you later.

The first notable characteristic is that the use of the GeneSearch BLN Assay without histology will have an impact on medical practice. This is because the device's target, analytical target, that is the detection of disease indicating at least micrometastasis in the sentinel node, combines tumor

staging categories that are separately reported and managed now.

Second, that is, it appears clear from our review that the GeneSearch BLN Assay does provide information about the presence of micro or macro metastatic tumor in sentinel lymph nodes.

Results from the clinical study indicate that the sensitivity of the GeneSearch BLN Assay exceeds that of frozen section consultation aimed at detecting micro and/or metastatic disease. However, the clinical study results also suggest that the specificity of the GeneSearch BLN Assay is less than that of frozen section diagnoses. It is certainly less than frozen section specificity commonly reported in the literature.

This is a matter of interest, especially because the design and analysis plan for the clinical study formally addressed neither the collection of frozen section diagnostic data, nor a comparison of the BLN assay and frozen section performance.

We will also note two items of practical interest from the submission. The first is that the rate of about 8 percent of which assays in which the clinical study yielded no reportable results. Second is the absence of submitted data that might establish

the amount of time needed to perform the assay in a realistic clinical setting.

Lastly, we will return several times to the safety and effectiveness issues of the GeneSearch Assay associated with true and false results from which the values of the assay focusing on predictive values will be presented. Sensitivity and specificity values will also be highlighted, so you will be seeing this slide several times throughout the talk, so at this stage, it's not important to me to pay attention to the details of the slide.

Certainly, clinically immediate outcomes will result from the GeneSearch Assay results. As illustrated in this slide, certain foreseeable consequences of assay test results will likely occur, and we hope to provide estimates to percentages in each of the four assay categories in order for you to assess the consequences of these estimates. We hope to highlight in later talks the particular consequences in each category.

We seek panel insight and advice to help us weigh these benefits and risks, and come to a decision about safety and effectiveness. FDA seeks the panel's advice concerning the proper trade-offs and conclusions to draw about the safety and

effectiveness of the GeneSearch BLN Assay.

Our next presenter is Dr. Horbowyj, who will discuss the submission from a clinician's perspective.

DR. HORBOWYJ: Good morning. This presentation highlights sentinel lymph node dissection in breast care with focus on the technique, risks, and benefits.

Breast cancer, as you know, is the most common noncutaneous cancer in women in the U.S.,and the second leading cause of malignancy-related mortality in the U.S. Survival is improving, and depends, amongst other factors, on treatment, which is, in turn, guided by various factors, such as disease stage at presentation. Current T&M tumor node metastasis staging for present cancer nodal status is based on clinical and histological evaluations. Surgical staging of the axilla is the most important predictor of clinical outcome.

Here is an example of a consensus treatment guideline for invasive breast cancer. I realize that there is much information here, and I apologize that it may be difficult to read. However, I would like to point out that in addition to other factors, the presence, as well as the size of lymph

node metastasis, micro compared to macro, may result in different treatment. For example, here you can see that for PN0 disease, which is no nodal metastasis, in this particular guideline, no adjuvant therapy would be provided. In the case where there would be micrometastasis, some additional treatment, such as adjuvant hormonal therapy may be considered. However, in the case of metastasis in the lymph nodes greater than 2 millimeters, another paradigm would be used in this particular case, which may then involve both adjuvant hormonal therapy and adjuvant chemotherapy.

The likelihood of axillary lymph node involvement is related to tumor size and location, histologic rating, the presence of lymphatic invasion. In a reported series of 2,282 women with invasive breast cancer, incidents of vascular and lymph node involvement increased with primary tumor size, as follows. From about 5 percent in the very small tumors, T1A, to 86 percent in T4 tumors.

Options for surgical management of the primary tumor in operable cases have evolved to decrease in rapidity of mastectomy. Current options include breast conserving surgery, post radiation therapy, mastectomy post reconstruction, and mastectomy alone. Selection is based on patient

preference, and suitability for breast conservation. Survival is equivalent with any of these options as documented in randomized prospective trials.

Breast conserving surgery, as you have heard, consists of lumpectomy, which is also known as segmental mastectomy, which removes the tumor with a margin of normal tissue. And in addition to this, axillary staging is performed. Axillary lymph node dissection aims to remove level 1 and 2 lymph nodes, level 3 lymph nodes are preserved unless gross disease is present. Sentinel lymph node dissection, or SLND aims to remove the sentinel lymph node, the first lymph node the cancer is likely to spread to from the tumor.

Extensive long-term outcomes are available on axillary lymph node dissection. Bland, et al, for example, analyzed the database of over 500,000 women treated for breast cancer and observed in 85 percent, 10 year survival among patients with axillary surgery, compared to 66 percent among patients without axillary surgery.

Axillary lymph node dissection, however, presents with risk of lymphodema, injury to or thrombosis of the axillary vein, seroma formation, impairment of shoulder movement, damage to the

brachial plexus with chronic pain and varying degrees of decreased grip strength, as well as chest wall pain.

Specifically as to lymphodema, the reported preference rate associated with axillary lymph node dissection is approximately 11 percent, with extremes ranging from 5 to 30 percent. Extensive surgery, radiotherapy, and advanced age are recognized risk factors for arm edema. Although the risk may decrease with time, it does not disappear completely. This picture demonstrates with the lymphodema in the patient's right upper extremity, which is illustrated with the increased size of the patient's right side. Lymphodema remains a quality of life concern for patients with breast cancer.

Sentinel lymph node dissection in the late 1990s was approach to decrease in morbidity, while maintaining accurate axillary staging assessment of the sentinel lymph node in patients with clinically negative axillary lymph nodes. In patient undergoing lumpectomy, SLND is performed during the same surgical session, but before lumpectomy. SLND is followed by ALND, if the sentinel lymph node has metastasis on pathological assessment. In order to decrease the false results of sentinel lymph node dissection, many

have used multiple modalities in order to find the sentinel lymph node.

In general, studies have restricted the use of SLND to women with T1 and T2 disease without evidence of multi-focal involvement, and without clinically positive lymph nodes. Data suggests that SLND is associated with less morbidity than ALND, and outcomes for comparative effects on tumor recurrence or patient survival are pending.

For example, as to morbidity, the ALMANAC trial is a prospective study of 1,031 clinically node negative patients randomized to undergo SLND or ALND. At one-year followup after surgery, results have recently been reported, and reported that the quality of life was superior in the SLND group. Arm function in time to return to daily activities were also better in the SLND group.

NSABP-32 is a randomized clinical trial comparing standardized SLND to conventional axillary dissection in clinically node negative breast cancer patients. Primary aims of this study are to evaluate if SLND alone is equivalent to ALND for overall survival and disease-free survival, as well as in the long-term control of regional disease, and for associated morbidity. This trial is designed to

detect just under 2 percent difference in survival between the two treatment groups.

As to micrometastasis, the American College of Surgeons Oncology Group Study Z-10 aims to estimate the prevalence and evaluate the prognostic significance of sentinel lymph node micrometastasis as detected by immunhistochemistry. It also aims to evaluate the hazard rate for regional recurrence in women with negative sentinel nodes by H&E staining, and to provide a mechanism for identifying women whose sentinel nodes contain metastasis detected by H&E.

Risks of false results for sentinel lymph node assessment may occur with false positive, or false negative assessment of the sentinel lymph node. In the case of a false positive sentinel lymph node, if ALND is performed, the patient has the risks of ALND, and the risks associated with intraoperative time, and anesthesia increased beyond SLND needs.

In the case of the false negative sentinel lymph node, if ALND is not performed, the patient has the risk of unrecognized under-staging, initial under-treatment, and associated decrease in survival, unless the false negative is identified, for example, during histologic evaluation.

So, in summary, advances in breast care

aim to minimize patient risk, and optimize benefits. Current T&N staging of lymph nodes is based on clinical exam, and tissue pathology. Treatment is based on the staging. Studies are underway to compare SLND and ALND associated with survival as just under 2 percent survival difference between these groups. And, also, studies are underway to determine the prognostic effects of micrometastasis. The unrecognized false results risk preventable compromise of patient care, and multi-modality evaluation can minimize false results.

Thank you for your attention.

DR. ROBINOWITZ: Good morning. I'm going to present an overview of the surgical pathology of sentinel lymph node biopsies, because Veridex has chosen this procedure as a comparator test for the validation of their test kit. I will be going over some of the information that's already been presented, but perhaps it will be a different viewpoint.

This is a photo micrograph through a longitudinal section of a normal lymph node stained by haematoxylon and eosin. The arrows depict the flow of lymph fluid from the outside of the capsule into the subcapsular space, through the node, and out to join lymph channels that flow to the next node in the

lymphatic system. Because cancer cells are first trapped and filtered out of the lymphatic fluid in the subcapsular space, the subcapsular space must be sampled to detect metastatic cancer cells by any analytic method.

FDA does not endorse any practice guideline, but we will use, as an example of current practices, the 2005 Multi-Disciplinary Evidence-Based Consensus Guideline published by the American Society for Clinical Oncology, ASCO, the recommendations for sentinel lymph node biopsy in early stage breast cancer. This incorporates recommendations of the American Joint Commission on Cancer, the College of American Pathologists, the Association of Directors of Anatomic and Surgical Pathology, and the National Comprehensive Cancer Network sponsored by NIH.

The guideline states that each institution must establish a policy on intraoperative assessment of sentinel lymph node biopsies or deferral to permanent sections. The sentinel lymph node biopsy procedure is very much a team effort with skilled involvement of multiple disciplines, and one must understand the strengths and limitations of each diagnostic method, and the particular institution's resources.

The directions for the pathologists are that all submitted lymph nodes should be counted and measured, the color noted, especially for blue dye, and to record the relative radioactivity uptake for each node detected by the surgeon.

It is the responsibility of the pathologist to systematically quantify and characterize the tumor burden in each sentinel node, and all other nodes that are submitted. This is important because the pathologic examination of axillary lymph nodes is a requirement for consistent categoric reporting using the AJCC cancer staging system.

This system for pathologic diagnosis is based on a gold standard of histologic examination of at least six axillary lymph nodes by permanent section H&E. The classification uses prefixes and suffices applied to the N of the TNM classification to document whether the diagnosis was made by histology.

In column one, I've listed the prefix P, that signifies that the diagnosis was made by axillary lymph node examination with H&E permanent sections. In column two, you see the designations of how to refer to the number of metastatic lymph nodes that are involved. The third column lists the suffices that

indicate whether the diagnosis is based on axillary lymph node dissection, or by sentinel lymph node biopsy, whether the metastasis are micrometastasis, if immunohistochemistry and/or molecular methods were used to make the diagnosis, and not haematoxylon and eosin histology.

Macrometastasis, again, are those metastasis that are greater than 2 millimeters in greatest dimension. They usually show histologic evidence of metastatic activity, such as proliferation, stromal reaction, penetration of vascular or lymphatic sinus walls. This is what I meant by characterization of the tumor. If any node metastasis is larger than 2 millimeters, the total number of tumor positive nodes determines the N category.

Micrometastasis are metastasis that are greater than 0.2 millimeters, but less than 2 millimeters in their greatest dimension. The lower limit accommodates the frequency of small tumor deposits identified in sentinel lymph nodes. Isolated tumor cells are single tumor cells or small clusters of cells less than 0.2 millimeters. They're usually detected by immunohistochemistry or molecular methods, but may be verified by H&E. They may be single foci,

multi-focal, or diffuse, in particular with lobular carcinoma. Usually the pathologist knows the diagnosis of the patient from the breast biopsy whether it'll be a lobular carcinoma, and will be alerted to this possibility.

Isolated tumor cells must be distinguished from mimics, such as macrophages and nevic cells, and as Dr. Kemeny mentioned, or Dr. Thomas mentioned the possibility of iatrogenic unintended causes, for example, from needle biopsy of breast tumor days to weeks before sentinel node biopsy. The pathologist assesses the morphologic features, and this is not possible with morphologic methods, with molecular methods.

This is a diagram to illustrate the Veridex sectioning plan for sharing alternating slabs of sentinel lymph node for histology, and for the Veridex test. For orientation, remember that lymph nodes are shaped like a lima bean. The Veridex plan differs from the ASCO plan in that the cross-sections are made perpendicular to the long axis, rather than parallel to the long axis. The Veridex plan results in more slabs of tissue per node.

Also, the Veridex slabs differ by being 1.5 to 3 millimeters thick, rather than 2 millimeters

thick, as called for by the ASCO guideline. And the ASCO guideline implies that one is embedding the entire node. Veridex supplied a Table of Instructions for the recommended number of slabs based on the longest dimension of each node. The minimum number of slabs would be two. For nodes 20 millimeters long or greater, at least 10 or more slabs would be necessary.

ASCO provides a protocol for a recommended limited step sectioning sampling of a complete lymph node. Again, it's a guideline, it's not mandatory, but if one follows that protocol and makes two microscopic sections from the face of the block, and then one or two sections at 200 to 500 micrometer intervals into the block, it is expected that virtually all macrometastasis will be detected, most micrometastasis, and in some patients, isolated tumor cells or clusters, particularly if immunohistochemistry is utilized. There is more yield with the step sections than with superficial serial sections that limit sampling to the upper levels of the block.

This is a photo micrograph of a subcapsular area of the lymph node containing multiple macrometastasis of proliferating metastatic adenocarcinoma surrounded by fibrous stromal reaction.

The label LN indicates the background lymph node, which is easily distinguished from the cancer.

This is a photo micrograph of another node. Within the white circle, we see approximately 15 isolated tumor cells stained brown by immunohistochemistry stain for cytokeratin. There is no evidence of metastatic activity.

The choices for intraoperative examination in the ASCO guideline are gross inspection of the cut surfaces of the node, cytology of node imprints or cell smears, and frozen section histopathology. Permanent section histopathology is considered the definitive pathologic diagnosis. The proviso that evaluation of sentinel lymph nodes is more likely to be accurate on the basis of paraffin sections, than frozen sections. And this is because frozen sections have basic limitations of, the microscopic features are not as detailed, thorough sectioning is a hazard, versus the risk of significant potential diagnostic tissue being lost, and incomplete sections may miss the subcapsular area. Finally, prior freezing may compromise the quality of the final paraffin section histology.

The ASCO guideline estimates the expected results that one would find from an intraoperative

examination of the sentinel biopsy. For every 100 patients considered for sentinel node biopsy, 25 percent will be positive by permanent H&E. Of that 25 percent, 16 to 17 of the 25 will be positive by frozen section, and 8 to 9 of the 25 will be false negative by the frozen section. Overall, 75 percent of the 100 patients will be negative by permanent H&E. When the frozen section is negative or suspicious, the recommendation is that the finding should be reported as not diagnostic for tumor, and deferred for paraffin section.

A brief review of frozen section practices from other peer review literature estimates that the sensitivity is good for macrometastasis, an average of about 80 percent, and the specificity shows that false positive frozen sections are rare. The College of American Pathology Quality Systems recommends that confirmation of frozen sections be done with permanent sections, and there should be monitoring of any discordant results.

IHC analysis was not recommended as a routine method by the ASCO guidance because of insufficient evidence, particularly for isolated tumor cells, or micrometastasis. And, finally, the ASCO guideline recognized that molecular approaches are

highly sensitive, may permit evaluation of relatively large amounts of tissue, but in October 2005, it was considered investigational. Also, that the tissues examined are destroyed making it not possible to identify the cells that were the source of the augmented signals for tumor marker messenger RNA, such as the differential between micrometastasis, isolated tumor cells, and macrometastasis.

Thank you for your attention, and now Dr. Reeves will continue.

DR. REEVES: Good morning, again, everyone. My review of the submission has focused on the intended use population and setting, analytical issues, clinical validity, and clinical utility. Here we have again the proposed intended use. The GeneSearch BLN Assay is a qualitative in vitro test for the rapid detection of clinically relevant metastasis greater than 0.2 millimeters in lymph node tissue removed from breast cancer patients. Results from the assay can be used to guide the decision to excise additional lymph nodes, and aid in patient staging.

We have noted from the intended use that intraoperative use of the assay is not noted, suggested use in other settings. We note, also, that

the sponsor's choice of defining clinically relevant metastasis as greater than 0.2 millimeters in size is controversial, particularly for metastasis between 0.2 and 2 millimeters. The clinical benefit or risk of metastasis between 0.2 millimeters and 2.0 millimeters lacks outcome data for long-term survival, which may become clearer pending ongoing clinical studies. We further note that the use of the assay as a substitute or as an addition to current intraoperative or subsequent permanent section histological procedures is absent.

In the performance of the assay, the instrument fluorescent signal is converted to cycle threshold values using instrument-specific software present in the Cepheid Smart Cycler instrument. CT values of the external positive and negative controls are compared with an acceptable range of values for each of the three markers using assay-specific software present in the Smart Cycler instrument. If controls are not in their specific acceptance range, the assay is deemed invalid, and could be repeated with another purified RNA sample from a particular patient, though the patient could be flagged as invalid, even if repeat tests give valid results.

Once controls are within the acceptable

range for each marker, CT values are compared with previously determined cutoff values for the two cancer markers. The strategy of the sponsor's assay is to designate a specimen positive, when either of the two cancer markers is below the cutoff value for their respective marker. The CT values of the internal control gene are not examined at this point.

A specimen is designated negative when the CT value of both cancer markers is above its respective cutoff, and the CT value of the internal control is examined for placement above or below the respective cutoff line. If below its respective cutoff, the sample preparation and processing implies adequate amplification of the internal control gene from the specimen. If all three markers are above the respective cutoff values, the sample is again deemed invalid, and could be repeated again with another RNA sample, though, again, the subject would be flagged as invalid, even if the repeat tests were valid.

As part of the clinical study, a reproducibility study was performed at three sites using two operators per site. All operators used a sponsor-provided contrived specimen composed of human axillary node tissue homogenate supplemented with an in vitro transcript of both cancer markers at high or

low levels. One specimen was designed to be negative for the two cancer markers, and starting at the assay RNA processing step, operators tested each of four specimens in duplicate using three different lots of the test kit.

The percent coefficient of variation of CT values was calculated for analysis. The coefficient of variation of CT value for both markers was less than 7 percent for inter-run, inter-run, inter-site, inter-operator, and inter-lot analysis. This reproducibility appears acceptable for an assay of this type.

In the clinical study, 34 of 421 subjects, or 8.1 percent, had failures of the external controls or internal control gene. Assay results from these subjects were classified by the sponsor as assay negative for purpose of performance calculations, and the sponsor has stated that such results were intended as part of the intent to diagnose population. Exclusion of invalid assay results indicate that the clinical sensitivity and specificity were not statistically different than when included.

The sponsor has made no statement regarding the classification of subjects with regard to the disposition in routine clinical use. Do

invalid assay results necessarily imply deferral of a decision to proceed, or is immediate retesting with purified RNA from the same node tissue, with the subsequent delay in a decision appropriate? Or do such results imply some other course of action?

In the absence of other intraoperative histology result, gross significant organ observation or clinical observation, no information to guide a decision to immediately proceed to further dissection would be available, and deferral would appear clinically reasonable. And deferral also does not necessarily imply a second operation, unless the permanent section histopathology report indicates a positive result.

The sponsor has designed the assay to detect metastasis greater than 0.2 millimeters, but has utilized H&E categories visualized on permanent section histopathology. The histological categories include negative histology, negative with clusters, negative with isolated tumor cells, or metastasis greater than 2 millimeters. The cutoff CT values for the combination of each marker was based upon an empirical distribution of sensitivity and specificity pairs that maximize specificity with a particular sensitivity. A finer amount of detail could have been

used from actual metastasis that were recorded and correlated with CT values of at least two of the three markers, rather than a correlation to categories of sizes defined in current histological practices.

This slide illustrates the modest correlation of CT values for mammoglobin on the left, and cytokeratin on the right, where the CT values on the X axis, the recorded size of the metastasis is - I'm sorry, the CT value is on the Y axis, the recorded size of the metastasis on the X axis represented in a log rhythmic scale. There is a modest correlation for both mammoglobin and cytokeratin. The lines here represent the boundary between 0.2 and 2 millimeters. And you notice in both of these plots, there is not a large amount of data in either of these two regions. There is more out there at greater than 2 millimeters metastasis. The use of the assay uses a combination of both markers, each with separate CT values correlated with the size ordered histological categories to find an appropriate CT value.

During assay development, the failure rate of external positive and negative controls, and of the internal control gene was noted. Training of assay technicians was undertaken to reduce or eliminate this failure rate. A study was performed by the sponsor

attempting to find if the failure rates could be reduced with cumulative experience reflected in the total number of assay runs. This is a graph that I had to copy out of the submission, so I apologize for it being a little grainy.

In the top graph, the initial failure rate starting here of approximately 15 percent, was reduced with cumulative time after approximately 20 cumulative runs. However, the failure rate remained at a low but fairly steady level approaching approximately 4 to 8 percent, even after repeated cumulative runs, as high as 90.

The lower table indicates the failure rate in the current clinical study when technicians with minimal experience, moderate experience, or extreme experience with PCR-based assays are stratified after their initial training, and the rate of failed assay runs calculated. Even highly trained technicians continue to have a failure rate of approximately 6 percent. This information, though limited, indicates that training can reduce, but does not eliminate the occurrence of failed assay runs during actual use, and that the failure rate is modestly significant.

The sponsor has indicated that the assay is designed to be completed in approximately 30

minutes. No information has been provided with regard to the actual measured times of assay completion in the submission. Additionally, no information has been provided with regard to trending of positive and negative controls with overall time, or with regard to cumulative assay experience. It is unclear if the time for assay completion during use meets user or clinician expectations.

As regards the intended use population and setting, the clinical study was performed in a clinical setting of sentinel lymph node biopsy on breast cancer patients who would appear to qualify for sentinel lymph node biopsy; that is, they're female, obviously, breast cancer patients 18 years or older, and had a diagnosis of invasive breast cancer, and were scheduled for sentinel lymph node biopsy. Since the assay is designed for intraoperative use, positive results suggest immediate intraoperative followup with full axillary node dissection in the absence of any other intraoperative histology results. Negative assay results suggest no further dissection of axillary lymph nodes in the absence of such other intraoperative histology.

