U.S. FOOD AND DRUG ADMINISTRATION

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CLINICAL CHEMISTRY AND CLINICAL TOXICOLOGY

DEVICES PANEL

OF THE

MEDICAL DEVICES ADVISORY COMMITTEE

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MEETING

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WEDNESDAY,

DECEMBER 6, 2006

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The meeting convened at 8:00 a.m. at the Holiday Inn Gaithersburg, Two Montgomery Village Avenue, Gaithersburg, Maryland, Bernard W. Steele, M.D., Chairperson, presiding.

PRESENT:

BERNARD W. STEELE, M.D. Chairperson

CINDY L. GRINES, M.D.Consultant

ANN M. GRONOWSKI, Ph.D. Voting Member

STANLEY S. LEVINSON, Ph.D. Consultant

MURRAY H. LOEW, Ph.D. Consumer Representative

SANTICA M. MARCOVINA, Ph.D. Consultant

ALAN T. REMALEY, M.D., Ph.D. Voting Member

ROBERT D. SHAMBUREK, M.D. Consultant

MICHAEL Y. TSAI, Ph.D. Consultant

KAROL E. WATSON, M.D., Ph.D. Consultant

WILLIAM E. WINTER, M.D. Consultant

THOMAS E. WORTHY, Ph.D. Industry Representative

RUIWEN ZHANG, M.D., Ph.D. Voting Member

FDA PARTICIPANTS:

VERONICA J. CALVIN, M.A. Executive Secretary

ALBERTO GUTIERREZ, Ph.D. Director, Division of Chemistry and Toxicology

FDA PARTICIPANTS (continued):

STEVE GUTMAN, M.D., M.B.A. Director, Office of In Vitro Diagnostics

CAROL C. BENSON, MT(ASCP), M.A. Associate Director for Chemistry

COURTNEY D. HARPER, Ph.D. Associate Director for Toxicology

DOUGLAS WOOD, MT(ASCP) MCSE Division of Chemistry and Toxicology

GUEST PRESENTER:

PARVIN P. WAYMACK, Ph.D. Research Chemist, Centers for Disease Control and Prevention

PUBLIC SPEAKERS:

RUSSELL G. WARNICK	Berkeley HeartLab, Inc.
KENNETH FRENCH	Atherotech, Inc.
NEHEMIAS MUNIZ	Quantimetrix Corporation

SAMIA MORA, M.D., M.H.S. Harvard Medical School

JAMES OTVOS, Ph.D. LipoScience, Inc.

GUEST SPEAKERS (continued):

H. ROBERT SUPERKO, M.D. Fuqua Heart Center for Prevention, Piedmont Hospital

WILLIAM CROMWELL, M.D. Medical Director, Division of Lipoprotein Disorders, Presbyterian Center for Preventive Cardiology, and Wake Forest University School of Medicine

PAUL ZIAJKA, M.D., Ph.D. Director, The Florida Lipid Institute and Chief Medical Officer, Atherotech

HERBERT K. NAITO, Ph.D., M.B.A. NorthStar Consulting Service

ELIZABETH SCHILLING, CRNP University of Maryland Medical Center

C-O-N-T-E-N-T-S

P-R-O-C-E-E-D-I-N-G-S

(8:09 a.m.)

CALL TO ORDER

DR. STEELE: Good morning.

I would like to call this meeting of the Clinical Chemistry and Clinical Toxicology Devices Panel to order.

My name is Dr. Bernard Steele. I am the chairperson of the Clinical Chemistry and clinical Toxicology Devices Panel.

I am a clinical chemist and toxicologist, and I am the director of the Core Laboratory at Jackson Memorial Hospital, a 1,500-bed county hospital in Miami Dade, Florida. And I am the director of the driving-under-the-influence laboratory for the County Miami Dade.

I am also a member of the University of Miami School of Medicine.

If you haven't done so already, please sign the attendance sheets that are on the tables by the doors, and I will note for the record that the voting members present constitute a quorum, as required by 21 CFR Part 14.

At this time, I will ask the panel members to introduce themselves, give their area of expertise, position, and affiliation. I will start at the corner with Dr. Gutierrez.

PANEL INTRODUCTIONS

DR. GUTIERREZ: I'm Alberto Gutierrez. I'm the division director for chemistry and toxicology in the Office of In Vitro Diagnostics at CDRH.

Dr. LOEW: I'm Murray Loew, the consumer representative, and a faculty member in electrical and computer engineering and biomedical engineering at George Washington University.

DR. GRINES: I'm Cindy Grines.

I'm an interventional cardiologist at William Beaumont Hospital.

DR. WINTER: I'm William Winter. I'm a professor of pathology and pediatrics at the University of Florida. My background is clinical chemistry and pediatric endocrinology.

DR. WATSON: I'm Karol Watson. I'm a cardiologist at UCLA, and director of the Center for Cholesterol and Hypertension Management there.

DR. LEVINSON: Hi, I'm Stanley Levinson, and I'm a professor of pathology and laboratory medicine at the University of Louisville, and I'm chief of clinical chemistry at the Louisville VA Hospital.

DR. REMALEY: My name is Alan Remaley. I'm a clinical chemist at the National Institutes of Health. And I do research at the Heart Lung and Blood Institute on HDL metabolism.

DR. TSAI: I'm Michael Tsai. I'm

a professor at the University of Minnesota in the Department of Laboratory Medicine and Pathology, and I do research in the cardiovascular disease area.

DR. MARCOVINA: My name is Santica Marcovina. I'm a professor of medicine at the University of Washington in Seattle, and I'm director of the Northwest Lipid Metabolism and Diabetes Research Laboratories.

DR. SHAMBUREK: I'm Bob Shamburek. I'm with the intramural NHLBI. My area interest is lipids and in vivo lipoprotein metabolism.

DR. ZHANG: I'm Ruiwen Zhang. I'm a toxicologist certified by American Board of Toxicology. I'm a professor of pharmacology, kinetopharmocology and toxicology, at the University of Alabama at the Birmingham School of Medicine.

Also I'm the director of cancer and pharmacology over there. I'm

responsible for kinetico (phonetic) pharmacology, toxicology and clinical trials over there.

DR. GRONOWSKI: I'm Ann Gronowski. I'm an associate professor at Washington University School of Medicine in St. Louis.

I am a clinical chemist with a specialist in endocrinology and reproductive physiology.

DR. WORTHY: I'm Tom Worthy. I'm the industry representative. I'm a consultant on in vitro diagnostics. My background is in lipid chemistry and amino assay.

DR. STEELE: Okay, at this moment I have a couple of announcements or pieces of information.

For the panel, please turn off your mikes when you are done. And two, we can only have four mikes on at one time, so please turn them off when you are done.

The second thing is, for the

audience there will be no outbursts.

And finally I would like to remind you, take a moment right now and take out your cell phone and turn it off, or any other device you might have. It would be much appreciated by everyone.

Ms. Calvin here is the executive secretary, and would like to make some introductory remarks.

CONFLICT OF INTEREST STATEMENT

MS. CALVIN: I will read into the record the conflict of interest statement.

The Food and Drug Administration is convening today's meeting of the clinical chemistry and clinical toxicology 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 members and consultants of the panel are special government employees or regular federal

employees from other agencies, and are subject to the federal conflict of interest laws and regulations.

The following information on the status of this 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 meetings.

These interests may include investments, consulting expert witness testimony, contracts, grants, CRADAS, teaching, speaking, writing, patents and royalties, and primary employment.

Today's agenda involves a discussion of general issues concerning lipoprotein, HDL and LDL subfraction assays. 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.

Dr. Thomas Worthy is serving as the industry representative, acting on behalf of all related industry, and is

employed by Worthy Consulting.

Dr. Parvin Waymack, who is a guest speaker with us today, has acknowledged scientific collaborations with firms at issue.

This conflict of interest statement will be available for review at the registration table during this meeting, and will be included as part of the official transcript.

We would like to remind members and consultants that 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.

Before I turn it back over to Dr. Steele, I would just like to remind you that transcripts of today's meeting will be available from Neal Gross & Company. Their contact information can be found on the table outside the meeting room.

Also information on purchasing videos of today's meeting is also outside on the table.

Presenters to the panel who have not already done so should provide FDA with a hard copy of their remarks, including any overheads.

Dr. Steele.