Use of the assay in conjunction with other current intraoperative histological procedures, such

as frozen section, histology, or touch imprint histology was not explicitly evaluated in the current clinical study; that is, the specific use and performance criteria for these other procedures was not designed into the study. However, performance of the proposed assay with these two other procedures was compared on subjects in which one of these other procedures was also performed.

With regard to the clinical validity and study design, the objective of the study was to gather data necessary to support assay performance initially estimated from previous studies ensuring that the assay was safe for use. Safety was defined as the lowest percentage of false positive or false negative rates possible. Effectiveness was defined as effectiveness in the user's hands.

The assay sensitivity was hypothesized at 70 percent or better at the lower confidence limit, and the assay specificity was hypothesized to be 90 percent or better at the lower 95 percent confidence limit. Though no specific safety outcomes or criteria were specified, the implied safety criteria from these outcomes were a false positive rate of 10 percent or better, and a false negative rate of 30 percent or better. Effectiveness outcomes were implied from the

specifications for the lower limits of sensitivity and specificity, and by the posterior probabilities of success. The final success or failure of both patient accrual for Bayesian interim analysis, as well as for the assay success or failure, centered upon the assay's ability to meet or exceed the limits specified. No information has been provided regarding secondary objectives, as stated in the clinical study protocol.

I missed a slide, I apologize. A prospective study of patients with previously diagnosed invasive breast cancer who are 18 years of age or older, had a previous diagnosis of invasive breast cancer, and who were scheduled to undergo sentinel lymph node biopsy was undertaken in at least five sites in the United States, as noted in this inclusion and exclusion criteria list. Eleven sites ultimately participated. Sentinel lymph node tissue identified by standard locating techniques was removed using each site's specific intraoperative procedure. Each removed node was cut as described in the node cutting scheme. The clinical site used alternating tissue slabs for histology and the proposed assay. Patient tissue destined for the proposed assay was pooled and processed intraoperatively. Permanent

section histopathology was evaluated by site pathologists and by a panel of three pathologists.

This, again, is the sectioning plan used at the sites to prepare the slides for H&E evaluation, and it appears to be more rigorous than the currently recommended ASCO guidelines. While differing from the ASCO recommendations in the orientation of the first cut, perpendicular compared to the ASCO-recommended meridional cut, double sets of three sections at three levels separated by 150 microns were performed for each tissue slab. A positive assay result differed from an - I'm sorry - when positive assay results differed from an initial negative histology result, one set of sections from the opposite block face of the tissue block was to be utilized.

Site pathologists prepared final permanent section mounts for routine patient management decisions. Each site determined the number and level of sectioning for these decisions. They, additionally, prepared sections of tissue slabs from tissue slabs for central pathology review using the node sectioning scheme, again. Sections were cut at 4 to 6 microns from three sections spaced approximately 150 microns apart in each 1-1/2 to 3 millimeter thickness tissue slab. IHC evaluations were performed

by site personnel using its usual methods when H&E staining was negative. Additional H&E sections, and IHC sections from the opposite block face that were destined for the proposed assay were also prepared using the same sectioning scheme.

Okay. Central pathologists were responsible for reading the slides prepared from sections made at the site on closely adjoining sections to those sections made and used by the site pathologist. When agreeing in result category, at least two central pathologists reviewed slides. When disagreeing, a third pathologist reviewed slides. At least two of the three must have agreed to give a final evaluation, but I must emphasize that this result was the final central H&E result. Final histology results, the overall results from H&E and IHC was the more positive of the final central or final site histopathology result. So I emphasize again, there is a final central and final site evaluation.

In the table on the lower portion of the slide, site pathologist's review of H&E stained permanent sections when negative, but positive on the central H&E slides had to be confirmed by at least one central pathologist, but the final H&E

characterization was placed in the final site as the final site result. If the final H&E site result was negative, but unconfirmed, the final site evaluation was undetermined. Other evaluations by site pathologist review when negative, but also negative on central pathology review from their slides did not require confirmation, and the final site H&E result was negative.

If confirmation was not possible on review, it is not clear to me from the protocol and discussions with the sponsor if further review by another central pathologist of the site slide was undertaken, and the result was the best two out of three results to be categorized as the final site result. This is a rather complicated figure attempting to describe the logic of the way the slides and site pathologists utilize each of their evaluations. Obviously, the central pathology results are going to be using permanent sections, and when positive, that is, greater than 0.2 millimeters, there is a final categorization of the central H&E results as positive, and when negative, the final central results is negative.

Likewise, for the site pathologist, when the site pathologist - I'm sorry - when the site

pathologist recognizes this as a positive result, he then has to reconfirm that positivity by submitting it to a central pathologist, and he then decides whether he can agree whether it's positive or negative. If he does not agree, that determination for the site pathology H&E result is labeled undetermined. And if it's positive, it's unclear to me whether that then becomes the site positive result, or that becomes an undetermined result since - I'm sorry - I apologize. He confirms it as positive, so in both cases, you've got two pathologists confirming the positivity. However, it gets further complicated here for results that the site pathologist reviews as negative. It's unclear what portion of the time a central pathologist is going to review and confirm that negative result or not. Obviously, if the central pathologist takes a look at it, and he determines that it's positive, it's unclear to me whether the site pathology categorization is going to be undetermined, or positive, or negative.

All right. I need to go back again to emphasize the frozen section results over here on the left-hand side of the screen. In essence, the frozen section result in terms of the regular determination for permanent section histology was done essentially

separate from the permanent section histology result, and a diagnosis was made as positive or negative on the basis of whether the metastasis seen were greater than 0.2 millimeters in size, and categorized with frozen section result as positive and negative.

For a comparison of the assay result using either site or central final H&E result, or final histology result, a positive or negative result need only be made. A patient final histology was classified as positive, if positive by either site pathology, or central pathology, or both using the higher of the metastasis category. If multiple nodes were removed from a patient, a positive histopathology in any tissue slab from any removed node caused the patient to be classified as node positive. A patient classified as negative, if negative by site and central pathology review from all tissue slabs, from all removed nodes, was utilized to classify the patient as negative.

For analysis purposes, the sponsor chose to categorize the final histology result in these six categories. Items A, B, and C represent a positive result in three different categories, greater than 2 millimeters, greater than 2 millimeters where the size was actually measured, and that was classified as a

macrometastasis, for nodes just greater than .2 millimeters with no specified size, it was still classified as positive. Metastasis between 0.2 and 2 millimeters were considered positive. Again, the negative categories are D, E, and F, where there was metastasis seen either as clusters, isolated tumor cells, or no metastasis seen at all.

Regarding the clinical utility and effectiveness, 421 enrolled subjects, 94 percent of whom were diagnosed with invasive ductile carcinoma or lobular carcinoma participated from 11 clinical sites. Of the cancer subjects, 95 percent had Stage 1 or Stage 2 disease. The mean and median number of lymph nodes removed was 2.9, and 2 nodes per patient. The overall cancer prevalence to lymph nodes, as detected by histology, was 29.1 percent; that is, 121 subjects in 416 subjects. The prevalence of positive lymph nodes or lymph nodes with metastatic cancer ranged from 14.3 percent to 45.5 percent by clinical site.

When information was available, approximately 75 percent of subjects had estrogen receptor positive tumors, and 67 percent had Her/2 negative tumors. Mean tumor size of all subjects was 1.9 centimeters, mean subject age was 60.3 years. Of note, true positive subjects tended to have slightly

larger tumor sizes, 2.6 centimeters, than the overall mean tumor size of 1.9 centimeters, and tended to be slightly younger, approximately 58 years, than the mean subject age of 60. Five subjects with final histology results of undetermined were excluded from the total 461, leaving 416 subjects with a defined final H&E histology result used for calculation of device performance.

The role of immunohistochemistry evaluation was examined by comparing H&E histology categorization with the final histology categorization resulting from an H&E, plus immunohistochemistry evaluations. The observed agreement in the six histological categories was 95 percent. The number of subjects who differ between H&E, and H&E plus IHC represented 4.8 percent of the subjects, but only one subject was significantly changed by IHC from negative to positive with micrometastasis. Other changes in categorization were within the three negative categories, or from negative to the undetermined category.

This appears to support a conclusion that IHC evaluations did not significantly change H&E evaluations, and that data also suggests that H&E evaluations alone in the study are reliable

evaluations to determine the final histological status of subjects.

The agreement of site and central pathology final evaluations was 91 percent, with a confidence interval that ranged from 86.7 to 95.4 percent. So the apparent greatest disagreement possible is 13 percent, while the least disagreement was approximately 5 percent.

While it's perhaps remarkable that a pathology review did as well as it did, there is some disagreement, and that is less than 100 percent agreement. The sensitivity of the proposed assay was 87.6 percent, with a 95 percent confidence interval from 80.4 to 92.9 percent. The specificity is 94.2 percent, with a 95 percent confidence interval from 90.9 to 96.6. When acting on an intraoperative GeneSearch Assay result, in the absence of any frozen section histology result, at least 3.4 percent and up to 9.1 percent of women who will, or would be, true negative histologically will be managed as SLND positive. At the same time, at least 7.1 percent, and up to 19.6 percent of women who will or would be true positive histologically must either await histology result, or be managed as SLND negative.

The risk of metastatic breast cancer in

the face of a positive test is high, at least 78 percent, and as much as 92 percent. The risk of lymphogenous spread of breast cancer is sufficiently high that the assay could provide a clinical rationale for Level 1 axillary node dissection. The clinical cost of that predictive value is that as many as 14 percent, and as much as 21 percent of test positive subjects, who are absent histologically detectible metastatic cancer, though they test positive, will be missed. When test negative, the risks of the absence of histologically detectible breast cancer is high, at least 92 percent, and as much as 97 percent. The clinical cost of that predictive value is that as many as 5 percent, and as much as 8 percent of subjects of test negatives will have histologically detectible metastatic cancer.

The apparent immediate consequence of an assay true positive, as reflected in the 86 percent positive predictive value, is that there is an 86 percent risk of histologically detectible metastatic cancer that should be verified by permanent section histology. That risk could support the decision to proceed to axillary node dissection, but is tempered by a lower risk than some, or even many, would find comfortable.

Since the assay detects metastasis greater than 0.2 millimeters in size, and since there is still clinical uncertainty regarding the benefits and risks of metastatic disease for metastasis sized in this range, it is unclear if the assay, when used alone, would provide any clinical benefit in the long term. The immediate consequence of an assay negative is reflected in the negative predictive value. It's 95 percent risk of histologically undetectable breast cancer that perhaps should also be verified by permanent section histology. That risk could support a decision that no axillary node dissection need take place, but the consequence of an assay false negative, as reflected in one minus the negative predictive value, is that there is a 5 percent risk of histologically detectible metastatic cancer, even though assay negative. The patient could fail to have an axillary node dissection that could be histologically significant; that is, metastasis greater than 2 millimeters in size, and it would be detected by permanent section histology, if done.

The assay itself does not provide information as to the size, macrometastatic or micrometastatic. When found positive and histologically significant by permanent section, the

patient would return for a second surgery, if deferral takes place. When found positive, but of unclear histological significance, or between 0.2 and 2 millimeters in size, the need for a second surgery becomes unclear. Is it clinically meaningful to remove cancer that may not be sufficiently significant to alter a patient's survival?

The consequence of assay false positives as reflected in one minus the positive predictive value indicates that there is a risk of 14 percent of the absence of histologically detectible cancer, though assay positive. The patient could proceed to axillary node dissection that is potentially preventable if histologically unconfirmed, either intraoperatively or at permanent section evaluation. Even when confirmed by permanent section histology, the discovery could potentially be made after the surgical decision was made, arguing that the surgery was unnecessary. Thus, there is some potential for surgical over-treatment and subsequent morbidity.

Even in the face of surgical over-treatment, the clinical benefit to removing lymph nodes that are currently undetectable histologically is uncertain. At the current time, the lack of long-term clinical benefit and the morbidity from surgical

over-treatment suggests that making a decision when test positive must be carefully considered, since there is an average risk of 14 percent, and as much as 21 percent.

I would now like to turn to staging, since sentinel lymph node biopsy procedure is a staging procedure with some built-in assumptions, to avoid the morbidity associated with full staging of axillary node dissection.

The sponsor has provided a summary table categorizing the number of positive nodes by assay, and by histology. In the cells highlighted in green, assay false positive subjects are shown, in which staging indicates by assay at least pN1, but by histology would be pN0. The percentage of false positive subjects relative to the 295 histology negative subjects represents approximately 6 percent. These subjects are characterized or over-staged in the assay.

In the cells highlighted in dark blue, the false negatives are shown in which the staging indicates by the assay that they're pN0, but by histology they're at least pN1. The percentage of false negative subjects here relative to the 121 histology positive subjects represents approximately

12 percent, and these subjects are over-staged by the assay, even though the slide says - no, I'm sorry, they're under-staged.

This total misstaging error of approximately 18 percent, or 12 percent plus 6 percent, is reflected in the greatest amount of disagreement, the lower confidence interval here, for the overall agreement, so these estimates are reflected in the complement of the overall agreement.

Misstaging can commonly lead to errors in the type of subsequent chemotherapy considered for breast cancer patients. Since the assay is designed to detect metastasis greater than 0.2 millimeters, and the clinical benefit of micrometastatic disease on cancer staging is uncertain, the misstaging error may not be as large as represented by the analysis. If staging is completely due to macrometastatic disease, then the maximum misstaging error estimate may be more representative, but the assay, itself, does not reveal disease as micrometastatic, macrometastatic, or some mixed disease; therefore, the use of the assay in the absence of histology could lead to some modest misstaging errors when macrometastatic disease is present, but perhaps a lower misstaging error when micrometastatic disease is present. This could, in

part, support a conclusion that the use of the assay for staging purposes should be discouraged in the absence of histology information.

Thank you for your attention. Following next is Dr. Gene Pennello, statistician.

DR. PENNELLO: Good morning, panel members. My name is Gene Pennello. I am in the Office of Surveillance and Biometrics, and I was the statistical reviewer for this device. And I would like to present to you an overview of the statistical design and analysis of the pivotal study.

DR. TAYLOR: Dr. Pennello, before we go on, we're about five minutes from the end of the scheduled time for the FDA, so maybe if we take 10 minutes or so and wind it up, would that work for you?

DR. PENNELLO: I will try to do the best I can. I'll try to go through this --

DR. TAYLOR: We can always erode the question time, but we do need to stop very promptly at 12:00.

DR. PENNELLO: Okay. Fine. So my outline is to talk a little bit about the study design, although you've heard some of that, do two analyses, one on all subjects, and one on subjects who had frozen section results for the purpose of comparing

frozen section with the BLN Assay, and then look at variability by site in the performance of the assay, and then summarize.

Very briefly, the study design, there was actually two studies, as you heard. There was a cutoff study that preceded the pivotal study, in which the cutoffs for the two markers, the CK-19 and the MG, and also the internal control were set. And immediately following that, there was a pivotal study, primary endpoints, as you heard, were sensitivity of 70 percent, specificity of 90 percent, and the transition in the pivotal study was seamless. Both studies were conducted at the same investigational sites.

A Bayesian interim analysis plan was designed. A Bayesian analysis would combine data from the trial with prior information, but in this case, no prior information was used, so the Bayesian analysis is very consistent with a non-Bayesian or frequentist analysis. And you heard from Dr. Berry the stopping rules for success for these interim blocks, the initial plan was to look at every 50 subjects starting from sample size 200, to up to 700 patients, and to stop and declare success if the probability of sensitivity greater than .7 given the data is at least

98.5 percent, and the specificity target value was met with probability at least 98.5 percent. There is provision for stopping for futility, otherwise continue. But in reality, there was only one interim look at sample size, N equals 412, that's a typo there.

There were other amendments submitted, and there was extensive discussion with the sponsor about whether any of these amendments or the changes to the interim analysis plan could have compromised the ability of the statistical analysis. We don't, at this time, have any strong reason to believe that that was the case.

I will skip this slide in the interest of time. You've seen these estimates before. The estimate of sensitivity was 87.6 with a given confidence interval here. This is the proportion of reference test positive subjects that tested positive by the assay. The sensitivity was 94.2, and that's the proportion of reference test negative subjects that tested negative by the assay, and the confidence interval indicates the specificity was no greater than 97 percent.

As Dr. Reeves mentioned, we're also quite interested in the predictive values. These are

looking at the data the other way. For positive predictive values, the proportion of assay test positive patients that were reference test positive, and that was 86 percent with a given confidence interval, and the negative predictive values the proportion of assay negative subjects that are reference test negative, and that was 954.9. The primary analysis was the Bayesian analysis, so what I just presented there was frequentist conference intervals, but the primary analysis was based on this Bayesian decision rule for success, and both hypotheses were met, the success criteria were met for both the endpoint sensitivity and specificity. And I note the prevalence in the study was 29.1 percent.

An interpretation is that the prevalence of disease increased from 29.1 percent to 86.2 percent if you tested positive, that's the positive predictive value. But that also means that 13.8 percent of test positive subjects might undergo ALND that was unsubstantiated by subsequent permanent section H&E. Another way to look at it is 4.1 percent of all subjects would undergo unsubstantiated ALND.

Now if you suppose that all subjects with positive permanent section H&E received the ALND, then the number of surgeries would increase by that 4.1

percent, so from 29.1 to 33.2 percent when using the assay, and the confidence interval on the latter rate of surgery is given there.

On the other hand, the prevalence of non-disease, or disease less than .2 millimeters is 70.9 percent, and that increases to 94.9 percent if the patient tests negative according to the data. That's the negative predictive value, but that means that 5.1 percent of test negative subjects are not referred to needed surgery, unless and until disease is detected in the permanent sections. And 3.6 percent of all subjects are not referred to the needed surgery until it's detected in the permanent sections.

This slide stratifies the results by histological category. There were six histological categories, so three positives and three negatives. The first category here is the macrometastasis category, and there were 94 subjects there with a sensitivity of 97.9. There were many fewer in the other two categories, a positive which meant .2 millimeter size or greater, but not known if it was greater than 2.0, and then the micrometastasis category, which had a 56 percent sensitivity, a confidence interval that was wide, but I would note is below the macrometastasis confidence interval.

There were three histological negative categories, clusters less than .2 millimeters, isolated tumor cells less than .2 millimeters, very few data points there, preponderance of the data were in the no tumor seen category, and the specificity was 95.6 percent. The study was not powered to show anything within these categories. It was powered to look at overall, but I thought these results were interesting to present. And as you can tell by the sample sizes within the categories, the overall results were more or less driven by the first histological positive category and the last histological negative category.

I wanted to present a receiver operating characteristic curve analysis. I hope you don't - because I think it provides a different perspective of the performance of the assay without having to depend on the cut points that were chosen in the study, so I hope you all are somewhat familiar with it. What an ROC curve does is it maps out all the sensitivity and specificity pairs as you move the cut point across the range. And so, the Y axis is sensitivity, the X axis is 1 minus specificity of the false positive rate. A perfect test would look like this blue line here. That would be the curve that you would see, and the

area under the curve is a summary measure, and this particular case for the perfect test, the area under the curve is one. For a completely random or non-informative test, the curve would look like a diagonal line, and the area under the curve is .5, so tests are in-between these two curves.

For the marker CK-19, was used as one of the two markers in the assay, the ROC curves looks as follows. It has an area under the curve of .94, with a given confidence interval, and the blue dot here, or the blue circle indicates the performance at the cut point that was chosen for the assay, for this particular marker. It's not the performance of the assay, because it combines two markers, but it's just for that marker. This is the second marker, the MG marker. The area under the curve is not quite as good, .88, and that's the performance at the given cut point.

Now I want to turn to an analysis of subjects on which you had frozen section results. These are the assay sensitivity, specificity, and predictive values, and the frozen section sensitivity, specificity, and predictive values. And looking at the differences here, the difference in sensitivity - now this, again, is at the cut points chosen - were 10

percent, and the confidence interval indicates there is a significant increase in sensitivity over frozen section. The specificity, however, decreased 3-1/2 percent, and the confidence interval straddled zero, so it's borderline significant difference.

Positive predictive value is minus 7.0. That's larger than, for example, the specificity; however, it's based on less data, so the confidence interval is wider, and it's not statistically significant. Negative predictive value was 3.7 percent difference, a statistically significant difference.

Now what I'd like to do here is present the performance of frozen section, the performance of the BLN assay at the cut points that they chose, and that's the red dot and the blue dot, respectively. And then as a frame of reference, the ROC curve for the CK-19 marker in this study population. And from this curve, I would interpret this curve as to mean that well, the assay and frozen section appear to be - they have different sensitivities and specificities, but they appear to be operating at different points on either the same, or maybe a similar ROC curve. And with the assay, you would have the opportunity, if you wanted to, to change the cut point such that it might

be operating at a very similar place to frozen section, as compared to what it is now.

This is variability by site, and sensitivity and specificity - now this is on all subjects, as opposed to just those with frozen section - the sample sizes among the sites were variable. Among those that had at least 10 subjects on which to test sensitivity and 10 subjects on which to test specificity, the range in sensitivities was 7,200, the range in specificities were 84.400. The sponsor data Breslow-Day test for a statistically significant heterogeneity among the sites, and the odds ratio, that's the odds of testing positive given a subject that's diseased over the odds of testing positive and giving not diseased. The P value is .066, so it's borderline significant. This is an asymptotic test, meaning it's approximate, so there are some limitations to that test given that the sample sizes in the some of the sites were fairly small.

To summarize, for the analysis of all subjects, the hypotheses sensitivity .7, specificity .9, were both met according to the primary analysis. And I'm giving you, once again, the sensitivity, specificity, and predictive value estimates and confidence intervals. The rate of ALND surgeries

would increase from 29.1 percent to 33.2 percent if this assay was used, as opposed to not using anything at all during intraoperative consultation. And the bulk of the subjects were in two of the six histological categories.

When in comparison with the BLN Assay, here are the differences, again. And two were significant, one was borderline significant, one was non-significant. And I would, again, point out that frozen section and BLN appear to be operating at different points on the same or similar ROC curve. The variation over sites was borderline significant.

Thank you very much for your attention.

DR. TAYLOR: Okay. Thank you. Dr. Reeves, in the interest of time, is it possible to proceed with clarification questions from the panel at this point, or do you feel there are vital issues you need to wrap-up here? I've glanced through your slides from your final presentation. Most of those already have been seen, but I think it's important the panel have a chance to ask for clarification, and the FDA is sort of about 10 minutes over the scheduled time, so your choice.

DR. REEVES: We can start with the questions.

DR. TAYLOR: Okay, great. That's very helpful. Thank you. Dr. Gollin.

DR. GOLLIN: The FDA Executive Summary says that the proposed assay has been described as an additional intraoperative evaluation of lymph node status, and provides the same information as subsequent permanent section histological evaluation. Do you consider that statement to say that the test should be an adjunctive test to frozen section, and/or permanent section histology, or as a replacement for frozen section, and/or permanent section histology?

DR. REEVES: I think it probably could be either.

DR. GOLLIN: As a replacement test?

DR. REEVES: Either as a replacement, or as an additional test. It's difficult from that wording to decipher which is - what is desired.

DR. TAYLOR: So that, perhaps, is a question that we come back this afternoon and ask the sponsor?

DR. REEVES: I'm sure that the sponsor would be glad to respond.

DR. TAYLOR: I'm sure they would. Yes. Any other questions? Yes?

DR. WHORTON: In your last slide, you

suggested that there was borderline significance among the sites on sensitivity under specificity. And, furthermore, since it looked like they had been higher for the larger sites, my question is, in the event that there are inter-site differences in sensitivity, and/or specificity, what does that do to the overall estimate of sensitivity and specificity, specifically with respect to the confidence interval, which is based on pure binomial distribution, in light of the fact that the sites may have different site specificities?

DR. REEVES: I'm afraid I'm going to have to ask Dr. Pennello to respond. That's more statistics than I get paid to understand.

DR. TAYLOR: Do you need the question again, Dr. Pennello, or are you comfortable with it?

DR. PENNELLO: I think I can answer that question.

DR. TAYLOR: Okay.

DR. PENNELLO: The analysis treated the sites as fixed. Now if there's variation in the sites, and you consider the sites as a random sample from a population of the sites, that would be called a random effects analysis, and if there was variation among those sites, that variation would be

incorporated into that analysis, such that the confidence intervals for the sensitivity, specificity, and predictive values would be larger than they would be for the fixed effects analysis, which is what was presented. I hope that - is that what you're asking?

DR. WHORTON: You raise another issue. You say they considered these sites as being fixed; therefore, the inference it's only related to these specific sites, and not the universe of --

DR. PENNELLO: Right.

DR. WHORTON: Okay. Second, do you know whether that's true? And, nevertheless, there is differences among those six sites. What does that do to the assumption of pure binomial distribution in the computation of confidence intervals for all the data when it's combined? Is that binomial distribution proper?

DR. PENNELLO: Well, if each of the sites had different performances - now the test that was conducted, the Breslow-DAY test, was borderline significant, but if each site had a different binomial proportion, then when you pool them over, then you wouldn't get a binomial distribution, so that's -- the analysis wouldn't be quite correct.

DR. TAYLOR: Do we have another? Yes, Dr.

Kemeny.

DR. KEMENY: I'm just having a little trouble with the statistics, as far as the false positives, because you have a slide where it says that 13.8 percent is the possible false positives; meaning, someone would have an axillary dissection and something wouldn't be there. And yet, Dr. Pennello said it's 4.1 percent undergo unsubstantiated, so which one is it?

DR. REEVES: I think Dr. Pennello means that when you take that 13.8 percent and apply it to the whole population, to the whole 416 in the study, so if you take -- is that correct? You take 13.8 percent of 416, and you get 4.1. At least that's my understanding.

DR. PENNELLO: The difference -- I actually presented them both ways, and it's -- there are different denominators. The 13.8 percent is the proportion of test positive subjects that were reference test negative, but you can also look at it as among all the subjects, how many were - let's see. What happened there?

DR. TAYLOR: Are you comfortable?

DR. PENNELLO: That should have been 13.8 percent.

DR. TAYLOR: Yes, we figured it out ourselves up this end, so we're okay.

DR. PENNELLO: I'm sorry.

DR. TAYLOR: Thank you.

DR. NETTO: One last question on the issue, though. What's the 21.2 percent, the maximum 21.2 percent in that same setting?

DR. REEVES: It's the upper confidence limit of the one minus the positive predictive value. That's the maximum false positives for 95 percent confidence.

DR. TAYLOR: Good. Do we have other questions from the panel? Okay. Thank you. There are some questions that the FDA has for the panel. Do we present those now, or do you want to do that after lunch?

DR. REEVES: Whatever the panel's preference is.

DR. TAYLOR: We will do that straight after lunch. We all have the written questions at this point, anyway. What I'd like to do now is break for lunch. We're going to reconvene promptly at 1:00. I would remind all the panelists that there is no discussion of the issues among panel members, or among anyone else, so you can talk about the food, but

that's about it.

We'd also like you to leave the room as expeditiously as possible. The FDA will secure the room during this break, so if you wish to leave anything in the room, such as computers, that's fine. If there are personal belongings that you need, then you should take them with you, because the idea is that you don't come back in the room until 1:00. Thank you.

(Whereupon, the proceedings went off the record at 11:49 a.m., and went back on the record at 1:01 p.m.)

A-F-T-E-R-N-O-O-N S-E-S-S-I-O-N

(1:01 p.m.)

DR. TAYLOR: Again, I'd like to call the meeting back to order. Once more remind public observers that while this portion of the meeting is open to public observation, public attendees may not participate unless requested to do so by the Chair.

The next part of the meeting, from reviewing the materials and presentations this morning, I'd now like to go around and ask, to be sure that each panel member has an opportunity to ask questions either of the sponsor, or of the FDA, or for each panel member to make any comments, so we can do this in a left to right fashion, or we can do it randomly. What's the preference of the panel? Maybe we could start with you then, Dr. Whorton.

DR. WHORTON: I would like to pursue, if I could, the question that I raised in the latter part of the early session, and that's all the confidence intervals that have been discussed have assumed what is known as the binominal distribution, which assumes you draw a random sample of the population, and compute the false positives or false negatives using a specific formula for a simple random sampling, and almost all of these intervals are based on that, as

was stated by the sponsor.

Whereas, if you have several sites with several locations in these clinical trials, as long as there's no significant differences among the locations on the primary outcomes, you probably can get away with the simple random sample assumption. But once those sensitivities or specificities slightly change among the sites, that means the sensitivity is location specific. In order to get an overall estimate, you had to consider the inter-site variability in these confidence intervals, and the net effect is to widen the length of the intervals. And I don't know the implications of that, but I suspect it will widen those intervals, and I'd be curious to know how wide that would be, and what implications that would have in terms of the conclusions.

DR. TAYLOR: Does anyone from the sponsor wish to respond?

DR. BERRY: Hello, my name is Scott Berry.

DR. TAYLOR: Would you put the microphone on, please. Thank you.

DR. BERRY: My name is Scott Berry, and I'm a consultant to the sponsor. You're absolutely right, if the sites have different rates, then the assumption - it's not the binomial assumption that's

at fault, it's that you assumed the same binomial parameter at all of them. The binomial is still a safe assumption, it's just it's different every site. You would have then 11 different intervals for each site for sensitivity and specificity, and the data available to inform each one of those is then less because you're not borrowing them all, pooling them all together. And so the intervals would become larger, yes, but you would have 11 different intervals. And there are great ways to do that statistically to do some borrowing, the appropriate amount of borrowing, but the intervals will become larger, but you will also have 11 of them, which opens up interesting questions about the differences in efficacy.

The other thing to point out was, though, that it was not statistically different there using a 0.5 level. There is no evidence that they are different, and that this is needed.

DR. TAYLOR: Go ahead.

DR. WHORTON: Correct. So there are two issues. One is, if these sites are considered stratum, therefore, fixed inference for those particular sites, then you would compute the variates, use the binomial with each of these sites, and then

add them up to get an overall margin of error for the overall population proportion. If, however, there are samples from the population, so the population of inference is larger, then the inter-site variability has to be included, much like the cluster analysis. It is possible to take each of those stratum and compute the variance, add those up in order to get an overall confidence interval for the total population, not necessarily specific, gets into each one of them separately. You can for each site-specific sensitivity, there's a way to get a weighted estimate of your overall margin of error by adding up the variances with each one of them.

DR. BERRY: I agree, but then the question becomes how do you interpret that it becomes a population mean, and you can also address the population variation at that point, too, so that -- and there are sophisticated techniques to do that. I completely agree.

DR. WHORTON: Would you think that since -- the other is, there was nothing built into the design to really evaluate the significance of the different locations. Maybe there's no power analysis, as we need so many in this site, and so many in this site, in order to be reasonably certain there is or is

not a site difference, so you may be underpowered as far as the significance of the inter-site variability. Given that there were more samples in some of the smaller locations, it may be, in fact, that that significance level would go down appreciably, and you would have the site differences that are now significant at the .05 level. But, nevertheless, if they are, then that inter-site variability, albeit within a site for the stratification, I think would wind up having a wider margin of error, and that may have a little bit more of an uncertainty about where that sensitivity and specificity really is.

DR. BERRY: Yes, and it's the sensitivity that probably becomes the issue, because the specificity has a much bigger sample size with that, and it's the 30 percent positivity rate to address the sensitivity. And just looking at the numbers, though, there aren't clear sites - well, this site is clearly different, this one is different, and they weren't significantly different, so yes, your estimates may become a little bit larger, but in the approaches that statisticians use, there can be some borrowing across them, and so I think the confidence intervals become slightly larger, yes. But I don't think it would be a large difference.

DR. WHORTON: But one of the ones had like an 85 percent rate, also had the smaller of the sample sizes in terms of number of subjects, so the binomial variability within that site, P times Q, and P being less close to one, is going to give a bigger P times Q, divided by smaller sample size, it's going to give a larger number that you add with others. And I'm just curious to how wide the actual --

DR. BERRY: If you were to simulate this situation, you're going to find the smaller sites are going to deviate the most, just because of the sample size. I don't think the data is surprising that that happened at the one site, or becomes slightly lower the smaller sites are. And if you tell me, I know the sites are different. I want you to model that, that one site, then, is certainly going to have the biggest variability because it has a smaller sample size. Absolutely. And you're right, it is closer to a half, which is the most variability.

DR. TAYLOR: I'd like to see if there's any other panel members have concerns or comments on this particular issue? Colin, anybody, this issue?

DR. BEGG: Not on this issue, no.

DR. TAYLOR: Okay.

DR. BEGG: I accept the answer. The

confidence interval would be a little bit wider, but probably not much.

DR. TAYLOR: Right. Thank you. Dr. Ernstoff.

DR. ERNSTOFF: I have one question for clarification, and another question of how the sponsor might deal with certain situations. The first question for clarification - there were a couple of numbers that were thrown out in terms of how frequently the test failed, either because the internal or external controls failed. And one number that was thrown out was 8.1 percent. Is that the number that we're dealing with? Is it 8 percent of tests that actually failed, that there's no information for those women?

DR. TAYLOR: Go ahead.

DR. VARGO: Yes. In the validation study overall, the number of patients that had a final test result of no test result due to invalids was 8 percent.

DR. ERNSTOFF: So how do you deal with that? I mean, if the tissue is completely homogenized and no tissue is left available as the proposal, there would be 8 percent of women that would have no information about their nodal status.

DR. VARGO: Not exactly. For every reaction done, every sample tested in the assay you have two types of sample remaining of the exact same tissue, leftover homogenate, and leftover RNA extract. Now in the study itself, it was up to the operator whether they wanted to go through the efforts, it wasn't being used for patient management, and we didn't mandate it to retest those tissues, retest with homogenate or RNA extract, so we don't have good data at the clinical sites on how often you can get a valid result after an invalid result. We do have anecdotal evidence from retesting in-house the samples sent to us, and you can get a valid result a good proportion of the time. So what we say to the operators is, if you - and a lot of times what happens is, or a number of times what will happen is something like they forget to spin down the tubes before they go into the Cepheid. Cepheid tubes have a little window at the bottom where you need to centrifuge to get the reaction down to the bottom of the tubes. If you don't spin it, your external controls are not going to work. That's the whole purpose of them, is to say something went wrong here.

There's nothing wrong with the extract. Potentially, there is nothing wrong with it, and what

happens, we've seen it a number of times where the operator does this, and it's a one-time mistake, typically, one-time learning. And they go oh, crap, got an invalid, I didn't spin it down. They can go right back to that extract, starting at that point, and redo it. Now whether or not that information would be available intraoperatively would definitely be a surgeon-specific/patient-specific thing, because it's going to take minimally another 15 minutes, or I should say 18-19 minutes to have the Cepheid rerun, but you would not lose the final result. You wouldn't get it intraoperatively, most likely.

And the other thing to keep in mind is that 8 percent we feel is worse case scenario before we had the opportunity to learn from setting up 30 different operators, literally, at least, teaching them how to do the assay. We learned a lot about how many people don't know how to pipette. That is actually the most difficult part. We have changed our training procedures to have pipetting certification, so to speak, during it, and I showed you some data indicating that those invalid rates have been reduced dramatically in new sites. And I will apologize that some of the data was not available in the FDA PMA, and is new data presented here today, and I should have

made that very clear at the time that I presented that.

DR. ERNSTOFF: Thank you.

DR. TAYLOR: Okay.

DR. ERNSTOFF: Another question?

DR. TAYLOR: Before we move on from that point, I'd just like to pick up the same point from any other panel members, and I think a couple of issues on the same point, so Dr. Gollin. Then I'll come back to you, Marc.

DR. GOLLIN: I'm really concerned about this 8.1 percent. If this is a stand-alone test, and you're destroying an entire sentinel lymph node for RNA, and there is this 8.1, or 8 percent error with no results, I believe that this compromises patient care. And I am concerned, also, about your issue of training. You're not going to have the control when you sell these assays of training.

DR. VARGO: Yes, it's mandatory. It's mandatory. Sponsor training is mandatory when the test is sold to a site.

DR. GOLLIN: And so then they get a new technician, and it's mandatory. And in 10 years, you're going to be as stringent with this?

DR. VARGO: There's one of two options

that we're likely to pursue. One is that it's either mandatory, and that the sponsor needs to do that training, or we train the trainer, which is commonly done.

DR. GOLLIN: I just know how that in practical terms --

DR. VARGO: For the training aspect, if I can address your first concern about the 8.1 percent, a legitimate concern. Let's assume - for instance, take the study data itself, of that 8.1 percent of the 421 patients tested in the assay, that was based on the 421, including the five that had undetermined histology because the assay invalid is independent of anything to do with histology - of those 8.1 percent, four of them were permanent section histology positive, so the great majority of that 8.1 percent were, in fact, negative patients. Why would it be disproportional instead of randomly distributed? You would expect by chance of the 8.1 percent that a quarter of them would be positive, because the positivity rate - it was actually less than that. And one of the reasons for that is, your chances of getting an invalid result are greater for very small nodes, and positive nodes are often not very small. And secondly, for you to get an invalid result, no

matter how many other nodes you had, the way it works in the assay - say you had four nodes removed for sentinel lymph node procedure, if three of them had a valid negative result, and one of them had an invalid result, for conservation purposes, we feel as a safety factor to the patient, that is reported as invalid for the patient, even though there's three nodes that had a valid negative result. The reason is, you don't know what the status was of that last node.

However, if that same patient had four positive nodes, and one of them tested positive, and all the rest of them tested invalid, the patient is positive, and the invalids are ignored, because the patient is positive. It doesn't matter what the other nodes tested, so in that sense, the 8.1 percent in the study is not proportional to positive patients, if you're following my reasoning there.

DR. GOLLIN: Yes.

DR. TAYLOR: Same point, Susanne? You're still pursuing the same point?

DR. GOLLIN: No. Well, it's sort of the same point. You also -- I'd like to -- on page 153 and 4 of our book, it says that "high expression of either marker may lead to negative results for the internal control."

DR. VARGO: Yes, this is - I can't remember the exact number - I know what that is. Theoretically -- George would have to answer whether or not we actually have analytical data for this - but what can happen, theoretically, is remember that PBGD, which is the internal control, is lowly expressed. It is exquisitely sensitive to error, or to lack of enough proper reagents to have the thing happen right, to have the amplification occur properly.

If you have a very high level of target cancer, it can compete for those reagents, and you could push the PBGD into a negative result, which you don't care about, because you wouldn't have gotten the positive cancer result if you didn't have proper amplification. That's why the internal control is ignored for positive cancer markers.

DR. GOLLIN: Okay.

DR. VARGO: And if I could just make one point that I missed, because I couldn't read somebody's writing when they handed me a note, that is that you'd said the 8.1 percent concerns you because if you threw the whole node in, that's all you would have, and that is true. The site always has the option to maintain some tissue for permanent section histology, which would also reduce that potential.

Not that there's a guarantee permanent section will find it, but it would give you an opportunity to, at least, not miss grossly metastatic disease.

DR. GOLLIN: Okay. On the same point, my question - another question is, and it's a question to you, to the FDA, and to the pathologists on the committee. Does the intraoperative node cutting for the assay, or taking a whole node instead of cutting it in half like they did on your testing, or cut it into pieces like they did on your testing, does the intraoperative node cutting for the assay compromise the histopathologic diagnosis of metastasis in these cases?

DR. VARGO: I'm going to, if I may, ask Dr. Palazzo to answer that question, because I think it is a pathologist question.

DR. TAYLOR: Go ahead, Dr. Palazzo.

DR. PALAZZO: Thank you. Five seconds addressing the previous question. I think it's at the discretion of the PA present or surgical pathologist when he gets a really tiny lymph node, and we do have rare cases in which even after embedding the tissue, there's no tissue there, and it's only fiber adipose tissue. I think it's the discretion that if the lymph node is really tiny, and you have to dissect the fat,

you might be better off not even doing frozen, and just sending whatever you think may be a lymph node or not as a permanent section. That's a given, and it does happen, not very often, but depends on the size and the quality of the lymph node, so that in that case the assay would probably not be run. You would end up with other fiber adipose tissue, lymph node aggregates in the permanent section.

The second thing, I do not think it does affect that. We have discussed this morning that some of the metastasis show up in the capsule. Instead of a longitudinal section, the assay picks a section for H&E and the assay in a vertical fashion, so in terms of the amount of capsule that you're capturing there, it's actually more than in the other one. If you perceive the lymph node as some sort of a sphere, you're only doing a tangential section, even if you go deep, we'd always be missing components, the more superficial and external aspect of that sphere that would also include capsule. So in our experience doing it both ways in terms of when we get the permanent sections, I don't think the quality of the presence of the metastasis is really compromised.

DR. TAYLOR: Okay. Thank you. Dr. Netto has a question on the same point, I think - comment.

DR. NETTO: Yes. So I guess this is the time to ask - so it's been presented, offered as a stand-alone option. You keep saying it's the discretion of the PA and the resident to decide whether to use it as PCR plus frozen, PCR plus permanent.

DR. VARGO: Yes.

DR. NETTO: So I think that's probably one of the most important points, is this going to be offered as a stand-alone to replace frozen section at potentially discretion of the PA, replace permanent sections, and then the issue of 8.1 percent being invalid becomes extremely crucial, versus is this going to be presented in a systematic way as a side-by-side or not? And I don't think until now we know which way this is going to be offered.

DR. VARGO: The way the sponsor views it is this; that the assay - we certainly have data to support stand-alone intraoperative decision making. We feel the data strongly support that if permanent section histology is alone done on the node, and it is negative, when the assay was positive, that the assay's status remains positive.

We believe that there is a minority of times where morphological information is useful, so

keeping some of the node for permanent section histology could augment the assay positivity result. The other advantage of using both permanent section and the assay would be, if you wanted to find out in a particular case, and you were very keen on knowing the morphology, which isn't the most critical component, but it can be useful information, you would be able to spend your efforts cutting more thoroughly into those nodes that were assay positive by histology, and spend your efforts where you're more likely to have productive results, as opposed to those where you already tested half the node and it was flat out negative, if you see what I mean. So we see that, certainly, the two could complement each other very well, providing the patient with much more the node examined than most labs, except for Milan, can afford to do with today's personnel and the time consuming aspects of very deep histological cutting.

DR. THOMAS: Can I go back to Dr. Gollin's question about - you asked - Dr. Gollin. You asked whether --

DR. TAYLOR: Hold on. Just a moment. Just finish the point up here, please. Thank you.

DR. NETTO: Yes. AT least in my view, I don't think that you present the data that shows that

as a stand-alone test, it will -- if you have a positive stand-alone test, and you do not have the confirmation by histology, you just mentioned that you will count this as a positive. And I know where you're coming from, you're saying because if you see it on the frozen section, and you don't see it on the permanent, the difference is you saw it. As opposed to your test that has potentially up to 13 percent - we discussed several figures of false positive - you can argue these are true false positive, or non-true false positive, but if we're looking at 13 percent potentially false positive, I mean, in the worst case scenario, 21 percent even of the cases based on the predictive positive value, so to go and say that these should be counted as positive, similar to when you see it on frozen section and you don't see it on permanent, I think that's a little bit not convincing to me.

DR. VARGO: I'd like to have Scott come up for a second to address the methodology used by the FDA in determining the false positive rate as being 13 or so percent.

DR. TAYLOR: It's okay. Yes, come. And then we'll get to Dr. Leitch next, and then Dr. Thomas.