DR. STEELE: Next, Ms. Carol Benson, associate director for chemistry, followed by Dr. Courtney Harper, associate director for toxicology, will give division updates.

DIVISION UPDATES - CHEMISTRY

MS. BENSON: Good morning. My name is Carol Benson, and I'm the associate director in chemistry branch in the Chemistry and Toxicology Division.

Today I'd like to give some updates of happenings in the chemistry branch on newborn screening, diabetes, cardiovascular disease, asthma, on CLIA, and safety.

When there is no predicate device, the device is automatically classified into class III. FDA can use the de novo process to classify a Class III device into Class I or II for special controls.

In August of 2004 FDA used the de novo process to classify a device for newborn screen, the Neogram amino acid caritine and acylcarnitines tandem mass spectrometry kit into Class II.

Likewise, in May of 2005 our sister branch, Immunology, classified a

device for gene mutation detection for cystic fibrosis into Class II with special controls.

And in January of 2006 this year another device was cleared for gene mutation detection for cystic fibrosis.

In the area of diabetes, recently we have revised the guidance for whole blood glucose monitors, and that is available on our OIVD web page.

Also on the OIVD web page are alerts about diabetes, blood glucose monitors, such as counterfeit reagent strips, and falsely elevated glucose results due to interferences of maltose galactose, and oral d-xylose solutions.

We have had some PMA approvals for Class III devices with continuous monitoring sensors. The two companies are the Medtronic and the Dexcom.

We've been involved with initiatives through the Juvenile Diabetes

Research Foundation and their efforts to promote research on the development of technology for diabetes monitoring, and their desire to make this technology more widely available.

In the cardiovascular area two new analytes were cleared for use, the diaDexis PLAC test and the CardioMPO test.

The indications for use for the PLAC test is that is an immunoassay for the quantitative determination of the lipoprotein associated phospholipase A-2 in human plasma to be used in conjunction with clinical evaluation and patient risk assessment, as an aid in predicting risk for coronary heart disease.

The CardioMPO test has an indications for use that it is intended for the quantitative determination of myeloperoxidase in human plasma, to be used in conjunction with clinical history, ECG and cardiac biomarkers to evaluate patients

presenting with chest pain that are at risk for major adverse cardiac events, including myocardial infarction, need for revascularization or death.

In the area of asthma, we've used the de novo process in April of 2003 to classify -- to evaluate a Class III device and to classify it into Class II for the breath nitric oxide that is used in the monitoring of treatment for asthmatic patients.

It has a special control guidance document, and that's available on our web page.

In the area of CLIA we can talk about the test categorization, the CLIA waivers that have been done for 2006, the draft guidance for CLIA waiver, and the database.

If we look at how the tests have been categorized since FDA has been doing the categorizations for almost seven years,

we can see that by far the majority of the tests are categorized as moderate.

The tests that have been categorized as high has remained about the same over these past years, a little around 200. The number of waived tests has seen some increase in the past two years.

The number of CLIA waivers that we've done in 2006, some examples are presented here. We have the glycosylated whole blood hemoglobin. We've done some drugs of abuse waivers for two companies, Branan and Acon.

We've done a microalbumine urine test for Bayer. We've added some chemistry tests to a table top clinical analyzer, the Abaxis Piccolo.

We've waived a whole blood TSH.

And the last one is the most recent, which is the Lead Care II blood lead testing system.

To help you understand how tests

are waived, there are three processes that a test can be waived: by regulation for nine generic tests, if the device is cleared by FDA for home use, and if it meets the statutory criteria with valid scientific data.

The draft CLIA waiver guidance was prepared through comments that were received from the CLIA committee. The guidance helps manufacturers to understand how they need to demonstrate simple; how they can demonstrate insignificant risk of erroneous result through failure alerts and fail-safe mechanisms, and demonstrating insignificant risk of erroneous result through accuracy.

The CLIA database is available from a link from the OIVD web page. It's updated twice a month, and it's downloadable, so you can prepare those charts that I showed you a few slides ago, or you can massage the data to find out how

a test is categorized.

If the test is not in the CLIA database, the default is high complexity.

In the area of safety, from our home page we have on some safety tips for laboratorians, such as false elevated HCG for pregnancy tests; falsely elevated triponin tests.

We have a link to Recalls. It's a searchable database for classified recalls of IVDs.