DR. BERRY: Yes, there were -- the term "false positive" there is the positive predictive value in the false positive, and they both - they go different ways on the table, so that the traditional false positive rate is if the subject is negative, how likely is the test to say positive? That was the 5.8 percent, which we believe was the value too high, comparing to frozen section, I believe it came down a little bit, but that's the 4 or 5 percent number. The 13 percent number is a very different - it's not the false positive rate, it's the one minus the positive predictive value, which is not false positive. It is the probability - the positive predictive values, if you give the test to an individual, and that individual tests positive, what is now the likelihood that they are actually positive? That depends on the prevalence, and the false positive, and the false negative rate of the test itself, but it's not, itself, a false positive rate. And the prevalence is incredibly important in that. And Gene talked about - Dr. Pennello talked about going from 29 percent up to about 86-87 percent in the positive predictive value based on that result, so the naming of that as false positive, though, I think is using a strange terminology for that.

Now as a mathematician, I can't tell you what to do with those numbers, but let's make sure that we're referring to false positive in the right way, and positive predictive value, both of which are very important, but are very different numbers. To say that it has a false positive rate of 13 percent, I believe, is mislabeling it, very much so.

DR. TAYLOR: Is that some help to you, George? Yes. Dr. Leitch, and then Dr. Thomas.

DR. LEITCH: I guess I have a followup to that point. If it's 17 people out of 121 plus 17, okay, so if you take all the people that are said to be positive - okay - not the whole population, not the 415, but the --

DR. BERRY: Reference test positive.

DR. LEITCH: The ones that are said to be positive, that's where you get that 14 percent number. Isn't it?

DR. BERRY: Yes.

DR. LEITCH: So when you're talking to a patient about it - okay - she's in the 100, but then if she turns out to be in the 30 that have a positive node, then within the 30 that have a positive node, 14 percent are going to be falsely positive.

DR. BERRY: The condition on them having

the positive test result for the BLN assay, or that they actually are positive on the reference test?

DR. LEITCH: If you take all the people that are said to be positive by your assay - okay - and you take the people that are positive by H&E - okay - and the difference of that was 17 people that weren't positive on H&E, is my recollection of the data.

DR. BERRY: Right, the 13 percent.

DR. LEITCH: Okay. So it's 17 over 128 or something, and so that's where you get the 14 percent number. Isn't it?

DR. BERRY: Yes.

DR. LEITCH: Okay. So that's what you're talking to a patient about. Okay. You're not talking to them about the 4 percent, because when they're starting out in the operating room, they're in the 100, and so the 4 percent is that. And for doctors or the community of the world, you can say well, 4 percent is not very much, but then for the person who's in the operating room, and now the surgeon is down to the node is positive on the test, then that group of people, there's a 14 percent chance it is falsely positive. And then to that patient that means a 14 percent chance she's getting axillary dissection

when she doesn't need it. So that's the discussion you have to have with a patient; whereas, the 4 percent doesn't sound like very much when you're talking about the whole group. It's as you narrow these things down to the person that you're operating on, that's where it becomes more complex.

DR. VARGO: Dr. Julian will respond to that, because you are asking a surgeon decision.

DR. LEITCH: Yes, and I still have my pathology question.

DR. BERRY: I agree with the numbers you all said, the interpretation was right.

DR. TAYLOR: I think this issue is obviously an issue we just need to try to get all the comments on in one go, before we turn to something else, so yes, Dr. Julian.

DR. JULIAN: Right. Thank you, Dr. Taylor. The whole issue of false positives and false negatives, obviously, is a conundrum, and that's why I brought out the Milan data, because if you look at the Milan data, and how they processed their lymph node from start to finish at incredibly small increments, they were doing essentially an anatomic homogenization of the lymph node. They increased the positive node rate from what we see in the United States, which

actually averages around 26 percent in most studies, and that even extends into the United Kingdom because they do the nodes the same way as we do here. But if you take that lymph node and you process it to the Nth degree, using the micro sectioning, you've increased that lymph node positivity by 6 percent. Okay?

I think this assay, and I truly believe it, that this assay is picking up those positive lymph nodes that we are missing pathologically by the limited, but intense sectioning that we still do, but it's still limited, because it is not going through the entire lymph node. And that is the only clinical trial that one can use to compare the type of lymph node processing to approximate what is being done on a molecular level. Granted, it is, at times, a very tough issue to bring clinicians, to bring the leap forward to believe from something that they can see, which is touchy and feely, to something that they have to believe based on something that is now molecular in its nature. But we are moving into the 21st century, if we, in a way, still held the gold standard of radical mastectomy and didn't crack that barrier, we would still be doing things as we did in the past, and so I think that it's time to look at that and say yes, we're moving to a more intense analysis of a lymph

node; and, therefore, picking up the metastasis that previously were not identified.

DR. TAYLOR: Dr. Leitch, finish with you, and then I promised we'd go to Dr. Thomas, and then we're going to go back to George.

DR. LEITCH: So then you're raising another question, and that is the clinical significance - let's say you're right, and you could be right, that, in fact, it is picking up real tumor cells. So then it comes to, is it valuable to the patient then to have an axillary dissection based on that information? Maybe it's valuable for prognosis, and that's another study, actually, to say let's take all the people that are assay positive, otherwise negative, what's the outcome of that group of patients, just like it's being done with immunohistochemistry, because it is the same question, really. And so, for patients you might say now, a lot of people would say if my patients are IHC positive only, I'm not going to do an axillary dissection on that patient, if they're IHC positive only. And so when you're in the operating room and you've got to make a decision on that, you don't have the Memorial Sloan-Kettering nomogram to work with, you're just sitting there with the assay to make that decision,

and I think that's where the problem comes. I think you may well be right, that it could really be tumor cells, but the question is what is the clinical significance of those?

DR. JULIAN: If I recall correctly from the way that the study was set up, this is really based on H&E, and not IHC analysis of the lymph nodes to have that cutoff. But you're correct in saying what do we do with that .2 to 2 millimeters of micrometastasis? Well, currently, we have guidelines which are established. They're the best that we have at this point in time to tell us that if on H&E we find a .2 millimeter metastasis, the recommendation is to go to the operating room and do an axillary dissection. That's what this assay was based on, start to finish. That standard may change into the future based on the outcomes of clinical trials, like NSABP-32, which is looking at IHC in the lymph nodes, and looking at micrometastasis like the Apizog Z-10 trial, like the trial in the Netherlands, which is being conducted at this time to look for micrometastasis. That data is not going to be available to us in any near foreseeable future. We're probably looking at outcome data based on those trials, which have an enormous population of patients.

NSABP B-32 has got 4,000 women with IHC negative, or with H&E negative nodes. Z-10 I think has the equivalent. In Europe, they have several thousand are going into that. That's the population of patients that if you want to try to derive outcome data for, that you're going to need to empower, I think that's probably something that will be unattainable in the current status of clinical trials with this kind of technology, so I think you have to make that stipulation and move forward.

DR. TAYLOR: Okay. I think we've still got a couple of comments on the same issue, and I'm keeping track, so Dr. Netto, and then Dr. Thomas, and then Dr. Kemeny.

DR. NETTO: Yes. Just to answer your point that you raised about moving to the 21st century, and being married to the slide. As a molecular diagnostician, I'm not necessarily that married to the slide. I'm a surgical pathologist, too, so I can - I don't have a problem leaving the slides, and adopting a PCR assay. The problem I have is I still have to practice evidence-based medicine, even if I'm a molecular pathologist, so looking at those false positives, we do not know, based on this study, that these false positives are, indeed, over-sensitivity,

meaning they're detecting true cases of metastasis, like we're seeing on the -- I don't think the data shows that.

Actually, can argue the other way around. Why are we having just a much lower sensitivity and picking up micrometastasis with this test, than it is by H&E slides, so I don't think this is overly sensitive test; and, therefore, these false positive are in reality not true false positive. I'm not convinced. It may be that a lot of these cases are truly, you just didn't pick them up on the slide, but I don't think that we proved that, nor did we prove the point that Dr. Leitch is saying about long-term. You definitely need the long-term. You do need that for the IHC to show, and up until now people didn't adopt what's isolated tumor cells, even when we tell them there is, it's not going to affect clinical management, so we definitely need to do the same thing for molecular-based technology, and show that positivity alone with that, without slides, is significant, need to be actionable information, and that's the problem I have.

DR. TAYLOR: Okay. You're commenting to Dr. Netto's question? Go ahead.

DR. VARGO: Yes. So I just wanted to

point out that can we say definitively that 100 percent of the 5.8 percent false positives not backed up by permanent section histology are, in fact, true positives? And the answer is no, we can't do that. But I think logic compels - the data is very compelling in a logical manner that given the differences between the site slides and the central slides in very adjacent tissue, and having a "false positive" rate of 4.2 percent, comparing very nearby tissue, it is logical that, at least, a good portion of that 5.8 percent false positives are due to sampling differential.

In terms of long-term outcome studies, fortunately, because of the early catching of breast cancer nowadays, and the very good detection of metastasis and having it out and good treatment, adjuvant therapy, et cetera, to do an outcome study, which really the only important cases of it are going to be assay positives histology negatives, which in this study of 421 were 17 patients, not all of whom, even if they were true positives, are going to recur, not all of them are going to have axillary involvement, further axillary involvement. And you have to assume that if it was a patient with gross metastasis, the chances are they would be concordant

with histology, so it's highly likely that in those cases they are -- so then you're down to probably, we sort of figured it out, 20,000 patient trial, five to ten year outcome. And we think it would be a disservice to the patients to hold off on applying this tool, giving this tool to the pathologists to use for waiting for outcome studies like that. It's just not practical, which is a good thing, because cancer does have lower recurrence rates over time now.

DR. TAYLOR: Okay. We're going to need to keep moving here. Dr. Thomas, is the same point that you're wishing to address?

DR. THOMAS: It's just answering a question that Dr. Gollin asked. I think her question --

DR. TAYLOR: Go ahead.

DR. THOMAS: -- was very simple, and that is, in this study, is the putting of every other section in for the assay, does that compromise the histology? Was that your question?

DR. LEITCH: I was asking if in the future they were to cut the node in half, not necessarily section it like they did in their assay, in their testing of it, I was wondering if, say even cutting it in half to use as an adjunctive test, if that would

compromise the histopathology.

DR. THOMAS: Well, obviously, if you put all the lymph node in, you're more likely to see it on the histology, than if you put half.

DR. TAYLOR: Okay. Dr. Kemeny.

DR. KEMENY: I'm just going back to the issue that Dr. Leitch brought up. I mean, again, we have to think about what's clinically significant. When we're talking to the patients, I mean, you really do have to look at the 14 percent, rather than the 5 percent, because we have to talk to the patients about what we're doing with them. And what's very important to patients today in our society - why, I'm not absolutely sure - but is that they don't get an axillary dissection if they don't need it, because women feel very strongly about this. And it's almost superseded the cancer treatment, to a certain extent, so we have to tell them what the chances are that we're going to do an axillary dissection on them when they don't need it. And from what I can tell, it looks like it's around 14 percent from the data that we have. And if you're saying what looks like a false positive really may be a positive, then I would still say is it a clinically significant positive, because, as I was saying before, if it's too - you can't tell

me what size those micrometastasis are. You just know that there's X number of cells there, but it could be 3.1 millimeter lesions, 3.1 millimeter lesions probably aren't clinically significant, the way we're working with things right now. So that's one of my concerns.

DR. TAYLOR: Okay. This is a comment to Dr. Kemeny?

DR. PALAZZO: Yes, this is subjective, and I attempted to discuss some of that. The assay seems to pick up the number of cells. I think one has to be careful when interpreting single isolated tumor cells, and most pathologists are very strict on these, that they have to be discohesive and very, very few cells, that you can barely see, on H&E most of the time not, and only on IHC. The updated AJCC published earlier this year and written by Dr. Colin in the archives, specifically suggests that if you have the few single cells, just because of the number of cells in the lymph node, they recommend the lymph node to be regarded as an N1 positive lymph node, so I think the concept of the isolated tumor cells, single tumor cells, when you are referring to a finding multiple foci, one has to be very careful from the pathology point of view that some them, if you cut deeper, say

that happens that one focus becomes more than one, become two, they're fused, and then from single tumor cells, decohesive, they become a micrometastasis. Many of them, the single isolated cells, most of the time, or many times are just seen with IHC, and not just with plain H&E.

DR. TAYLOR: Okay. Then Dr. Siegel had a point. And I'll come back to you, Dr. Leitch.

DR. SIEGEL: I wanted to go back to Dr. Vargo's comment that why it was inappropriate, if you will, to not do, or why she couldn't do a long-term survival study. Evidence is beginning to emerge from sentinel node biopsies in melanoma patients, that those patients that are molecular positive, but histology negative, do not act as if they're histology positive; that is, their survival and their time to recurrence follows as if they're negative, so the concept that molecular positivity is really positive is not probably true, at least in melanoma. And so can you comment on that?

DR. VARGO: Yes, I can. And I don't know if there's any other experts in the background here who may have a lot of melanoma experience, but my understanding is, in fact, the results of those studies are not as you state, but that if the patient

has H&E positivity only, they have not so great survival. If they have PCR only positivity, they also have not so great survival, and if they have both positivity, it's worse than either one separate. That is my understanding of that data set.

DR. TAYLOR: Could you give your name, please?

MR. BACCUS: My name is John Baccus, and I am a member of an affiliate of Veridex.

DR. TAYLOR: Thank you.

MR. BACCUS: In terms of the studies that I'm familiar with looking at melanoma sentinel lymph node analysis with PCR, most of those studies have been done very similarly to how the early studies were done in breast sentinel lymph nodes, where they look at - they're not asking a question of how many cells are there in terms of the PCR data, but they're just saying is there something there. And if you do that in breast sentinel lymph nodes, then you'll find that there is something there in a fairly high proportion of histologically and IHC negative nodes, on the order of 20 to 30 percent of the nodes have something there. That's why we have gone the approach of actually applying a cutoff that correlates to a certain number of cells, is to avoid that type of issue.

DR. TAYLOR: Okay. Are we still on the same point, Dr. Palazzo. Okay.

DR. PALAZZO: Dr. Siegel, I think that you can compare them, but from what I know from breast cancer and a little bit of melanoma, they're really, really, really two different tumor models. From the primary cancer gene expression profile, how they metastasize, and how we interpret lymph node. I do understand your point of this data, preliminary data on melanoma, but I really think they're such a different tumor models, how they grow, how they metastasize, time of recurrence, and so forth, so I think it's a little bit difficult to really compare the molecular significance of cancer in the breast compared to melanoma.

DR. SIEGEL: I'd certainly accept that, and I like the previous speaker's suggestion, also. I think that's true, but we, again, come down to the problem, without a long-term study, we don't know, and so it's not really evidence-based, getting back to Dr. Netto's point.

DR. BLUMENCRANTZ: On the same point, Peter Blumencrantz, Principal Investigator and Consultant to Veridex.

DR. TAYLOR: Yes.

DR. BLUMENCRANTZ: There was a nice symposium on melanoma and PCR presented at the Society of Surgical Oncology not this year, prior year. Kelly Masters and some other names may be familiar, given some nice summaries on this effective PCR. And, quite frankly, that presentation differed in opinion. They showed a very nice slide looking at the three survival curves, looking at H&E, PCR negative, PCR only positive, or PCR H&E positive, and there were three distinct lines with the worst survival code for PCR, so I think there is some presented evidence in the literature that the PCR alone may actually have a survival disadvantage.

DR. TAYLOR: Okay. Thank you. I'm going to go back to going around the table. You've been very patient. This has been an important issue, and obviously, we've spent time on it. I just want to see if there are other issues that we need to uncover. We can always revisit this issue, and we do need to get to the FDA questions at some point, so let's see if we can keep going. Got another point or question?

DR. ERNSTOFF: The other question that I had, we're in a time where we're shifting technologies, clearly, and the current guidelines and plans for therapeutic intervention for women with node

positive disease is based on old technology, if you wish, which is H&E technology, and so recommendations beyond the axillary node dissection, which has to deal with adjuvant therapies, are based on size of lymph node involvement, number of lymph nodes involvement, extra capsular spread, things that are found on H&E, but will not be found on your assay. And I was wondering whether you can comment on how you would advise the medical community to deal with those kinds of issues.

DR. VARGO: Well, clearly, the final say will be advisory panels and consensus documents, and not from the sponsor. Our viewpoint is yes, you may not get all the information morphologically, and you won't get all the information morphologically that you get out of histology, but this assay offers the opportunity to test more of the node, and I think it's more important to get it right more often than to get the details on the ones that are right, and so I see the advantages, again, outweighing the disadvantages.

DR. ERNSTOFF: The current recommendations of adjuvant therapy in node positive women differs by whether it's micro, macro, and number of nodes. And as you mentioned before, you dichotomize the patient, so if they have four sentinel nodes removed, three of

which fall into a category of not evaluable, one is positive, you're going to call that patient positive, but we will never know whether it's two, three, four lymph nodes that are positive. And adjuvant therapies are impacted, decisions are made, people's toxicity and survival might be impacted on that information.

DR. VARGO: And that example - I mean, that's a fair example to give, but one must remember that that example, first of all, didn't happen in the trial, it was a theoretical. And even if it did happen, it's going to be extremely rare. And, again, I would weigh that possibility against the much higher likelihood that you are going to get valid results on all the nodes, and you will get a better assessment of what their true metastatic status is.

DR. TAYLOR: Okay. Thank you. We're going to go ahead to Dr. Lichtor.

DR. LICHTOR: I guess the only comment, the question I really had was the issue of taking patients back to surgery for a second procedure. In other words, there seems to be some comment that it's important to make a decision at the first surgical procedure, and although I'm a neurosurgeon, but it's not unusual that I would have to take a patient back to surgery. And I guess I don't see that that is a

problem. In other words, it's better to make sure you're doing the right thing, rather than doing something for haste or convenience, which seems to be one argument that's made, and I just wonder what your thoughts were. In other words, I think for your patients you should give the best treatment based on all the information, and not make a haste decision based on some incomplete information, if you will. Just wonder what your thoughts were on that.

DR. JULIAN: Dr. Lichtor, I'd be happy to try to answer that question for you. When you're in the operating room and you're performing the lymph node biopsy, to start with, you've obviously got nice tissues and it's fairly easy in most surgeon's hands to make that small incision, go down inside, take out on an average three sentinel nodes, and that's what most of the trials are showing that we collect. Obviously, it varies from one to maybe even five sentinel nodes that you remove at that time, if they are deemed to be negative at that time, by whatever method one uses. Nodes, unfortunately, does not just leave an empty hole, as we know. You have scar tissue, you have tissue that could be bound the inner costal brachial cutaneous nerves that provide sensory patterns to the arm, to the side of the chest, and

these are things that we have tried as we have evolved axillary dissection to minimize their destruction, to provide better care, and provide less parasthesias, and allow a patient to have an operative result after an axillary dissection so they're comfortable. The problem when you go back in and you have the scar tissue, is that you may end up sacrificing those nerves. Chances are you won't sacrifice the motor nerves because you're going to try to be as very careful as you can for that, but you also have issues of removing lymphatic tissue that may be caught up in this material, as well, even though you're trying to preserve it, so you have the risk and run the risk of increasing parasthesias, numbness in the axilla, also increasing the risk of axillary, or arm lymphodema.

Unfortunately, there are really no great databases that have looked at this across the board. This data is trying to be collected in a prospective way, and again, it's something that will probably have to come out in time. But those are the issues of trying to get back to do.

DR. TAYLOR: Thanks. We're in agreement, Dr. Leitch, with that comment?

DR. LEITCH: Well, you know, you can have more scar tissue, but I pretty much, if I need to go

back, we schedule it and go back. You can always say there's inconvenience of operating room schedule, if you plan to do five cases in a day, and then two of them have to have axillary dissection you hadn't planned, then that can keep your patients from the end of the day from getting to have their surgery, so there's a lot of things that go into surgical planning that might cause you a difficulty. I think you -- actually, there are some technical issues about completing the axillary dissection when you've already torn it up a little bit to do the sentinel node, that your anatomic borders have been somewhat disturbed by that point, so I think there's technical issues any time you do a surgery, so I don't think that that's the main rationale.

Obviously, for patients it's nice to be finished which whatever they're going to have done, but if I told a patient that there was a 14 percent chance I was going to do an axillary dissection on her and she didn't need it, I think most patients wouldn't accept that.

DR. TAYLOR: Okay. Dr. Kemeny.

DR. KEMENY: I agree with that, and I really don't think you can say that there's increased lymphodema if you do a delayed axillary dissection

after a sentinel node. There's no study that's shown that.

DR. JULIAN: I would agree with you, and I didn't say there would be, I said there could be, with that. And you run the risk of that, though, because you are going to interrupt more tissue than you would probably have done with your first axillary dissection because of the scar tissue, that you have to peel it off of the underside of the axillary vein. And we can get into a diatribe on that, but you run the risk of that.

I guess the other issue is that we keep going around on the 14 percent possibly not needing the axillary dissection. And I can only say that if we go back and we look at the existing data, and utilize, again, the information from the Milan node processing, and feel that there are positive nodes, and truly believe that that 14 percent is not 14 percent false positive, then I think by overlooking a positive sentinel node, and not going on to complete an axillary dissection where you may have another 10 to 35 percent of positive non-sentinel nodes, could be doing the patient a disservice, as well.

DR. TAYLOR: Okay. I think we've discussed this point. I'll come to you in a second,

sir. I'd like to move on. I'll come to you, and to Joan London at the end, if that's okay. Dr. Thomas.

DR. THOMAS: I'd kind of like to talk a little bit more about the site-to-site variability, especially considering that the invalid results were more often or predominately related to operator error. And I'm from Kansas, so there's going to be a solo practitioner out there in Dodge City, Kansas who you can sell this test to, and I need to know that it's going to work as well in that setting as it is at M.D. Anderson, where they do hundreds in a month.

DR. VARGO: And that's exactly what we think is one of the benefits of the assay, and that is that in these, I hate to call them podunk places, I don't know what another good term is, but --

DR. THOMAS: It's not podunk.

DR. VARGO: -- small town places.

DR. THOMAS: Isolated.

DR. VARGO: Isolated places that may not have, first of all, a lot of cases coming through where the pathologists have a great deal of experience seeing this stuff over and over again. You are going to get more variability in the accuracy of your final histology result, forget about frozen section or touch prep. Your final histology result is less likely to

be as accurate. And Dr. Palazzo can speak to that from his experience with various pathologists' experience, and how accurate or non-accurate they are. And there's plenty of literature indicating that the histological differences are pretty good from pathologist to pathologist, depending upon experience, et cetera.

The thing to remember about the performance differences as talked a lot from Dr. Whorton and Scott Berry, Dr. Berry, is that they're talking about sensitivity and specificity. And that is calling, for our calculations, we're calling negatives - excuse me - invalids as if they're negative, as a worst case scenario, you're not getting any information, rather than excluding them. Right? So we're taking the hit, so to speak.

As I mentioned before, one of the nice things about the assay is you are going to get an invalid result. You're not going to get a false positive, because somebody is sloppy and contaminates something. You're going to get an invalid, so it's a very nice failsafe for people who may not be as experienced to not get false positive results with the assay. You will get an invalid, which will not give you a false positive result. Correct?

The other thing that I can point out is that most of the operators in the study were people that we trained who were histo techs or med techs, not molecular biologists. And as I mentioned, the hardest thing for them to learn was the pipette, so these were -- there were a number of people who had never used a pipetter before. We wanted to train people who were the people that you would have access to, to run an assay, not molecular biologists, not your MD pathologist, but people that you would be more likely to be able to get in any environment to be able to run this assay. And the performances that you see are based on training of those types of people, not selected micro biologists.

DR. THOMAS: I didn't presume that. You still haven't convinced me that somebody who does one once a month is going to be able to have as good a result as somebody who does more. And, also, for the pathologist out in Dodge City, if he or she reads a pathology slide and has a question, they can send the slide to me, I can look, or somebody else can look, or they can send the image by telepathology. You've lost the chance of doing that once you've consumed the tissue.

DR. VARGO: And I agree with you. And I

think in places that have very low surgery rates, that it may not be worth their investment or time to do this methodology, if they are very spaced out.

DR. THOMAS: Or it might be dangerous.

DR. VARGO: It could be, if you do it so seldom. The other thing to keep in mind is on a practical basis, just as the guidelines today are followed depending upon site personnel availability, skill set availability, if you had a grossly metastatic, clinically obviously metastatic node, you probably are not going to run the assay on it. You're going to run a quick frozen section, and even the H&E done later is really like okay, let's just do it because it's what we do. So practical considerations are going to be taken into account.

Also, there are risk factors that are already known before you go in to do your breast surgery or your sentinel lymph node surgery for an increased risk of lymphodema. If somebody is coming in with those high risk factors, again, you may not want to do any intraoperative result, and you may want to be very conservative about a very thorough, if at all possible, permanent section result, because that person has an increased risk of the side effects of unnecessary, so all of it has to be taken into

account. Nothing is black and white, and this assay would not be used any differently than any other test where you choose to use it where it is appropriate for your patient, for your surgery, for your personnel, et cetera.

DR. TAYLOR: Okay. Thank you. One member of the panel hasn't yet had a chance to ask questions. This is Dr. Gulley, do you have any comments or questions at this point? And then I want to move on to the FDA questions, and then we can come back to any other panel-related issues.

DR. GULLEY: I have no questions.

DR. TAYLOR: Okay.

DR. GULLEY: Actually, I have no questions at this point.

DR. TAYLOR: Okay, no questions. I shall come back to you, Ms. London. Don't worry. Yes, Dr. Begg.

DR. BEGG: I would like to actually come back to some of the things that we've already talked about, because for me, it's the primary concern I have. If you look at the comparison with frozen section, the data that you presented in the 319 cases, this was on page 158 of the report, for the BLN Assay, there were 13 patients who were false positives, and

four that were false negatives. And for the frozen H&E, there was five false positives, and 13 false negatives, so that the two tests are, in essence, if you believe the gold standard, have, in essence, the same number of failures, but in the case of the BLN Assay, there are more false positives and less false negatives, and vice versa. And so what I was really going to ask was what's the trade-off in the different errors, because you seem to be implying a false positive error is less of a concern than a false negative error.

Now I think in the earlier discussion, you've kind of answered that by saying, in essence, if I understand your correctly, you don't really believe the false positive errors. Now it's fair enough to have that opinion, but if you set up an experiment like this where you have a gold standard, and then at the end of the day you say you don't really believe it, it kind of raises the question, what are we really testing here? And when the question is posed to you well, one could design a different study with a clinical endpoint, but you're not comfortable with that because it would take too long and so forth, I'm sort of left wondering where is the evidence here for making this decision?

DR. TAYLOR: Go ahead.

DR. VARGO: Moving into molecular pathology is an extremely difficult business, and the reason is, primarily - and we're looking at other areas, and we're always having to compare ourselves against histology because that is the answer. Whether it's a prognostic test, was the final answer correct? Was it read right? And you know you get differences if you send it out, so we're always going to be living with the fact that the standard against which we have to compare ourselves is not perfect.

I will not say, though, that we don't believe histology. On any given - I mean, there are cases even with two out of three pathologists, if you had five out of nine pathologists it could flip for certain samples, but those are the rare ones. I'm not worried about those. But in any given piece of tissue, two out of three pathologists are likely to be correct with what is going on on that piece of tissue. So we're not discounting that, and you notice that I'm not saying that the assay negatives are false. The assay negatives are false in the sense on the nodal basis. If another piece of tissue on that same node was found to be positive by histology and confirmed in two out of three pathologists, it was, in

fact, positive, and the assay did miss it. Whether the assay missed it because it didn't have it, or missed it because it truly has some lack of sensitivity for certain types of cells that were there and it didn't get, doesn't matter, it would have missed it. But the same is not true for positives.

When you have a histological section that is negative, and you have a histological section, another level that is positive, the negative one is not wrong for that section, and the positive one is not wrong because the second level didn't back it up. And I think you have to, because we don't have any way really around this, to use some logic to adjust, as Scott showed in his slide, to adjust the true specificity of what the assay is likely to be based on an imperfect comparator. Those statistical methods are not illegitimate. We're not claiming the adjusted specificity, but you have to take them into account, or advances in this field are going to be extremely long in coming. I don't know any way around it. All I can tell you is that the preponderance of data I think that we're showing, especially comparing histology levels to histology levels, and that is a 4.2 percent difference in positivity with site pathology "false", when, of course, it's not falsely

positive. It's just not backed up in different levels. Of course, the assay, which is testing half of the node that histology doesn't get to test, is going to have positives that are not detected by histology because histology didn't get a chance to test our tissue.

DR. TAYLOR: Okay. I'm going to have to exercise the Chair's prerogative here, and move on at the moment. There may be a chance to come back. I'd like to ask if either the industry representative, Dr. Allard, and then the consumer representative have questions or comments for the sponsor.

DR. ALLARD: Yes, thank you, I would. We're comparing - when we talk about false positives and false negatives, we're comparing to a standard, but the standard is not a perfect standard, as Dr. Begg has pointed out. And I think an important point here is when we compare the test to central pathology, that's one comparison. But we can also compare the site pathology with central pathology, and we can ask how often is pathology wrong itself? How often is the gold standard not so gold? And when I did that, according to the information that was provided to me by the FDA, I came up with a false positive and false negative rate there, too. Now I did it by simple

arithmetic, and I'm sure that statisticians do a much more sophisticated analysis, but the false positive and false negative rates that I saw using the test that's been reported today, of course, are 13.8 percent false positive, 5.1 false negative. But if you compare site versus central pathology, the false positive rate is 12 percent, and the false negative rate is around 3 percent, simple arithmetic. Now I wouldn't take those numbers to the bank, but the point is, there is a false positive and a false negative rate that's already associated with what you do today, so I think that the question becomes what additional risk does the test add to the current practice, not the simple comparison of test versus central pathology. I think it's a little more complex than that, and I don't think you're going from zero percent false positives today to 14, you're going from some number, which according to this data is 12, to 14, as opposed to zero to 14, so I don't think the increment here of false positives and false negatives is nearly as large as it may have seemed.

DR. TAYLOR: Okay. Thanks. Joan London, do you have comments.

MS. LONDON: I do. I'd like to move a second from the scientific aspect to the

communications aspect, specifically the chapter on labeling in our book, and I'm going to refer to page 122 in just a minute. When a woman and her family, or a man in a few rare cases finds out they have cancer, it's an emotionally charged time, and the doctor may explain to them what's going on, and their mind is just flipping flopping around. I'm wondering if either in the packaging or as adjunct material there could be some explanations what is false positive, what is false negative, so that whoever the health providers are who talk to the family, they have some assistance in explaining what all this is.

There is some very helpful material in our packet with abbreviations, and I looked through the labeling chapter and didn't really see any kind of explanations. And I'm wondering, that may not be the appropriate place, but if you are developing any materials, or what your way of communicating what all this means to the patient in patient language, where that stands.

My second quick point, and this is just a tiny little nitpick - on page 22 in the label - 122 in the labeling chapter, you have "technical and consumer support can be reached 8 a.m. to 8 p.m. Eastern time". Is that Monday through Friday, is that Saturday and

Sunday, is that holiday? It might be clarified very simply right there.

DR. TAYLOR: Okay. Thank you. Those are good comments. I want to ask Dr. Reeves now to come forward with the FDA directed questions, and then we will resume the discussion. And we will, obviously, pick up some of these same issues again as we go through these questions. I think you're going to show them as PowerPoints. Is that correct?

DR. REEVES: That's correct, yes.

DR. TAYLOR: Thank you. There are also copies of these available on the table outside, and hopefully most people have a hard copy.

DR. REEVES: Okay. Question one - Is the inability of this test to distinguish size of metastasis, micro versus macro, relevant to the safe and effective use of the test? If so, how should this issue be addressed?

Question two is a long one. The BLN Assay detects histological metastasis greater than 0.2 millimeters with the following performance characteristics; the sensitivity is 87.6 percent with a confidence interval from 80.4 percent to 92.9 percent, and the specificity is 94.2 percent with a confidence interval from 90.9 to 96.6 percent. For

prevalence of 29.1 percent node positive patients, 8 percent invalid results treated as negative, an estimated time of 30 minutes. The predictive value of a positive result is 86.2 percent with the stated confidence intervals, the point estimate of false positive results in any patient tested is 14 percent. The predictive value of a negative --

DR. TAYLOR: We'll consider one at a time.

DR. REEVES: I thought -- I understood I was just to read the questions off. Is that incorrect?

DR. TAYLOR: Does everybody have a written copy of these questions? So let's just -- we'll deal with -- if everybody's got a written copy, some of these questions get in -- they're really overlapping a little bit, so I think everybody needs to have seen all the questions. So if everybody's got a written copy, we could actually save some time here by going back to the first one.

DR. REEVES: Okay, fine.

DR. TAYLOR: All the panelists have got written copies. Right? So let's just put the first one up, and then we'll --

DR. REEVES: My apologies for going on.

DR. TAYLOR: No, that's not a problem. So

question one then from the FDA is up on the screen before you. Does anyone from the panel wish to comment, or address this issue? Okay, Dr. Netto.

DR. NETTO: I do believe that's an important matter, the inability tell the micro from macro metastasis, and it ties into what's been previously said, because some of these findings are currently guiding therapy, so this, again, brings immediately the issue, is this going to be a stand-alone, or not stand-alone? And there is a difference between not doing a frozen section at all, and saying well, anything is better than nothing. It's not true, anything here is going to force the axillary dissection in case false positive, so I do think that's an important distinction, because it's going to affect --

DR. TAYLOR: Yes, this is the same question that you raised, isn't it? Does anybody else have comments on this? Dr. Leitch.

DR. LEITCH: Well, it raises the issue of the axillary dissection. We've kind of already talked about it. I think the other issue is systemic therapy, and it may not actually make as much difference as you might think about a person getting systemic therapy. But if you wanted to look at a way

it could be addressed, and we've talked about this a little bit in this session, and I think it'll come up later in the questions, of do you use up the whole lymph node for this assay, and all of the sentinel nodes for this assay, or do you do it more like was done in the study, taking some sections that you reserve aside probably for permanent pathology, and then the stuff that you do the assay on, so if you had positivity, you might have a prayer of quantitating the size of the metastasis on the sections that you've reserved aside for the permanent, so there is some way to try to deal with it. It doesn't address the question if you took the only piece that has any lymph node in it, then you don't, and you could kind of try to guess that.

The other thing, which I had wondered about was doing the other axis, taking the tissue on the other axis, and we didn't get to address that question really, but if you might have a chance of seeing more the overview of the node if you took it in the long axis, and kept one slice, some slices in that axis, and then the other would be submitted for the assay to help you deal with this issue, if you're going to do.

DR. TAYLOR: Okay. Dr. Kemeny.

DR. KEMENY: I just think it's important for people to remember that the standard of care really isn't to do a frozen section after - on doing sentinel lymph node biopsies. A number of us don't do that. I mean, there are people that do do it, especially maybe in larger institutions where everything is set up, but a number of us just do sentinel lymph node biopsies, and do permanent sections, and don't do frozen sections, so this -- we can't put this down as standard of care. It's not the standard of care at this moment.

DR. TAYLOR: I think that's a fair point. Any other comments from the panel on question one, because otherwise question two is a real monster, so we -- could I ask you to put question two up for us, please, Dr. Reeves.

DR. REEVES: Do you want me to --

DR. TAYLOR: No, I don't think you need read this one. It's up there. We've all got a copy of this, so --

DR. REEVES: Fine, thank you.

DR. TAYLOR: -- the issue is, do we have comments or questions from the panel to the points of this particular FDA question, which really is given the above performance numbers, is this device safe and

effective as a stand-alone? Dr. Leitch.

DR. LEITCH: Again, I think we have to define what we mean by stand-alone. Do we mean we use up the whole sentinel node for this assay intraoperatively? Is that what we mean by stand-alone? Do we section it up and reserve some aside, and my thought on that, that it would be as a permanent section, that the set aside would be for that. What do you mean when you say "stand-alone"? And I think if it's going to be adopted, you have to have a definition of that, what you mean when you say "stand-alone."

DR. TAYLOR: Yes. Okay. Any other comments, same point? Dr. Netto.

DR. NETTO: Yes, I fully agree. I mean, it's stand-alone plus permanent probably would be more of an idea, but the issue is, is also what you do when there is a conflict, going back to if it's positive, then only on the molecular, and negative on the permanent, how you're going to count that patient, and without having the evidence for that, that's going to become also an issue, so I think that's that.

DR. TAYLOR: Dr. Gollin.

DR. GOLLIN: I don't think you can call it stand-alone if you're saving part of each sentinel

node for permanent section histopathology. I think it's used as an adjunctive test to permanent section histopathology.

DR. TAYLOR: Okay. Dr. Thomas, same point, same question?

DR. THOMAS: Same question. I'm going to challenge the time. I know you showed nicely that it could take 30 to 40 minutes, but in a very busy frozen section laboratory where you might get five or six at the same time, it's hard to believe that you can turn this thing around in the time that's necessary. And at my institution, that's 20 minutes per frozen.

DR. TAYLOR: Okay. Any other comments or questions on this one, because question three is sort of similar to this, and it deals with some of the same issues. Do you want me to come back to you at the end of all the questions, or do you want to do it after each question?

MR. ST. PIERRE: Actually, I think the FDA would like to clarify what they meant by stand-alone to help the discussion.

DR. TAYLOR: They would. I have a question for you. The definition here, is this - are we talking about the sponsor's definition of stand-alone, or are we talking about the FDA's definition,

or the panel's definition? We need to work that out.

DR. BECKER: It's the definition that we meant when we composed the question --

DR. TAYLOR: Okay.

DR. BECKER: -- that I can recite for you now, which is that we were talking about --

MR. ST. PIERRE: Bob, can you introduce yourself.

DR. BECKER: I should do that.

DR. TAYLOR: Yes, give your name.

DR. BECKER: Okay. I'm Robert Becker. I'm the Division Director for Immunology and Hematology Devices. By stand-alone, we meant that it is used, in this case, in an intraoperative consultation without frozen section simultaneously being used. That is without reference to whether there would be permanent sections afterward, but in the context of the intraoperative consultation, it would be --

DR. TAYLOR: So stand-alone intraoperative is the definition you're giving. Doesn't mean stand-alone, period. It's just stand-alone as an intraoperative test.

DR. BECKER: Stand-alone intraoperative.

DR. TAYLOR: Does not assume that the H&E

permanently is done away with.

DR. BECKER: That's correct.

DR. TAYLOR: Okay. Thank you. Does that help everybody?

DR. SIEGEL: That assumes that the whole node isn't used in the procedure or the test.

DR. BECKER: It doesn't address that. It only indicates that there's not another test being used in the intraoperative consultation mode. That's in contrast to the subsequent question. I hope it'll be clear at that point.

DR. GOLLIN: But I think the labeling needs to define that very clearly.

DR. TAYLOR: Okay. Thanks, Bob. All right. Let's do question three, and then we'll come back to Dr. Pierre at the end to make sure we've at least tried to address the FDA's concerns here, because these are a little complicated.

Question three is another long question. Thank you. I don't think, again, we need to read it. The numbers are there. And, again, the question addressed to the panel is given the above performance figures, is this device safe and effective as a stand-alone replacement for frozen section consultation? And I guess there's a subtle difference here between

question two and question three. So does anybody have comments on question three, as separate from question two? Sorry, we're slow readers. It'll take us a few minutes. So I think the issue here is - I'm having problems with stand-alone addition, versus stand-alone replacement, because I don't see how you can have a stand-alone addition. Bob.

DR. BECKER: We were speaking of the context with question two, in which you would not have been carrying out a frozen section as part of an intraoperative procedure, but you would have, instead, be introducing this test as the only test used in an intraoperative procedure, so that in contrast with question three, if there was already an intraoperative procedure being done, frozen section, you would now be marrying this test up with the frozen section as part of that procedure. So let me make it slightly clearer, I hope.

In question two, you may be in a service which does not carry out intraoperative consultation, does not do frozen section, and one might introduce this test as a device for a new service to provide intraoperative consultation, and it would be the stand-alone test used for the purpose of that consultation.

In question three, you would already have an intraoperative consultation service running, likely based on frozen section, and this test might be used to replace the frozen section component of that intraoperative consultation using it in lieu of frozen section.

DR. NETTO: May I?

DR. TAYLOR: Yes, please, George.

DR. NETTO: Yes. So I guess the more important issue here is not is this better or worse than frozen section, given the fact that some places they don't do the frozen section at all, so it's not a problem that you've got a test that is as good, or not as good. The issue is more what will happen with a positive result, so it takes us back to the 14 percent, places that elected not to do frozen section, that means they didn't accept that rate of false positivity, or false negativity, whatever in frozen section, so why would they accept that high rate. And so I think to say that it's replaced or not replaced frozen section, it's going to depend on the place, but at the same time, you still have to deal with the 14 percent positivity that may be false positivity.

DR. TAYLOR: Any other comments on questions two and three, because I'm sort of looking

these as just two sides of the same coin, Dr. Becker. So any comments on two and three? Yes, Dr. Siegel.

DR. SIEGEL: I think it would be very dependent on the standard of care in every different institution, and I think it will be very confusing, for example, to third-party payers, as the example given, if you split a lymph node and there was gross metastasis, you might decide just go ahead and do an H&E and be done with it. Whereas, the other one node or 12 nodes, however many you have, where you didn't see that you might want to do the molecular test, and then you would be submitting a bill, if you will, for both frozen for one, and molecular for the other. And I think people would have confusion about why you're charging different things, and so forth and so on.

DR. TAYLOR: Practical issue. Right? Anything else? Dr. Leitch.

DR. LEITCH: Well, I just think we need to be clear from this question that you wouldn't be doing both things. You wouldn't be doing frozen section and this assay in the same procedure. And to make it clear that if you do the assay, then you have to accept the results. If you're going to use it, then you have to accept the results. And if people might be inclined to say well, I'll do a frozen section to

verify it. For example, some people will do a touch prep, and then if that's positive, do a frozen section to verify that. If they're the types that never -- if you want to reduce as low as possible the chance that you have a false positive, then there are some steps people go through to avoid that, if they intraoperative evaluation. But I think if you're saying you're going to use this assay, you wouldn't do frozen section as "the backup", you'd just either do frozen section or not, and you'd have to accept the results of this assay. And, again, it's going to get back to that question of the informing of the patient about that 14 percent issue when the assay is positive for that given patient. And, again, I think it's very possible that that does represent cancer cells, but, again, just as we are reluctant to base a lot of treatment issues on immunohistochemistry, I think the same would apply for the circumstance of assay positive, H&E negative.

DR. ERNSTOFF: Can I just get a clarification?

DR. TAYLOR: Yes.

DR. ERNSTOFF: Maybe both from Dr. Kemeny and Dr. Leitch. Do you discuss with your patients what the false negative or false positive rates of H&E

are, permanent H&E? You say that we don't sample the whole node, we sample 50 percent of it, and it's possible that you would have a positive node? I don't know whether that data is available.

DR. KEMENY: No, I don't discuss that. You mean as opposed to immunohistochemistry? That, I do talk about immunohistochemistry, but H&E is the only game in town, so what --

DR. ERNSTOFF: I mean, but you don't discuss with patients that H&E, which is "the gold standard", can be falsely negative because of a sampling error, and that the pathologist, at least on this side of the pond, doesn't sample the entire node, only a part of the node. We don't go through that. I don't go through that in melanoma patients in great detail, but I do tell them that it could -- it's conceivable that there's a sampling error.

DR. KEMENY: But, again, it is the only game in town, so I mean, in other words, there's no other way to do it, so that's the way they do it. I don't know if one needs to go into a discussion with patients about how they're sampling their lymph nodes, because they don't know how --

DR. ERNSTOFF: But it's the same --

dR. KEMENY: What you do talk to them

about is what immunohistochemistry means, because when that came along, then that was something new, and then you do have to talk about that.

DR. ERNSTOFF: But it's the same question about whether you're going to ultimately take that patient back for a lymph node dissection or not, based on a gold standard. And the gold standard, as we've talked about, is slightly off gold in terms of the accuracy of actually telling you whether that node is positive or negative. You're making a decision based on that gold standard.