You can also use the Maude database to get redacted medical device reports.

And the LabSun and the MedSun are two interactive postmarket surveillance efforts that provide interactive communication between FDA and the users of medical devices.

MedSun is for hospitals and nursing homes and other health care facilities. The LabNet is for people that

are using in vitro diagnostic devices in their laboratory.

Thank you.

DIVISION UPDATES - TOXICOLOGY

DR. HARPER: Hello, my name is Courtney Harper, and I'm the associate director for toxicology in the Office of In Vitro Diagnostic Devices, and I'm going to give you a very brief update of the recent new and novel devices, and things that are upcoming in the toxicology branch.

As all of you know, the toxicology branch is responsible for reviewing and regulating a wide variety of toxicology type devices, including tests for drugs of abuse.

But I thought today that I would focus on some recent novel and upcoming type toxicology and in vitro diagnostic devices, including a lot of devices that are indicated for uses that are useful for personalized medicine.

For those of you that are not that familiar with the concept of personalized medicine, it's an upcoming initiative, and is certainly very important in FDA's critical path.

In terms of increasing new and novel medical products that will increase the availability of new drugs and new products for patients.

And the idea of personalized medicine is choosing the right drug or the right therapy or the right treatment in the right dose for the right person.

And in order to do that, one approach is from the use of companion diagnostic assays. So these are assays that are used in conjunction with some sort of therapy or treatment for a patient.

Companion diagnostic tests are tests that are intended to select or guide drug or treatment therapy. And there are several potential benefits to the use of

these companion diagnostics for personalized medicine.

One might be to provide differential diagnosis of certain disorders in order to identify a specific patient subset that might be more likely to respond to that particular drug or treatment.

And this would provide ways to target therapy to the right patients.

Maybe even more importantly is the possibility to identify individuals who might be at risk for adverse events from certain drugs or therapies.

They can -- these types of diagnostic tests can also be used as adjunct tools for monitoring response to drugs, so that you can know if you are treating your patient in the right way using the drug that you have chosen.

And all of these are designed to advance the field of individualized medicine. And this will be to promote

treatment for individuals rather than populations. And this is a new field.

So in order to do this we have sort of three types of devices that we have seen and are seeing in increasing amounts in the toxicology branch. And these three types of devices are devices that are intended for pharmacogenetics, for therapeutic drug monitoring, and devices that are breath tests for a variety of indications.

Pharmacogenetics is the use of a patient's genetic information to guide drug selection or dosage. So far other devices that we have seen and talked most to sponsors about are devices that are for drug metabolizing enzymes. And a lot of these are genotyping assays.

The first pharmacogenetic assay that we cleared in the toxicology branch was the Roche AmpliChip Cytochrome P450 Microarray system.

This device was cleared in December of 2004 by the de novo process. This device is a microarray that's intended to detect 27 alleles of the cytochrome P450 2D6 gene, and three alleles of the cytochrome P450 2C19 gene. And this device is intended to help doctors select and guide therapy for drugs that are metabolized by these two enzymes.

Notably this was the first microarray that was cleared for clinical use in the United States. And this is an Affymetrix-based microarray.

We also reviewed in parallel the Affymetrix gene chip instrumentation system that is designed to read this AmpliChip microarray. This was also done by the de novo process.

And notably I'd like to discuss the FDA review time. In anticipation of an increasing amount of pharmacogenetic and genomic activity in the IVD industry, in

molecular diagnostics, over the past several years the Office of In Vitro Diagnostics has put a lot of effort into recruiting expertise in the area of genetics and molecular diagnostics, and informing themselves about pharmacogenetics and personalized medicine, in order to be ready for submissions such as this.

Through those efforts, and a lot of collaboration and communication in the field in general, and with the companies involved, the FDA review time for this device was actually three days.

Similarly about six months later our branch cleared another device for pharmacogenetic testing. The Third Wave Invader UGT1A1 Assay.

This assay was submitted in response to a labeling change for the drug camptosar. That labeling change indicated that certain patients with a STAR 28 allele may at increased risk for neutropenia when

ingesting drugs such as Irinotecan for cancer chemotherapy.

This assay is designed to attack the normal and one variant allele that UGT 1A1 in order to try and predict risk of this adverse event.