DR. KEMENY: And I'll let Marilyn answer, also, but I mean, certainly, when we're doing sentinel lymph node biopsies now, when before in the yesteryear we used to do axillary dissections on everybody, so now we're doing the sentinel lymph node biopsies. Yes, you do explain to them about sentinel lymph node biopsies, what you're doing, and that there is an inaccuracy of it. But, as I was saying before, I find it's just an interesting commentary on people in our society at this point, that sentinel lymph node biopsies actually came mostly from women in the community, rather than from scientific data. And most women want sentinel lymph node biopsies, they don't want to hear about anything else.

DR. TAYLOR: Dr. Leitch.

DR. LEITCH: So we do talk to them about that, because that's the whole discussion of immunohistochemistry, that there is inaccuracy with lesser processing of a lymph node, and that's one of the advantages of the sentinel node, is that you do process it in more detail, both for H&E and for immunohistochemistry. And then you have to talk to them about well, we do this immunohistochemistry, but we may not know the value of it. I mean, it can help us for lobular to recognize something we might miss, that it really is clinically significant, but it may show us data that we don't know how to handle. And then we're sitting there with them, and saying well -- they are saying - because the patient is going to look at the report, and they're saying well, they're saying that there are these cells, and then you say well, are you going to do an axillary dissection for that? And then I say well, by the current staging system, that's not considered node positive. Is it possible that has some prognostic significance for that patient? Yes, it is possible it does, and that's what we're waiting for this data on the studies. We're also waiting for on sentinel node, if the person is negative on the sentinel node, and they don't have

an axillary dissection, what's the probability that later on disease shows up in their axilla? Well, it looks very low in the early followup studies, but that data is still generating, also. So yes, I can explain that stuff to the patients when I talk to them about sentinel node. I still talk to them about the false negative rate of sentinel node, because the data from those studies is continuing to come out, so that's part of the discussion. And this would be really, to me, in the same line with immunohistochemistry, but I wouldn't be in the operating room deciding to go forward. It's kind of like doing a mastectomy on a frozen section. The patient in their mind is already thinking well, it's going to be benign. I don't have to worry about it, but if they go in and you're saying well, I might do an axillary dissection, and it could be that in the end you didn't really need it, but that's the data we have in the surgery, and it'll spare you an extra trip, they may want to do that.

The other thing people should remember about the extra trip is the patient may have other reasons to come back for an extra trip, if they have a positive node, maybe they're going to have to have a port put in, so then they would come back for a port. Maybe they have positive margins on their lumpectomy.

They're going to come back for that. Now you might say well, they could have that under local, as opposed to general, but nevertheless, it's another trip to the operating room. And the morbidity of breast cancer relative to anesthesia is pretty low, except for really high risk patients, and those patients we make decisions about not doing axillary dissections on those patients. So those are all the questions that go on in the discussion, and because I do tell people all that stuff, I would feel obliged to tell them what the data is that has been reported here. And so, if I was going to propose to them having the assay intraoperatively, the patient would have to weigh to them the value of one surgery, possibly one surgery, because I can't promise them it'll just be one surgery, based on the other things they may need, versus the possibility of having an axillary dissection when they wouldn't have had it if we didn't do the assay.

DR. TAYLOR: Okay. Question four also deals with safe and effective, again in a slightly different context, but it overlaps, so I think we ought to look at question four, too. Then we'll come back and look at safe and effective on each of these four questions. So question four is a short question,

and we thank the FDA for a short question. Right? The answer may be no shorter, however. The question is - are there sufficient data to establish safe and effective use of the test for tumor staging, or any aspect of tumor staging in breast cancer patients? So anyone on the panel want to comment? It's not dissimilar to your question, Marc, so maybe you should start.

DR. ERNSTOFF: I think it's an appropriate question that needs to be asked. We have the data, and we'll have to make a decision once we, I think, have a full discussion.

DR. TAYLOR: Okay. Any comments or discussion from anybody else?

DR. ERNSTOFF: Dr. Thomas has a question.

DR. TAYLOR: Oh, I'm sorry. I didn't see you.

DR. THOMAS: Well, if size is related to the information you need to make decisions, then that's not included.

DR. ERNSTOFF: Size is included. If you look at guidelines, people with positive nodes are going to have adjuvant therapy. Question of the kind of adjuvant therapy might be impacted by size, so one has to make that decision. It would have been nice

not to have a qualitative, but a quantitative assay that could say cutoff number two is if you've got macroscopic disease, and maybe that data exists within the databases, maybe it doesn't.

DR. KEMENY: Clarification. I'm not exactly understanding this question. Are we asking whether we should use this assay for looking at the rest of the lymph nodes, is that what you're asking?

DR. TAYLOR: I'm not asking anything.

DR. KEMENY: No, is that what this question is asking.

(Laughter.)

DR. KEMENY: I'd like an interpretation.

DR. TAYLOR: We're going to have Dr. Becker give us another interpretation.

DR. BECKER: It's a somewhat general question. I think you pretty much have the gist of it, that we're asking whether you can use data from this test to establish or to assist you in establishing the stage of disease for the patient in the formal sense of the staging systems that are in existence now, in particular.

DR. TAYLOR: So you want to know where we'd put it in a T&M system, whether we put this as an N suffix something or other. Is that the question

you're asking us?

DR. BECKER: That's right.

DR. TAYLOR: Okay. So that's easy. Now you can answer that one. Right?

DR. NETTO: So my question is, how -- would that be something that would deserve a molecular, like the staging system was suggesting, because that would be it. Correct? In the category of NI, when you say N0, then I, then MOL, molecular. And like the ASCO consensus suggested, so this will be something, if you use this test and it's positive, will be N molecular, but it's negative by slides. Correct?

DR. TAYLOR: Is that how you envision it being used?

DR. VARGO: We envision, minimally, that obviously the (molecular plus) would be used, which is already in the guidelines. WE think that the data support, as well, changing the status of the node to N1. So right now, the way it is in the guidelines, and it says right in the guidelines the reason for this, is there wasn't enough clinical data to support it. It's N0(molecular plus) just as it is for IHC. We believe that the data support changing that to N1, whether or not you'd also want a molecular after it to

indicate that was by molecular only, is a different subtlety that, perhaps, could be added to the guidelines.

DR. TAYLOR: Okay. Thank you.

DR. THOMAS: So what one would lose in the staging system is the size, micro versus macro, and any decisions that you might make based on that.

DR. ERNSTOFF: I think you lose any clinical decisions based on size or extra capsular spread, things that are only defined by H&E, mitotic rate that we haven't talked about, which is mostly on the primary, but clearly not indicated, I would think in a post adjuvant, neo adjuvant setting, but that's not what is being proposed here.

DR. TAYLOR: Any other pathologist input to that? Dr. Siegel, Dr. Netto, anybody? So how about a surgeon's input?

DR. LEITCH: Well, again, to get into the staging system, and if you want to be listed in the staging system, then there has to be data to support that that test has prognostic value. And, of course, that's the dilemma now in the staging systems, is how do you incorporate prognostic tests, whether they're like this, or other molecular tests, how do you incorporate them into the current staging system? And

that's why it's evaluated, but not every year, but it's sort of ongoing.

I would be surprised if the AJCC would accept this data as sufficient to describe the nodes as positive, prognostically positive, with the data that we have to look at, at the moment.

DR. TAYLOR: George.

DR. NETTO: Well, if it's been so many years we're doing IHC, and it's still stuck in the N0(I), so I don't think just based on the 400+, I don't think the data does show that these are truly positive. We go back to what we started discussing, so I don't see the rationale why we should jump to N1 and put (molecular), so I don't think the AJCC should do that based on just this.

DR. TAYLOR: Anyone else on the panel? I sense that you might wish to make another comment, Ms. Vargo.

DR. VARGO: Yes. Just very briefly, I just wanted to point out that for touch prep right now, for which you get no size information, when the touch prep is positive and permanent section is negative, that node stands as positive.

DR. TAYLOR: Okay. Dr. Leitch.

DR. LEITCH: Well, I don't know if I would

agree with that. I mean, from my pathologists, if they tell me a touch prep is positive, I ask them to freeze it. I make them kind of do two things for that, so that I have more documentation of that.

DR. TAYLOR: Or you wait for the permanent.

DR. LEITCH: Or I wait for the permanent. So I can't think of a case that I have ever done, where I did an axillary dissection for a touch prep that the permanent was negative.

DR. TAYLOR: All right.

DR. JULIAN: Dr. Taylor, may I?

DR. TAYLOR: Yes, go ahead.

DR. JULIAN: Okay. Actually, for NSAPB B-32 touch prep positive only nodes were classified as positive nodes for the study, and so we'll be following those out, as well. And, actually, in my clinical practice, if I have a touch prep positive node, as much as I don't like the idea of it, and I have an H&E that is negative, this is a discussion that goes on with the patient. And I can tell you that in more cases than not, the patient wants to know if she has any further non-positive, or non-sentinel nodes that are positive, so that's a discussion that, agreed, has to be undertaken, and, obviously, this is

where the art and the science of medicine have to merge.

DR. TAYLOR: That's not an intraoperative discussion, I assume.

(Laughter.)

DR. JULIAN: No, because -- no, you're right on that. Not on that. I'm sorry.

DR. TAYLOR: No, I was just trying to --

DR. JULIAN: But there has been the occasion - no, this is something that can be discussed ahead of time, as well.

DR. TAYLOR: Okay.

DR. JULIAN: But in the operating room, if I had a touch prep positive, yes, I've done the axillary dissection.

DR. TAYLOR: Okay. That's one of the issues that's clearly different practices.

DR. NETTO: Correct. It does differ according to the center, so some centers accept touch prep without confirmation with frozen section.

DR. LEITCH: I think it depends on the whole picture. Now if they cut the node, they see this white thing, they touch prep it, and you have more clinical data to support it, as opposed to not.

DR. NETTO: Plus you still do have the

permanent.

DR. LEITCH: Right.

DR. TAYLOR: Now, Don Pierre - I'm sorry, I didn't see you. Sorry.

DR. WHITWORTH: That's all right. Pat Whitworth, one of the primary investigators in the study.

DR. TAYLOR: Please.

DR. WHITWORTH: Just to throw another opinion into the mix; that question is really a question for the AJCC. The question there is not, is there clinically useful information here that might improve care. If you ask that question, really the AJCC will tell us current guidelines, certainly there's sufficient information to make the patient N0 N positive.

DR. TAYLOR: Well, they're not here today, so we can't really address them. As far as the FDA is concerned, they posed four questions to the panel. We discussed them. Is there any more pertinent discussion the FDA would like to hear before we, essentially, have another public session that we do have some individuals who indicated they wish to speak, so we should deal with any other FDA-related questions here before the panel then have a chance to

vote on this. So, Don.

MR. ST. PIERRE: No, we don't have any more issues.

DR. TAYLOR: Okay. On the panel - yes, Dr. Siegel.

DR. SIEGEL: I just wanted to ask the FDA sort of a point of information. I'm assuming that the test or the assay is sort of all or none phenomena as far as the FDA is concerned. So if I had my own way to prepare the tissue for probing, or I had my own machine to do PCR, that would be unacceptable. You have to have every point along the way. Is that true, or not?

DR. BECKER: We would expect the test to be sold and promoted as a unit, but there are adaptations that clinicians make from time to time in their own laboratory practice.

DR. TAYLOR: Did you get the answer you wanted?

DR. SIEGEL: Yes, and the answer is yes, you can split it.

DR. TAYLOR: Okay. The answer is yes, you can home brew, I guess, but then you're going to have home validate, so that's that issue.

DR. NETTO: Sorry, wouldn't that make it

NASR if we start taking pieces and adding some home brew portions to it?

DR. BECKER: No. What I'm saying is that we don't regulate the practice of medicine, and so there certainly are off-label configurations of other devices. I think that in the context of this one, you'd have to be looking at the specifics of the circumstances.

DR. ERNSTOFF: I think the question, though, was, if the FDA were to approve this, you would approve the device as it is presented here, that includes not just the reagents, but the actual hardware.

DR. BECKER: That's certainly true.

DR. NETTO: Yes, that helps. That clarifies it.

DR. TAYLOR: That's really what you were asking, isn't it? Yes. Any other comments from the panel? Okay. I think this would be a good time just to take a short break. We have a short 15-minute break scheduled, after which there's a public comment session, so we are now at 2:44, so let's try to keep this thing moving. We will take 15-minutes break. We will reconvene at 3:00.

(Whereupon, the proceedings went off the

record at 2:44 p.m., and went back on the record at 2:58 p.m.)

DR. TAYLOR: At this time, we have an opportunity for a second one-half hour open public hearing session, and my understanding is there are two persons who wish to have an opportunity to speak. Is that correct? Are they here? Yes. I would ask you to restrict your comments to 10 minutes or less, because we do need to move the process along. So please, if you'd come to the podium, introduce yourselves. So we have two people, is that correct? I'm just trying to get a sense of numbers as to how many people there are. Two. Correct? No more than two? Okay. So that will be 10 minutes each, and we need to read you a public hearing statement before we begin. We'd like you both to identify yourselves, please. Would you please stand and identify yourselves, just for the record, of course.

DR. WHITWORTH: I've got a slide coming up that'll help you with that, but it's Pat Whitworth.

DR. TAYLOR: Pat Whitworth, thank you.

DR. WHITWORTH: Surgical oncologist.

DR. BLUMENCRANTZ: And Peter Blumencrantz, also surgical oncologist.

DR. TAYLOR: Thank you. And are either or

both of you associated with the company? You have a financial interest in the company? We also need that for the record.

DR. WHITWORTH: It should be up there in a minute. I am a consultant, primary investigator, and my time, and travel, and consultation is paid for by the company. I have no financial interest in the performance of the company.

DR. TAYLOR: Thank you.

DR. BLUMENCRANTZ: I also am an investigator, primary investigator, consultant, but no other financial interest.

DR. TAYLOR: Thank you. Ms. Carlos will now the open public hearing statement. Thank you.

MS. CARLOS: Both the Food and Drug Administration and the public believe in a transparent process for information gathering and decision making. To ensure such transparency at the open public hearing session of the Advisory Committee meeting, FDA believes that it is important to understand the context of an individual's presentation. For this reason, FDA encourages you, the open public hearing speaker, to advise the committee of any financial relationship that you may have with the sponsor, its product, and if known, its direct competitors. For

example, this financial information may include the sponsor's payment of your travel, lodging, or other expenses in connection with your attendance at the meeting.

Likewise, FDA encourages you at the beginning of your statement, to advise the committee if you do not have any such financial relationships. If you choose not to address this issue of financial relationships at the beginning of your statement, it will not preclude you from speaking.

DR. TAYLOR: Okay. So then we should begin with Dr. Whitworth. Thank you.

DR. WHITWORTH: Pat Whitworth. My interest in this subject matter is based on my participation as a principal investigator, and also because of my participation as a principal investigator, in the first American multi-institutional trial studying sentinel lymph node staging funded by the NCI through a grant obtained by David Cragg at the University of Vermont in the middle 90s. That information was published in the late 90s, and the sentinel node staging approach has rapidly become a standard of care.

I also Vice Chair the American College of Surgeons and Oncology Group Committee Breast Committee

that was responsible for the Z-10 and Z-11 trials you heard about earlier, and I Chair the Board of Directors for the American Society of Breast Surgeons.

To start with, what we do know, and what I don't think is particularly controversial about this assay from what we've heard already today, it is more sensitive intraoperatively than frozen section. That gives two advantages; one, it avoids misleading the patient and her family, and telling them later something that they were relieved to hear wasn't true, is true. But, moreover, it reduces second surgeries, which are also distressing, but incur risks and costs above a single surgery.

The pathology laboratory has been stressed ever since we started doing sentinel nodes, and this reduces the burden on the expert pathologist in the frozen section test, and it also can be expected to reduce inter-institutional variability that exists currently.

Another thing we know is an "if", if this is more accurate than the reference pathology that was used as the gold standard here, if this new test is more accurate than anything we've had so far, and that's an if, it's going to lead to better, more effective adjuvant treatment, and survival. And I'll

show you some slides that I think address Dr. Leitch and Dr. Kemeny's concern that we might be detecting something that's not clinically important. I think there is reason to believe that we may be doing that.

But, furthermore, let me clarify, because some really important significant questions were asked. I don't think we can move from histology to molecular too rapidly, and I think we have to make small steps. And I think the questions about are we preserving tissue that's important to preserve, are important, so my recommendation, what I'm going to be talking about here for the next few slides, and trust me, there aren't that many, is use of the test as an intraoperative stand-alone replacement for frozen section where that's done, or, perhaps, as an addition where it's not been done because it couldn't be done, but to be used only as an adjunct to conventional pathology, so that the alternate slices which were used in the study are also preserved, saving extra capsular invasion information, and architectural information, and size information that people have been concerned about. Next slide.

The concerns about this assay have to do with this question - are the false positives truly positive? Now this is something we really -- it's an

important issue so let me just take a moment to comment on it. This would lead, if these false positives were truly positive, let's just be clear about these numbers, it would lead to a so-called unnecessary axillary dissection in four out of 100 women. That's if you are talking about women who - Dr. Leitch pointed out earlier that you're talking to this woman, you're giving her some information, and this is prior to the operation, and so the number you would be telling this woman is four out of 100 risk that you would have an unnecessary axillary dissection if this false positive is truly false positive, or if it's clinically unimportant. It would not be the 13 or 14 percent rate that applies only to people who have a positive test. We don't know that answer until after the results are back, until after the surgery is over.

Interestingly, whether you talk about this 4 percent out of 100 in the overall group, or 12 percent out of the ones that test positive, or 13 percent out of the ones that test positive, that happens to be the case with the site pathology that we're all using right now. If you send it all to a reference lab, as was done here, you'd get the same numbers.

In addition - this was a great question earlier. I'm sorry, I can't identify who asked it, and they said well, look, the frozen sections had five false positives, and the test had 13. I'll address that a little bit more in a minute, but no one here, no pathologist here believes that those five so-called false positive frozen sections were false positive. They were truly positive, but based on our definition in reference to an assay, they were called false positive. Everybody here knows they were not false positive, so those were truly, if there's such a word "false", false positives.

Now on the other hand, if false positives, so called in this study, because in my opinion the text things that our reference approach doesn't, if these false positive detect reference misses, and, again, if they're clinically important, then multi-disciplinary treatment is better informed, there is a reduction in false negative sentinel node staging, axillary dissections are done when they're needed, systemic adjuvant chemotherapy is given when that's needed.

Is there evidence that these false positives are reference misses? Well, there's only inductive evidence from this study, and from studies

that we've looked at before. Certainly, we see the expected result if the sensitivity of this new assay exceeds the reference test sensitivity. It would guarantee false positives. Everyone here knows that if you take the standard ASCO recommended approach to a lymph node, and then you go back through the rest of the tissue with serial sections, in every study of that, you will increase the detection of disease, and you will reduce the number of false negatives. It really leads to Dr. Leitch's and Dr. Kemeny's question from earlier, which I'll address in a moment, which is, are those clinically important?

We also know that laboratory cell suspension studies, whether it's the negative controls in the actual trial, or cell numbers in laboratory work, the negative controls result in a positive assay far less than 1 percent of the time. Next slide.

So if we accept that more sections yield more detection of metastasis, we know - leaving the clinical significance aside for a moment - we know that the current ASCO recommended approach to the lymph node does not give us 100 percent sensitivity. We don't know if it's 95, or 90, or 87, but we certainly know that the calculations here for specificity of this new test are under-estimated.

Next slide.

Earlier, you heard from a pathologist that if you use the ASCO guidelines and you go through here carefully, you'll find out how this node should be staged, but if perhaps you didn't go through carefully, you might call it negative, you might call it microscopic, you might call it macroscopic, but even if you use the recommended ASCO guidelines and you take perhaps three sections, and you take two or three slices at each section, you're going to miss these two metastasis right here, using the recommended guidelines. The only way to find those is to do either serial sectioning, as was discussed from the Italian group, or use something that assays all of that tissue. Next slide.

This is the thing that is absolutely shocking to surgeons, and we don't want to hear this, and we don't even want to know about it - conventional pathology, you're talking about every bit of tissue in the lymph node, sees about 5 percent of the lymph node, and that's just the way it is. This is three levels, two or three in each lymph node, five micron section for immunohistochemistry. And you heard earlier the ASCO guidelines say if you do this, you will find the majority of clinically relevant

metastasis, micrometastasis. What you didn't hear was that you will miss 10 to 15 percent of clinically relevant micrometastasis. I'm actually not saying this to my patients, they're already worried enough. I'm not going to add more scary information about the limitations of our current state-of-the-art, but I certainly want to see that pushed forward. Next.

This is a slide from 1995, and this is what caused about half of the excitement about sentinel node staging. Armen Giuilliano showed that his detection of macrometastasis with the addition of sentinel lymph node staging, compared to the gold standard at the time, axillary lymph node dissection, macrometastasis were detected at about the same rate, but micrometastasis, which we all felt at the time must be clinically significant, why in the world would chemotherapy be so helpful in these node negative patients, maybe some of that is because we are missing things.

DR. TAYLOR: You have about a minute, doctor. Thank you.

DR. WHITWORTH: I'm wrapping. This was something that made us think this was an advance. Let me show you the next slide. It looks amazingly like this slide, where we compare the detection of

macrometastasis with the new assay compared to frozen section intraoperatively, and the detection of micrometastasis, where you see a dramatic improvement. Next slide. Is that clinically important? Next slide. I'm sorry. These are old slides that I used to give with sentinel node staging lectures, courses.

We don't have a lot of information about whether these tiny metastasis are clinically important. In the old days when residents asked this question, they were told yes, you can do a whole lot more slices, and you'll find more metastasis, but it doesn't mean anything. Then two very large studies were reported with five year overall survival numbers, or eleven year overall survival numbers, and these represented 900 patients or so apiece. And what you see is that a more intensive evaluation of the lymph nodes after standard pathology says node negative, identifies a group of patients with a 10 to 15 percent decrease in survival, so we do believe that more intensive evaluation identifies patients at more risk.

DR. TAYLOR: We need to ask you to stop.

DR. WHITWORTH: That's it.

DR. TAYLOR: Thank you. The next speaker, please. Again, 10 minutes, if you would.

DR. BLUMENCRANTZ: Right. Peter

Blumencrantz, and I will have no slides and be quite brief.

DR. TAYLOR: Thank you.

DR. BLUMENCRANTZ: I'm not going to repeat a lot of things that have been stated, although I would agree with Dr. Whitworth's comments. I think there was a point made earlier by Dr. Allard about the reference of 14 percent relative to zero, and I have to come back to that, because it's not zero. There are a lot of things we do to patients, and I'm talking now more from the heart as a clinician. A lot of things we do for patients that we operate in less than a perfect zone. I don't know any screening tests we do that are 100 percent. And, in fact, we have most patients for something real simple for breast cancer, like mammograms, which have 85 percent sensitivity of picking up the cancer, and yet most patients walk out of the mammogram test and think if it's negative, I'm clean. They are not. We don't typically stress that.

Now when they come back to the office and see me, and they have cancer and say well, gee, my test didn't show it, then we first have to tell them, guess what, no perfection in any screening test we have. Doesn't matter if it's a colonoscopy, doesn't matter what it is. You have to accept that. It works

with pap smears and everything else you screen for, so the idea of somehow holding this number up, we're not truthful enough, probably, with patients. If you really wanted to drill down, none of us in clinical practice probably tell our patients in the ultimate detail about what the risks are, what they're about to undertake.

We cover things like - we skip metastis rate with mapping, but it's variable depending on the operator. You talk about a place with less experience, you talk - the surgeons here have a lot of experience, all of us have been in the trials. How about the surgeon who's just picking it up? They go take a course, they come back, and the credentialing people at their hospital, do they check out their ability to do mapping? Absolutely not, in most places. You take a course, if lucky, they actually take a course. And because they read about it or have seen it, they go and do it. Have they been benchmarked like we were in the trials, where we have to have a minimum of 85 percent success rate and a 5 percent miss rate? No measurement out in the community. So as you start to apply standards here as to what value there is in this test, I'd like you to just put that in that perspective of everything else

we do in medicine, and what standard are we holding this to?

The other issue is the business of IHC. I've heard it repeatedly stated that somehow this should be considered the MOL relative to IHC, and under the current AJCC Sixth Edition, that's the way it looks. It's an N0 with some modifier, suffix. The fact is that - and I'm an investigator, not the scientist, not the molecular biologist. My understanding going to this is that when that cutoff trial was designed, and you can have more expert technical people than me address this, I suppose, but this was designed with cutoffs to be conservative such that at the cutoff where the patients are declared positive by this test, that the absolute - and this is not a quantitative study as presented - but that that tumor burden in a node being analyzed by this assay would exceed anything we would normally consider an ITC. So to try to say that this test in a positive setting represents the equivalent of ITC, I'm not sure is fair to this analysis, so I would leave you to think about that, because I think we need to be considering this is something that's a tumor burden in excess of ITC.

DR. TAYLOR: Okay. Thank you. That

concludes the public comments. And at this point in the meeting, we'll hear final summary statements from both the FDA and from the sponsor. These are statements, they are not questions and answer sessions, and they're not interactive. And I would, again in the interest of closing the meeting on time, would ask that they keep them to 10 minutes. So the FDA would go first, so who is speaking for the FDA?

MR. ST. PIERRE: Actually, the FDA has no final statement. We think the discussion has been very productive, and very helpful.

DR. TAYLOR: Okay. So that rushes you folks. Are you ready for final statement, or do you need a moment or two?

DR. VARGO: Well, I guess --

DR. TAYLOR: Always ready.

DR. VARGO: Always ready, always ready, and we will also keep it brief. I would like to reiterate what Dr. Whitworth mentioned, which is, when you're counseling the patient, if you're planning on using the assay for intraoperative result, the counsel would have to be that the false positive rate is the 5.8 percent, the opposite, the one minus specificity. It's the entire population that has not yet been tested with the assay. That would be what the consult

would have to be, because you don't have an assay result yet on that patient.

Secondly, I'd like you to put it into perspective. The literature shows very compelling evidence that if you were able to, if it was practical, to do thorough histological testing every 50 microns throughout the node, that you will detect 10 percent or so, to 15 percent of clinically relevant metastasis, as by all the guidelines today, greater than .2 millimeters.

If you sat down to counsel a patient before they go in to have an axillary lymph node dissection, and you said those two things to them, I'm going to test your node as standard of care is with the histological methods, and the literature shows repeatedly that you have a 10 to 15 percent chance of having metastasis in the same node that we partially evaluated, and you're not going to get an axillary lymph node dissection that probably would have benefitted you based on outcome data, that also Dr. Whitworth mentioned, versus you also have a 5.8 percent chance that an assay that is new and doesn't have a lot of background yet to support the clinical meaningfulness of the 5.8 percent positivity that has not yet been supported by histological information or

outcome data, so you may undergo, you have a 5.8 percent chance of undergoing an axillary lymph node dissection that may or may not benefit you. Remembering that even if you have your nodes thoroughly tested, as Milan does, and the percent of false sentinel lymph node - able to predict what the axillary status is, even if you do thorough histological evaluation, and perhaps even thorough assay evaluation probably due to skipped metastasis or whatever, you still have a 5 percent chance - this was a large study done by the Italian group - of the remaining axillary nodes left in the patient of having metastasis. If you look at that percent done by the way the U.S. tests nodes, and they come up negative, you have about a 10 percent chance of still having axillary metastasis left in the body.

I think you have to carefully weigh the advantages of appropriately diagnosing the positives, and giving that patient a better chance of survival against the possibility that it's an unnecessary axillary lymph node dissection that may or may not be unnecessary. And it concerns me that that balance is not being looked at, because I think it would be a great disservice to the patients.

DR. TAYLOR: Okay. Thank you. I'd like

to ask Dr. Allard, as the industry representative, for any final comments.

DR. ALLARD: Thank you very much, Dr. Taylor. I do just have several, and I will keep them brief.

DR. TAYLOR: Please.

DR. ALLARD: First thing is, I'd like to congratulate the sponsor. I believe they have done an outstanding piece of work here, and I think it's on several levels. The first level is on bringing forth a test as a stand-alone device. And what many members here may not be aware of is, this is a very painful and agonizing decision that we make in industry. We often retreat into the use of our tests as adjunctive, and only to be used in conjunction with many other things, and in my mind, that demeans our industry, and I think that they've done a service to our industry by bringing this test forward as, in fact, a stand-alone device. I think that's a bold move that took some courage and corporate will in order to do that.

I think, also, the test is well constructed. We didn't talk today about the pre-clinical performance of the test, but I did look at that quite carefully, and I think the test has very good pre-clinical performance in terms of

reproducibility, accuracy, interfering substances, the kinds of testing that we would standardly do in our industry to qualify and validate, and verify a test of this type. So I think that they've done a very nice job here.

I was a little disappointed that they did not follow the Bayesian statistical plan that they outlined together with the FDA, but I want to be real clear, not because I think that flawed the study. I don't think it did. All it did, in my understanding, is to create a higher hurdle for them to cross, but I was disappointed only because the FDA has been very forward-looking, I think, in offering this to industry. It's something that I think can, in fact, shorten our time to market, and lessen our cost, and our burden, and I would love to have seen them to have pursued that as a paradigm for industry to follow in the future. Unfortunately, that wasn't done, but I don't think it decreases the value of the data.

And then lastly, I think the data, from my perspective - I'm a Ph.D. Researcher. I don't claim to treat patients - but certainly the data, from my perspective, I've looked at diagnostic tests now in oncology for 20 years, and I've been looking up and down sensitivity, specificity, and PVPPVs for many

years. The data here is very good. You don't see tests very often that have sensitivities of 88 percent, roughly, specificities around 92 percent. And I think it was demonstrated, in fact, most dramatically, by Dr. Pennello of FDA, who showed the ROC curve. And that was a very compelling ROC curve. I've been sliding up and down ROC curves for years, and we don't see curves like that, that are up in the 90s for area under the curve. That really is outstanding performance, and in my view, it provides compelling data. Now, again, as I said, I don't treat patients, so you may have a somewhat different view. But certainly, the data from a diagnostician researcher point of view is very strong for a test of this type, and I'm not saying these things because I benefit in some way. We're competitors, so take that for what it's worth.

Last thing, I'd just like to say, I do think that there are a couple of things that are very clear from this discussion, and I'm sure it will come out further, but I think that there are two things that are incumbent on the sponsor to clarify in their package labeling, and the first one is the volume of tissue that should be used, and that was talked about earlier. I think it cannot be left solely to the

discretion of the user, because if you used a 1 millimeter chunk of tissue, I don't think you could reproduce the data that was derived here in the trial. But what does that mean? Is it a 2 cubic millimeter, 3, where is it? I think guidance needs to be given. And it may be necessary to require, at least in the short term, that not all tissue be consumed in the process of running this test, so that there is tissue remaining for H&E staining. But I don't think that, in any way, is incompatible from what I understand with the use of the test and the way the sponsor is presenting the test to be used in the laboratory. So I think both are doable. I think they just need to be clarified in the package labeling. So those are my comments. Thank you very much.

DR. TAYLOR: Okay. Thank you. Ms. London, consumer representative, do you have any further comments?

MS. LONDON: Well, I would just like to go along with what you said a minute ago. And as a woman, and as the consumer rep, I feel very reassured to be in this room with such dedicated people, the sponsor, the FDA, and the members of the panel in doing this work. And I know that many, many people will benefit from it in the future.

Having said that, the patient is the bottom line, and I hope that there will be for the surgeons and the other healthcare professionals, grassroots informational materials to help this very, very scary and difficult situation be explained to patients so that they can make decisions with their partners, their physicians.

DR. TAYLOR: Okay. Thank you. This brings us to the point where the panel is ready to vote on the recommendation to the FDA for this pre-market approval. For those in the room not familiar with the process, the industry representative and the consumer representative do not vote, and I, as Chair, only vote if the others can't decide by themselves; that is, if there's a tie. So Ms. Carlos will now read the panel recommendation options; that is, the options that the panel has for pre-market approval application. Ms. Carlos.

MS. CARLOS: Thank you. "The medical device amendments to the Federal Food, Drug, and Cosmetic Act, as amended by the Safe Medical Devices Act of 1990, allows the Food and Drug Administration to obtain a recommendation from an expert advisory panel on designated medical device pre-market approval applications that are filed with the agency. The PMA

must stand on its own merits, and your recommendation must be supported by safety and effectiveness data in the application, or by applicable publicly available information.

The definitions of safety, effectiveness, and valid scientific evidence are as follows. Safety, under 21 CFR 860.7(d)1, there is reasonable assurance that the device is safe when it can be determined based upon valid scientific evidence that the probable benefits to health from use of the device for its intended uses and conditions of use, when accompanied by adequate directions and warnings against unsafe use outweigh any probable risks.

Effectiveness under 21 CFR 860.7, there is reasonable assurance that the device is effective when it can be determined based upon valid scientific evidence that in a significant portion of the target population the use of the device for its intended uses and conditions of use, when accompanied by adequate directions for use and warnings against unsafe use will provide clinically significant results.

Valid scientific evidence under 21 CFR 860.7(c)2 - valid scientific evidence is evidence from well-controlled investigations, partially controlled studies, studies and objective trials without match

controls, well-documented case histories conducted by qualified experts, and reports of significant experience with marketed device from which it can fairly and responsibly be concluded by qualified experts that there is reasonable assurance of the safety and effectiveness of a device under its conditions of use.

Isolated case reports, random experience, reports lacking sufficient details to permit scientific evaluation and substantiated opinions are not regarded as valid scientific evidence to show safety or effectiveness.

Your recommendation options for the vote are as follows. Approval if there are no conditions attached, approval with conditions. The panel may recommend that the PMA be found approvable subject to specified conditions, such as physician or patient education, labeling changes, or further analysis of existing data. Prior to voting, all of the conditions should be discussed by the panel. Not approvable - the panel may recommend that the PMA is not approvable if the data do not provide a reasonable assurance that the device is safe, or the data do not provide reasonable assurance that the device is effective under the conditions of use prescribed, recommended,

or suggested in the proposed labeling. If the vote is for not-approvable, the panel should indicate what steps the sponsor may take to make the device approvable."

DR. TAYLOR: So are there any questions from members of the panel as to these voting options before we proceed to asking for a main motion? Anybody from the panel have questions about the options available? Okay.

In that instance, then, I would ask the panel is there a main motion that would recommend either approval, approval with conditions, or not approvable for this PMA? Dr. Gollin.

DR. GOLLIN: I move that the test be approvable with conditions.

DR. TAYLOR: Is there a second for the motion?

DR. THOMAS: Second.

DR. TAYLOR: We have two seconds, both Dr. Gulley and Dr. Thomas. We have three seconds. All right. In this instance with this particular motion, before we vote on the motion, it's necessary to consider what the conditions for approval might be. So, therefore, I will now entertain a motion for the first condition of approvability. Is there a motion

for what the first condition might be? Dr. Siegel.

DR. SIEGEL: I would move for a condition for a post approval requirement to test other tumor types in lymph nodes because of the evidence presented that lymphoma gives, if you will, false positive data. So the question is what other tumor types, and other pathologic conditions in lymph nodes would also invalidate the test?

DR. TAYLOR: Does anyone wish to second that motion?

DR. THOMAS: Second.

DR. TAYLOR: We have a second from Dr. Thomas. Any discussion of the motion for this condition by the panel?

DR. ERNSTOFF: Is there a statistical sample size calculation that you would want to see?

DR. SIEGEL: Yes, I'll accept that as a friendly amendment, and ask the statisticians to provide that.

DR. TAYLOR: Okay. Is that friendly? Right. So we have an amendment condition, that there be a post market analysis as to whether or not lymphoma or other tumors interfere with the reliability of this assay, and there would be statistical advice as to the number of specimens that

need to be analyzed. Does that summarize where we are? Anybody else, comments, discussion?

DR. GOLLIN: And once that information is available, that the labeling should be amended to that effect. Can that happen, FDA, that the labeling can be amended once that study is complete?

MR. ST. PIERRE: Yes, that can happen.

DR. SIEGEL: I accept that.

DR. TAYLOR: I hope somebody is writing this down. It's beyond my recall here. Okay. Any other discussion? We have now a condition that requires for a post market study, the effect of other tumors on the reliability of the assay, statistical input, and amendment of the labeling. Dr. Robinowitz, did you have --

DR. ROBINOWITZ: I just wanted to see if I could take notes of all this.

DR. TAYLOR: Okay. That's okay, we're fine. So we're in position to vote on this first condition? So I'm going to --

PARTICIPANT: We vote each condition, and then we have to go back once we've got the conditions for approval. There may be other conditions before we vote.

DR. TAYLOR: So I'm going to ask each of

you in turn to indicate aye or nay, so Dr. Whorton.

DR. WHORTON: I vote affirmative.

DR. TAYLOR: Favor.

DR. ERNSTOFF: Affirmative.

DR. LICHTOR: Affirmative.

DR. TAYLOR: Dr. Thomas.

DR. THOMAS: Affirmative.

DR. GULLEY: Affirmative.

DR. TAYLOR: Dr. Gollin.

DR. GOLLIN: Affirmative.

DR. TAYLOR: Dr. Netto.

DR. NETTO: Affirmative.

DR. TAYLOR: Dr. Siegel.

DR. SIEGEL: Affirmative.

DR. TAYLOR: Dr. Kemeny.

DR. KEMENY: Aye.

DR. TAYLOR: Dr. Begg.

DR. BEGG: Affirmative.

DR. TAYLOR: Dr. Leitch.

DR. LEITCH: Yes.

DR. TAYLOR: So the panel is unanimous with regard to the first condition. Is there any motion for any additional condition? Dr. Gollin.

DR. GOLLIN: I move that it needs to be stated in the labeling that the test should be carried

out on a segmented lymph node, and how it should be segmented can be discussed between the company and the FDA, to be followed with permanent histopathology sections.

DR. TAYLOR: Is there a second to that condition?

DR. GULLEY: Second.

DR. TAYLOR: Second from Dr. Gulley. Any discussion from the panel of this second condition? Yes, Dr. Leitch.

DR. LEITCH: Well, I - and this may be - I don't know if this is a different one or just a tag-on to that, but ultimately, if this test really works as it's said to by the sponsors, where it could replace, I think the way to validate that would be outcome from a number of studies that continue to evaluate the node versus the H&E, and outcomes from that, if they want to take it to the level of being where the whole node is used for the assay, which may be the right thing to do, ultimately. I think what we're facing right now is the level of comfort of losing the architecture is not there, but if what the sponsor says is true about the ability to detect something that might be clinically important, there needs to be followup of that, too.

DR. TAYLOR: So Dr. Gollin, as proposer for this condition, do you have comment regarding Dr. Leitch?

DR. GOLLIN: Yes, I do. I was going to propose long-term outcome studies as a separate condition.

DR. TAYLOR: As a separate condition. Okay. So condition number two, then, relates to the recommendation in the labeling that the assay be performed on a segmented lymph node, such that there is residual lymph node that would be submitted to H&E permanent section evaluation.

DR. ERNSTOFF: Can I just clarify? We heard discussion about the size of the lymph node, and how it would be sectioned, et cetera, et cetera, so I think there are some details within that amendment that really needs to be worked out between the sponsor and the FDA.

DR. TAYLOR: How do we work that into a condition? Can somebody from the FDA give me advice on that? Don.

MR. ST. PIERRE: Yes. If you don't have the details, you can just say that'll be worked out between FDA and the company.

DR. TAYLOR: Okay, then let's do that. It

would take us a long time to work that one out. So we have a second condition, essentially that the node, should it be sufficiently large, would be segmented, and part of it reserved for permanent H&E evaluation. Any discussion? George.

DR. NETTO: I think part of the clarification on the details of what to section, and how much to put for PCR versus needs to take at least for the time being - that if tissue is not enough for both, that probably tissue for permanent should be that one that takes precedence over tissue for PCR.

DR. TAYLOR: Okay.

DR. NETTO: Until we accumulate enough data, and know that on the long-term it's really more powerful detecting clinically significant cases.

DR. ERNSTOFF: And, once again, if we're going to ask them to do that, some statistical analysis - I mean, is this going to go on forever, or do they have another four or five hundred cases that --

DR. TAYLOR: Well, that's probably condition three, so we're going to come back to that one. We're going to try and keep this one simple and pure, if we possibly can, and I'll rephrase it. Obviously, we have this being recorded, but the issue

would be that the assay be performed on a segmented lymph node, that there be sufficient remaining tissue for H&E permanent, and that the assay be compared to the evaluation, and that would be a subsequent condition. So do we have - are we in position to vote on condition two? So we'll go in the same process as before, please indicate individually. Dr. Whorton.

DR. WHORTON: Affirmative.

DR. ERNSTOFF: Affirmative.

DR. TAYLOR: Dr. Ernstoff.

DR. ERNSTOFF: Affirmative.

DR. TAYLOR: Dr. Lichtor.

DR. LICHTOR: Affirmative.

DR. TAYLOR: Dr. Thomas.

DR. THOMAS: Affirmative.

DR. TAYLOR: Dr. Gulley.

DR. GULLEY: Affirmative.

DR. TAYLOR: Dr. Gollin.

DR. GOLLIN: Affirmative.

DR. TAYLOR: Dr. Netto.

DR. NETTO: Affirmative.

DR. TAYLOR: Dr. Siegel. Voted with his feet. Kemeny.

DR. KEMENY: Affirmative.

DR. TAYLOR: Begg.

DR. NETTO: Affirmative.

DR. TAYLOR: Leitch.

DR. LEITCH: Yes.

DR. TAYLOR: Thank you. There is still a quorum, by the way, as far as the panel is concerned. Okay, so that's two conditions. I understand there's a third condition. Do we have a motion, Dr. Gollin, again? Number three.

DR. GOLLIN: I move that user training and more detailed precautions against PCR contamination in the operating room, and in the pathology lab, be specified in the labeling.

DR. TAYLOR: Is there a second?

DR. ERNSTOFF: Second.

DR. TAYLOR: Is there any further discussion of that condition? Absent discussion, we'll vote as previously. Dr. Whorton.

DR. WHORTON: Affirmative.

DR. ERNSTOFF: Affirmative.

DR. LICHTOR: Affirmative.

DR. THOMAS: Affirmative.

DR. GULLEY: Affirmative.

DR. GOLLIN: Affirmative.

DR. NETTO: Yes.

DR. KEMENY: Yes.

DR. BEGG: Yes.

DR. LEITCH: Yes.

DR. TAYLOR: Again, we have a unanimous vote for condition three. Do we have other conditions from the panel? Dr. Ernstoff.

DR. ERNSTOFF: Yes. I would like to see some followup data collected both in relationship to how patients are treated with this information, and what their participation in adjuvant therapies and potentially how there would be interaction with participation in clinical trials, so there's a number of subsets to that followup, but some sort of followup of how patients are cared for.

DR. TAYLOR: Is there a second?

DR. LEITCH: I'll second.

DR. TAYLOR: So the issue here is whether you would accept some sort of amendment that the FDA would work out with the company as to what that condition should be in detail.

DR. ERNSTOFF: Yes. I think we need to find out how these women are doing, lacking any other data. I think that's an important component.

DR. TAYLOR: Dr. Gulley.

DR. GULLEY: So some kind of clinical outcomes.

DR. ERNSTOFF: A database that gives clinical outcomes, 50 percent of patients went on to radiation, 50 percent had chemotherapy, whether potential interactions with participation in clinical trials. That's going to be a difficult situation because the clinical trials are going to have to adapt to this new technology.

DR. TAYLOR: Dr. Leitch.