Just like the AmpliChip and the Affymetrix review, the FDA review time for this particular submission was 10 days because of a lot of communication between our office, the device submitter, and the Center for Drug Evaluation.

In addition to those two assays that have been cleared, we have a lot of interest from other companies and other stakeholders in additional pharmacogenetic targets, including other cytochrome P450 enzymes, including genes that are involved in Warfarin pharmacokinetics and pharmacodynamics, and also genes that are identified in drug development programs as being target specific.

Another area of personalized medicine is the area of therapeutic drug monitoring. FDA has been regulating TDA assays that are commercially distributed for many years now. Therapeutic drug monitoring assays are intended to measure the serum and plasma levels of certain drugs in order to help physicians identify patients who may be at risk for toxicities from those drugs, or may be at subtherapeutic levels.

We have cleared assays for many therapeutic drugs, including cyclosporin, tacrolimus, sirolimus and zonisamide, and many others, and we have a lot of interest in companies that are developing assays for a lot of other drugs for therapeutic drug monitoring.

A few years ago the assays for cyclosporin and tacrolimus were down classified. They were originally Class III type devices, and we felt like there was enough information available to mitigate the

risks for those assays, and they were actually down classified, and are now Class II type assays.

And there is a special controls guidance document on our website that describes the type of information necessary to provide a submission for these types of assays.

In addition our office is also working on developing a general guidance for therapeutic drug monitoring assays to enable companies to more easily predict what types of studies might be necessary for introducing new types of assays on the market.

And finally I'd like to talk about another category of tests which are breath tests. These types of assays generally use a isotype labeled ingested compound, and then they measure exhaled breath to measure a physiological phenomenon.

A few years ago our office cleared one of these type of assays for H. Pylori infection, but we've been getting increased interest in development of these type of assays for many more types of indications. And those included some sorts of enzyme activity including metabolizing enzymes or gastrointestinal absorption assays, and a lot of other conditions.

Notably the FDA has determined these types of devices that include an ingested compound are combination products, and that the device is the primary mode of action. What this means is that companies may choose to submit one application that would include information about both the ingested drug and the device for measuring breath as a PMA, and the drug and the device components would both be approved together under that application.

This was communicated publicly in a jurisdictional update out of the Office of

Combination Products, and I've included that website link in my talk.

I'd like to thank you for your attention. If anyone has questions about devices that are regulated in the toxicology branch, please contact me.

Thank you.

DR. STEELE: Thank you.

Next we will have a presentation by Dr. Sousan Altaie on the critical path initiative in medical devices.

Dr. Altaie.

I understand she may not be here.

OPEN PUBLIC HEARING

DR. STEELE: We will now proceed to the first open public hearing portion of the meeting. Public attendees are given an opportunity to address the panel, to present data, information, or views relevant to the meeting agenda.

We have five speakers scheduled for this morning's session. They are

Russell Warnick, Kenneth French, Nehemiah Muniz, Samia Mora and James Otvos.

Each speaker has been allotted a maximum of seven minutes to speak. Since this will take over 30 minutes, we ask each speaker to be as brief as possible, and the panel to hold all questions until after everyone has presented.

I might add that I will -- or actually Ms. Calvin here -- will be keeping a clock, and at six minutes I will raise this notebook as a guide that you have one minute left.

At this time I will read the open public hearing disclosure statement. 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 sessions 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, at the beginning of your written or oral statement, to advise the committee of any financial relationship that you may have with any company or group that may be affected by the topic of this meeting.

For example, this financial information may include a company's or a group's payment of your travel, lodging or other expenses in connection with your attendance at this 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 do not choose to address this issue of financial relationships at the beginning of your statement it will not preclude you from speaking.

Mr. Warnick -- Dr. Warnick.

DR. WARNICK: Good morning. I appreciate the opportunity to present before this panel today. I should disclose that I am employed by Berkeley Heart Lab, which provides subclass testing in the context of cardiovascular disease management.

But I am speaking today primarily from the benefit of over 35 years experience in promoting improvements in lipid and lipoprotein testing.

In the Bay area we are quite familiar with earthquakes. This phenomenon is a result of opposing forces. The Pacific plate is continually driving against the North American plate. The movement is locked, and then when the force becomes overwhelming, then the plate moves and the consequence is an earthquake.