DR. LEITCH: Well, in this followup study, I guess amendment to that, or whatever, I would like to see the outcomes of axillary dissection from the nodes that are judged intraoperatively to be positive by the assay. What is the outcome of the completion axillary dissection to see kind of what the validation is for that, and that would help to answer the question and concern of "whether the person needs an axillary dissection."

DR. TAYLOR: I'm going to ask the FDA how much detail they want from the panel as to what this followup study should look like. Just hold on a second while we - I see you.

MR. ST. PIERRE: Actually, you don't have to put -- as long as you discuss them, you don't have to put it all in the recommendation, as long as your recommendation generally cover the specifics. And

we'll get the transcripts.

DR. TAYLOR: That's fine. And then you work it out with the sponsor?

MR. ST. PIERRE: Yes.

DR. TAYLOR: Thank you. Dr. Gollin.

DR. GOLLIN: I would like to recommend that a long-term followup study of the assay positive histology negative cases should be included in that study.

DR. TAYLOR: This seems to me it's part of the same condition for a followup study. No, I can't invite the sponsors to speak at this time. I'm sorry.

DR. NETTO: Just a question, it's a question about the process. So here we are suggesting conditions that do not cover existing data. I thought that's not part of --

DR. TAYLOR: Well, this is why I'm asking the FDA and the sponsors that they would work out a followup study.

DR. NETTO: So tying this in with a long-term followup study, basically, it's not part - it's like asking also for a frozen section study, for example.

DR. TAYLOR: Correct.

DR. NETTO: That's not part of the

existing study.

DR. TAYLOR: I agree.

DR. NETTO: So the condition here, it's really a different study, so that would take it away from approval with condition to not approval, if I understood correctly.

DR. TAYLOR: Could we have a read on the FDA? My understanding is that it's possible to ask that it be approved, and that the company collects data on performance subsequently to approval. Is that correct?

MR. ST. PIERRE: Yes, you can do that. The important part is that your recommendation of approvable is based on the data that you have in-hand, so you have sufficient data on-hand to say that the product is reasonably safe and effective. And then, so the conditions you're putting on are recommendations to the FDA to consider the other things that would help clarify the performance of the test.

DR. ERNSTOFF: I think to clarify my amendment, maybe, or my condition was, it wasn't a study as we were talking about, but a database that we're asking the company to collect to assure that the performance of the test is as -- hasn't shifted from

what we have been presented from today.

DR. TAYLOR: Yes. And to continue to monitor performance of the test against - permanence is what you're really asking for. I think the discussion that we've had reflects that. We're just all asking for slightly different things to be included in that, and under the context of the FDA process, they would need to work out with the sponsor as to what those conditions - what the condition would be in terms of detail. I don't think we're in a position to set that. Is that reasonable? I'm sorry, you missed part of that discussion.

MR. ST. PIERRE: Yes, I'm sorry.

DR. TAYLOR: Yes. I'll just rephrase it. The condition that we have now is condition number four, where there would be a post market database developed and maintained by the company that would be able to answer issues as to performance of the assay, both in terms of outcome, and in relationship to permanent sections. And that the FDA would work with the company to determine what that database should look like. Does that summarize it for the panel? Is that fair? And we don't specify what's in that database. Not our job.

DR. GOLLIN: I would actually like to see

a followup study that would then be used to amend labeling based on the data, amend labeling in terms of educating the patients and the users to the information that results from the collection of data in the database.

DR. TAYLOR: Well, if we get into a followup study, then we're getting beyond the point where we're able to approve the test, so that's slightly different. Is that correct, Don? Sorry, we're pausing for a second.

MR. ST. PIERRE: Yes. I guess if you're asking for a new study with new data, then that can't come under.

DR. TAYLOR: Correct.

DR. GOLLIN: How about having a database? I withdraw my request for a study.

DR. TAYLOR: That's what condition four was, was a database. We have a motion on the table. We're still discussing it. It is a different one, we're discussing motion four. It's been withdrawn. Okay. George.

DR. NETTO: So if it's not a study, if it's a collection of database, and you already approve the test, and the database find that the 14 percent that we talked about is 14 percent, and it's not

significant for the patient care, what can the FDA do in two or three years? Is somebody going to visit that database, re-analyze that database, is the company obliged to show us in three years that, indeed, those 14 percent, you know what, we're telling you that they're really truly positive, turn out they're really truly positive, turn out that this is a better test, turn out that these patients did bad and here's the data. I think then the test will be flying colors, but if in three years the data shows that this 14 percent are indeed false positive, you can think of a lot of ways for false positive. It can be contamination of any cells, it can other tumor, like they were suggesting, so the issue is just having a database, I think some of the panelists may be mistaking that condition as a green light into approval, and what's going to happen is, you collect that database, but you cannot retract later on, and go back and say this test - you already approved it as a adjunct to frozen section, so that's my fear. That's why I started saying maybe you're suggesting a study, and if you're suggesting a second study, then you're not really approving the test.

DR. TAYLOR: Well, the approval that we're discussing with conditions relates to it being an

adjunct to permanent sections, not an adjunct to frozen sections.

PARTICIPANT: Can I ask a question? The first condition we've already voted on sounds like another study to me, as well.

DR. TAYLOR: First condition?

PARTICIPANT: Testing lymph nodes in other diseases.

DR. TAYLOR: Well, it's maintaining a database, it's pulling that together. So, again, we need some advice as to where we go with further conditions. The condition that we're dealing with now is really talking about an extensive brand new study.

PARTICIPANT: Dr. Whorton has a comment.

DR. TAYLOR: Dr. Whorton, I'm sorry.

DR. WHORTON: Point of clarification - are we talking about things that may be conditions for the pre-market approval, or may be post marketing studies? I'm not totally clear, but it seems like worth --

DR. TAYLOR: Yes, I think there's confusion at this point. We have a pre-market approval with conditions, and one of the conditions that's come up is that there should be some post market studies, so the condition is that we will approve it, if there is agreement that post market

studies occur. The question is, what are those studies, how extensive are they, and do they - can you then go back and withdraw the approval? You can't. Yes.

MS. SHOAIBI: My name is Azadeh Shoaibi, and I'm part of the review team and epidemiologist with the FDA.

DR. TAYLOR: Yes.

MS. SHOAIBI: What I would like to draw your attention to is that today we are gathered here to look at this device, and evaluate this device based on the current data for the safety and effectiveness of the device. What ever data we decide to collect afterwards, it would be additional data, but we are here to evaluate this data, what is this data telling us, whether this device is safe and effective? And FDA does not normally recommend that the panel or any other evaluation will be based on the data that will be collected, and looked at later on. That could be additional data that would add to future evaluations, but I would like to draw your attention that we are here to look at the data as it stands right now. And all of the conditional approval studies that you are recommending or considering, these are additional data that may or may not add to whatever is available to

you today. So there are a number of issues related to safety and effectiveness that would not necessarily be appropriate to look at after the device has been approved, so I just ask that you look at this data as it stands today, and make your decision based on this data, and not look for future data to either confirm your decision today, or reject it.

DR. TAYLOR: So then we'll ask the panelists to vote with your comments in mind. That's fine. You're still on condition four here?

DR. NETTO: No. I'm still trying to understand the process here of the condition. So if we have conditions, because all these conditions, if you read through them, really because we do have some concerns about the existing data, the 14 percent or what have you, that affect safety and effectiveness of this test. And I think some of the panelists are thinking by collecting this data, yes, we will confirm it, but it's already too late then, that we assume that what you're proposing is correct, but we don't know that, because the studies have not been done, the outcome studies, the correlation to permanent studies on a wider base, so to go - if you approve it now, you already approved it, and then this data will be considered for another approval, maybe for totally

different thing, but it's not going to retract. That's my understanding.

DR. TAYLOR: Well, one condition that we went over, that it be performed as an adjunctive test on part of the lymph node tissue that's available in conjunction with permanent sections, that's not a study, that's a condition of use of the test.

DR. NETTO: And that's the only one of the conditions.

DR. TAYLOR: Correct.

DR. NETTO: And maybe there --

PARTICIPANT: PCR training, yes.

DR. TAYLOR: And user training is a second one, so there's --

DR. NETTO: Long outcome data looking at whether false positivity is due to other tumors, looking at where there contamination issues, so these are things that you need to know now, if you're going to approve, before you approve, not after you approve, so that's the problem I'm having.

DR. TAYLOR: Yes, that's a legitimate concern. So I think as the Chair of the panel, we need to revisit the conditions that we wish to attach. We have on the floor a proposal that this is approvable with conditions, and we've had discussion

of the conditions. We have gotten up to four conditions, two of those conditions, number one and number four, are more in the nature of post market data banks or studies, and the other two were conditions that were not dependent on further studies. They were, in fact, really related on to how the test is performed, so does the panel wish to go back and review those four conditions? I think we should go back and re-vote those three conditions. Is that agreeable?

DR. GOLLIN: What about four?

DR. TAYLOR: We haven't voted on four. So at this point, let's go back. We have number four still as an open item. We need to close that item, so let's go and vote on condition four with the discussion that you just heard in mind.

DR. THOMAS: Do we -- what is number four?

DR. TAYLOR: Number four is a long-term database that they would need to collect.

DR. NETTO: On outcome?

DR. TAYLOR: To be determined by the sponsor and FDA. So, Dr. Whorton, in favor or not?

DR. WHORTON: Does that mean they have to have the database before, or is it they begin the database --

DR. TAYLOR: This is post market approval. Post market.

DR. WHORTON: Post market.

DR. TAYLOR: Yes.

DR. WHORTON: Affirmative.

DR. ERNSTOFF: Affirmative.

DR. LICHTOR: Affirmative.

DR. THOMAS: Affirmative.

DR. GULLEY: Yes.

DR. GOLLIN: Yes.

DR. NETTO: Yes.

DR. KEMENY: Yes.

DR. BEGG: Yes.

DR. LEITCH: Yes.

DR. TAYLOR: So we now have four conditions attached. Are there additional conditions? Dr. Leitch.

DR. LEITCH: I guess the labeling, I suppose this would be, or perhaps education, but for its written down somewhere. I think the explication of the false positive rate needs to be very clear, both to patients and to physicians who would be using the test. And the arguments can be presented, as they've been presented here, that maybe it's detecting something that's below the level of other tests, and

that may be a true positive, even though it's called a false positive. But, again, this deal with the 4 percent and the 14 percent, that is the discussion that you have to have with the patient, and I think that needs to be there. And I think for people who use intraoperative evaluation of the sentinel node to make a decision, this test may be helpful to them, compared to using frozen sections. But I think they need to be prepared to tell their patients --

DR. TAYLOR: Well, we need a one-line condition.

DR. LEITCH: That there is clear information in the labeling about the false positive rate.

DR. TAYLOR: Is there a second?

DR. NETTO: Second.

DR. TAYLOR: Okay. So we have a one-line condition, that is clear information regarding the false positive rate. It's been seconded. Is there any further discussion?

DR. WHORTON: Yes.

DR. TAYLOR: Yes.

DR. WHORTON: It goes back to slide two, and that's where the 14 percent was. That was associated, as the sponsor set forth the confidence

interval, so that 14 percent is only a point estimator of those false positives. It can go as low as maybe 2 percent, or as high as maybe 30 percent. And in the spirit of reliability, I think the confidence interval issue needs to be considered at the same time you're talking about the false positive, 14 percent is not a fixed number.

DR. TAYLOR: So do you have an amendment to the condition?

DR. WHORTON: I'd like to - that in the reliability discussion, either marketing or otherwise, that the unreliability issue at least be clarified in the statements of false positive.

DR. NETTO: Lower and upper confidence.

DR. TAYLOR: So, again, we have to keep this reasonably concise as a condition. We've got a condition that states that the false positive issue needs to be stated in the label. You want to have an upper and lower confidence limit.

DR. WHORTON: Plus and minus the margin of error.

DR. TAYLOR: Placed in there. Okay. That's another half-line. That's fine. Dr. Gulley.

DR. GULLEY: Are we talking about the false positive rate, or the positive predictive value?

DR. WHORTON: I use the 14, because that's the one that clinically they continue to talk about. Whatever that is. Whatever it is, it's margin of error that needs to be at least kept in mind.

DR. GULLEY: Okay. I just want to make sure what we're talking about.

DR. TAYLOR: Okay. So with that context, are we able to vote on condition four? Five. I'm going to have a change and start with Dr. Leitch this time.

DR. LEITCH: Yes.

DR. BEGG: Yes.

DR. KEMENY: Yes.

DR. NETTO: Yes.

DR. GOLLIN: Yes.

DR. GULLEY: Yes.

DR. TAYLOR: Dr. Gulley says yes. Technical problem. Next. Dr. Thomas.

DR. THOMAS: Yes.

DR. LICHTOR: Yes.

DR. ERNSTOFF: Yes.

DR. WHORTON: Yes.

DR. TAYLOR: Okay. So that also is approved. We have condition - any further conditions? I'm sorry, do you have another condition? Okay, Dr.

Thomas.

DR. THOMAS: My microphone is not working, but I think there was some extensive discussion about clarifying that this was a stand-alone to replace the frozen section, and that wasn't clear in the labeling. And we discussed maybe making that clear, that the stand-alone was to replace the frozen section, but not the permanent.

DR. TAYLOR: There was an earlier condition that dealt with that issue.

DR. THOMAS: I don't think so.

DR. TAYLOR: Condition number two, basically said that you segmented the lymph nodal tissue, and you retained sufficient tissue to do permanents.

DR. THOMAS: Okay. I guess the language about it standing alone, I think that still could be interpreted, or still be confusing. You don't think so?

DR. TAYLOR: Does anybody else on the panel wish to second that?

DR. NETTO: I think it needs to explicitly say that it's not intended to replace frozen section. I mean, if you don't do frozen section, and you wanted to use this as stand-alone, then --

DR. TAYLOR: So do you want to second Dr. Thomas' --

DR. NETTO: Yes.

DR. TAYLOR: Hold on.

DR. NETTO: We already --

DR. TAYLOR: We need to have a motion on the floor, which we can then discuss, or at least a condition on the floor. So your condition was a statement to the effect of what?

DR. KEMENY: Isn't that a modification of the number two --

DR. TAYLOR: Well, it could be. Let's just see what she says.

DR. THOMAS: I think mine is different from Dr. Netto's. I think I was saying that it should not - that there should be explicit language saying what the stand-alone part meant, was that it was to replace or not to replace permanent section, but could replace frozen section.

DR. TAYLOR: Is there a second?

DR. GULLEY: Second.

DR. TAYLOR: Okay. So your feeling is that that's distinct from number two?

DR. GULLEY: I think that could be added into number two. I don't know how we do that.

DR. TAYLOR: Well, I don't think we go back to number two, because number two is done. You can vote on this as an addition to number two, subsequently.

DR. WHORTON: But it seemed like number two is to collect the samples, but you may not use them. I think she's saying it should not be --

DR. TAYLOR: Okay. Any further discussion? Dr. Leitch, your vote.

DR. KEMENY: Can you restate it?

DR. TAYLOR: Would you restate it, Dr. Thomas?

DR. THOMAS: I'll try.

DR. KEMENY: Is this a separation condition?

DR. TAYLOR: This is condition six. It's a separate condition.

DR. THOMAS: Just make it clear somewhere in the labeling that it should not replace permanent sections, but may replace frozen sections as a stand-alone.

DR. TAYLOR: And we had a second. Does the second still stand? Okay. Second from Dr. Gulley. Dr. Leitch.

DR. LEITCH: Yes.

DR. BEGG: Yes.

DR. KEMENY: Yes.

DR. NETTO: No.

DR. GOLLIN: Yes.

DR. TAYLOR: No from Dr. Netto.

DR. GULLEY: Yes.

DR. THOMAS: Yes.

DR. LICHTOR: Yes.

DR. ERNSTOFF: Yes.

DR. WHORTON: Yes.

DR. TAYLOR: So we had one dissenting vote, remainder in favor. Okay. We're up to condition seven, if we get that far. Does anybody else have other conditions they wish to add before we vote on the primary motion? Okay. So I'm going to call for a vote on the primary motion, which was approval with the six conditions that we've just been through. I certainly can't recite them verbatim at this point, so I don't intend to try, but I will go around the panel, and I will ask you to vote. And then I'll come back to each of you and ask you to make a statement about the reason that you voted the way you voted. So, again, we'll start with Dr. Whorton this time.

DR. WHORTON: Affirmative.

DR. TAYLOR: Affirmative.

DR. ERNSTOFF: Affirmative.

DR. TAYLOR: Affirmative.

DR. LICHTOR: Affirmative.

DR. TAYLOR: Affirmative. Dr. Thomas.

DR. THOMAS: Affirmative.

DR. TAYLOR: Affirmative, Dr. Thomas.

DR. GULLEY: Affirmative.

DR. TAYLOR: Dr. Gulley, affirmative.

DR. GOLLIN: Affirmative.

DR. TAYLOR: Dr. Netto.

DR. NETTO: No.

DR. TAYLOR: Dr. Netto, negative.

DR. KEMENY: Affirmative.

DR. TAYLOR: Affirmative, Dr. Kemeny.

DR. BEGG: Affirmative.

DR. TAYLOR: Dr. Begg, affirmative.

DR. LEITCH: Yes.

DR. TAYLOR: Dr. Leitch, affirmative. So we have one dissenting vote, and the remainder in favor, and the motion carries, along with the six conditions that were described. Now it's usual to ask each panel member just to make a comment as to why they voted the way they voted, so Dr. Whorton.

DR. WHORTON: I agree with the industrial rep. I think it's a time that things like this begin

to move forward, and I think the study, as bad as I tried to pick on it, it was fairly well done, and the results are at least clear in so far as the panel discussed. I think the conditions are prudent, and I think time will bear us out that that was, in fact, prudent. I think the motion that she made was proper, to leave the gold standard in place for the time being, and to preserve the sample, so I think, at this point in time, it's a well --

DR. TAYLOR: Thank you. Dr. Ernstoff.

DR. ERNSTOFF: Yes. I would also like to commend the industry for doing the study. I think it was very well designed. I think the data was excellent. I think that we're in a transition time in history, and what you're hearing I think from the panel is yes, let's proceed forward, but cautiously.

DR. TAYLOR: Thank you. Dr. Lichtor.

DR. LICHTOR: I just want to say that I think this PCR based lymph node analysis does appear to add some additional information to that obtained from routine pathologic analysis, particularly in addressing the sampling errors inherent in frozen section or permanent section analysis.

DR. TAYLOR: Dr. Thomas.

DR. THOMAS: I guess while I don't find

evaluating sentinel lymph nodes that burdensome, I think any test that might add information should be brought to market, and so I voted in favor, because I thought it would --

DR. TAYLOR: Dr. Gulley.

DR. GULLEY: I want to congratulate Veridex for doing a nice study, and for meeting both of its endpoints for the specificity and sensitivity. I think that I agree that we should do this in a step-wise fashion, and by adding in the condition of using the fixed permanent embedded sections, that will help to gain more data, and we can go on from here.

DR. TAYLOR: Dr. Gollin.

DR. GOLLIN: A test that identifies metastatic tumor cells in lymph nodes or in the peripheral circulation is extremely exciting to me, and as a member of a breast cancer family, I feel that it's really important to the population, and to the public health. And I think approaching this in a prudent fashion is appropriate.

DR. TAYLOR: Dr. Netto.

DR. NETTO: By voting no, I don't mean to say that the study is not an excellent study, and that I'm not excited about the technology. It's really the conditions. If I know all these conditions are going

to be taken care of, and it's going to pan out the way we think these conditions are going to pan out, then definitely my vote would be yes. My problem is that we already pre-market approval, and so these conditions are afterwards, how much really strength the FDA will have after that, if they show the unlikely event that it's the other way around, that it's similar to the IHC, then what happened with this test? Of course, I feel much better that it is with the conditions, and especially the fact that you're going to keep it parallel to permanent section.

DR. TAYLOR: Dr. Kemeny.

DR. KEMENY: I think it seems like an extremely good product, and the only thing that I was worried about is being taken care of in the conditions, that the false positives are clearly shown, and clearly explained to the patient, so nobody -- so everybody knows what's going on.

DR. TAYLOR: Dr. Begg.

DR. BEGG: Yes, I think that the -- I have some reservations here in terms of a lack of clarity about exactly the circumstances in which this test would be used, and also, some of the opinions of the panel members, skepticism about the logistical issues about applying this in clinic. Despite all of that, I

think the results are sufficiently encouraging to approve it at this time.

DR. TAYLOR: And Dr. Leitch.

DR. LEITCH: I also think that it's a test that does get us into modern examination of the sentinel node, which I think all of us who have done sentinel node technology would like to see as an important prognosticator for the patients. Obviously, what we're approving is not really that, we're just approving that utilization as a replacement for frozen section for intraoperative analysis of the lymph node, but my hope in the discussion about the conditions would be that the company would followup on some of these issues, so that we - let's say maybe the patient doesn't need to have an axillary dissection, even if they are positive for the assay, but yet, that may provide prognostic information about the patient that would be important for them. So I think it offers a chance to help laboratories handle intraoperative evaluation, and so for that reason, I'm allowing it to be approved, and hope that physicians will inform their patients properly about it.

DR. TAYLOR: So thank you. You've heard from the panel members as to why they voted the way they did. From my perspective as Chair, it was not

needful for me to vote, but I would like to thank the company for what I thought was an excellent presentation. I think the only concerns that I had have been well expressed. They were really how the test would be used. I think anatomic pathology does need to step forward and get into an area where tests can be made reproducible and standardizable, and essentially, sometimes even taken away from the microscope. It's a painful thing to say, but it's going to happen.

I think replacing an H&E permanent with a test where the data is sort of limited to 421 patients is perhaps not wise, which is why I think the conditions came out the way they did. I look forward to seeing the test in use, and hopefully the new data will emerge, and we'll see where we are. So thank you everybody, thank you all for attending, and we do have a closing comment from Ms. Carlos.

MS. CARLOS: I just want to remind the panel to leave all the materials on the table, and we'll take care of them.

DR. TAYLOR: Thank you. The meeting is adjourned. Thank you.

(Whereupon, the proceedings went off the record at 4:08 p.m.)