Scientific research transitions to clinical practice I believe in a similar manner. On the one hand we have push from ever-evolving research and technology.

Innovators develop new approaches. Early adopters are interested in using new technology. And of course we can't ignore financial incentives.

On the other hand we have the natural resistance to change, inertia in the organizations, and agencies. The time to achieve consensus, and vested interests. So when the push overcomes the opposition we have an earthquake, and practice can eventually change.

A lesson from history: John Gofman at the University of California Berkeley began this career as a physicist, purified plutonium for the Manhattan Project. Following the second world war he received his M.D. and organized the Donner Laboratory Research on coronary artery disease.

In the early `50s, using analytical ultracentrifugation he demonstrated differential relationships of

lipoproteins to coronary artery disease.

NIH convened a consensus conference in 1956 that reviewed the evidence and rejected his conclusions about the utility of lipoproteins, concluding that measurement of total cholesterol was adequate.

The consequence was that Gofman abandoned the lipoprotein field and went back to radiation. The more significant consequence in this context was that HDL was forgotten and largely ignored for almost two decades, until the mid-1980s, when it was rediscovered. The result was a lipid panel. Of course total cholesterol, triglycerides, HDL cholesterol and LDL cholesterol measures, became endorsed by the NCEP adult treatment panel guidelines. The lipid panel has been standard for longer than the career of many in this audience.

What is not widely appreciated is that the LDL cholesterol measurement, which

is the keystone of the guidelines, by either calculation or direct assay, can be quite unreliable, and these traditional biomarkers miss about half the patients at risk for cardiovascular disease.

We see here a study from the Berkeley Heart Lab database of over a half a million patient records, over 4,000 patients with known CVDs diagnosed within three months were pulled from the database. Of these patients, total cholesterol, the total cholesterol cut (phonetic) point identified only 23 percent; 39 percent had elevated triglycerides; only 11 percent had increased LDL cholesterol. That is, that cut point missed 89 percent of the patients with cardiovascular disease.

By contrast, the small dense LDL subclasses -- LDL 3A plus B -- identified 92 percent of the patients, missing only eight percent of the patients at risk.

The HDL cholesterol cut point

identified 40 percent of patients, missing 60 percent. And the large HDL fractions, HDL 2B identified 70 percent of patients with disease, missing only 30 percent.

Now, HDL is highly heterogeneous. I am going to hit very high points of a very complex story here. But a 2-dimensional electrophoretic method separates at least 12 or 13 different fractions of HDL. Most important are thee alpha one and alpha two species.

Considering a patient with coronary heart disease compared to a control healthy patient, there are very different observations among the subclasses. The pre-beta one, alpha one particles are low in coronary heart disease patients, whereas alpha two and alpha three particles can actually be elevated.

There are many different studies showing the differential association of subclasses. Expert opinion indicates that

the alpha one and alpha two HDL particles are much better at CHD risk prediction than HDL cholesterol.

The subclasses also much better monitor the effects of therapy.

LDL is also heterogeneous with at least seven fractions separated by a gradient gel electrophoresis method.

There is abundant evidence that small dense particles are more atherogenic, as indicated here. And a variety of studies have shown that LDL size can be an independent risk factor independent of triglyceride and HDL.

In one study LDL size as a better predicter of the stenotic change than LDL cholesterol.

So current LDL cholesterol, HDL cholesterol measurements, do not fully characterize cardiovascular disease risk in patients. The HDL cholesterol assay does not identify the differential association of

subclasses. LDL cholesterol assays can be unreliable. Subclass determinations can better characterize risk, facilitate prevention and treatment options are available.

So in conclusion, lipid panel has dominated the practice for over 20 years; fails to identify half the patients at risk. Lipoprotein subclasses can better characterize risk.

So, time for a new paradigm. Thank you.

DR. STEELE: The next speaker.

MS. CALVIN: I just want to remind you that when the yellow light comes on, that is your one-minute warning.

MR. FRENCH: Should I start? Okay. My name is Kenneth French. In the interest of full disclosure, I am the director of education at Atherotech that performs the vertical auto-profile technique, also known as the VAP cholesterol

test.

This test is used by roughly over 12,000 physicians nationwide, performing a little over a million tests per year at a cost of $4, and reimbursing around $34.

So that's the landscape which I'm coming from. I was asked to put together a presentation of clinical relevance, and I actually chose the opportunity to use the current national guidelines, and current recommendations to clinicians who are managing patients who are at risk for coronary vascular disease or dyslipidemia associated with diabetes, or thyroid stimulating problems, or patients with -- female patients with hormone problems.

The first one is probably the most familiar to most people. It's of course the National Cholesterol Education Program, the ATP III guidelines that was produced in 2002. I was quite pleased with this presentation that was delivered,

simply because it addressed more than just LDL, which is what we are here to do today is address more than just traditional risk factors.

So looking at the highlights, when I looked at the term, subclasses of LDL, well there is just more than one subclass. And a quote from the ATP guidelines said, emerging risk factors that can be measured include elevations in lipoprotein (a) remnants, hence, IDL is a portion of the LDL total. So it's a subclass of LDL, as well as small LDL, which is I think largely where a lot of the focus is here.

But I think the key here, that there was already a recognition that these can be measured, and can be used in clinical practice.

Metabolic syndrome, I think this is probably -- I could be wrong -- but I think this is rapidly increasing as the

number one risk factor in the United States, due to the fact that we are very savvy, and we love sugar, and we're losing our population in terms of exercise.

But I think it's associated risk factor have emerged as a coequal partner. That was referenced in the guidelines. And one of the real contributing factors to that is the small LDL that is associated with the triad or the dyslipidemia associated with this disorder.

And I think Gerald Grievens (phonetic) did some really good work where he's actually showing this triad actually predicts diabetes risk much earlier than the traditional hemoglobin A1C or glucose markers that we've been using for years.

But it does certainly warrant -- how do you address when you see this triad, is, you certainly lower the LDL goal. One of the things we are looking at is when you take more and more, you have for example a

patient who is borderline, we don't know when to treat. This could certainly be an opportunity to raise that patient's risk level, not just that dyslipidemia alone, but the metabolic syndrome as a whole. This is just the lipid portion of that.

And then treatment opportunities could change as a result of this.

Lipoprotein (a), you know, the guidelines express that the presence of an Lp(a) thus raises an option to raise a person's risk to a higher level.

Again, the emphasis is what do you do when you see this, or you have a patient who is intermediate risk, where there is a decision to maybe treat or not treat. An Lp(a) certainly warrants the ability for a physician to say yes, due to a family history, choose to treat these patients' LDL more aggressively.

Small dense LDL is a component of atherogenic dyslipidemia, which we just

discussed, with the metabolic syndrome. It's not exclusively as a part of the checklist for high elevations, but it's a large part of. And of course this changes the way the risk is associated with that patient.

And of course there are opportunities for therapeutic changes.

And of course the last is the remnant lipoproteins, a person with high serum triglycerides, remnants should be treated in addition to the lowering of LDL cholesterol. So here we see not only LDL being addressed, but we see the opportunity that we should be lowering remnant lipoproteins in addition to the lowering of LDL-C.

So that's a component of non-HDL, so again, this changes the patient's risk and therapeutic changes.

The next one is the working group in lipoprotein measurements, the document

from 1995, sponsored by the NIH, and National Heart and Lung Blood Institute. One slide, and I think it's quite important, and two bullets.

Proportional contributions of those two emerging risk factors, IDL and Lp(a), to the total LDL measurement would expect it to be higher in at-risk populations, and I think you are hearing that. And of course for all current and future methods -- I think this is why we're here -- when we look at these methods, the nature of these lipoproteins, in other words, when we look at LDL, we need to have measurements and methods that can actually differentiate what we are looking at, because not all LDL is created the same, nor is it treated the same. So they have very, very different pharmaceutical reactions to the different drugs that we have.

So the next group is just the NACB, or the National Academy of Clinical

Biochemistry. And this was a summary of the recommendations of the draft. I haven't gotten the actual final report. But it was very clear at the meeting that they felt Lp(a) is a unique animal in the risk factor, particularly useful in genetic predisposition.

They definitely did talk a lot about the HDL and LDL subclasses. I think one of the things we need to remember is, the sizing of LDL is directly related -- there is a direct relationship to the Apo B concentration. So I mean it's knowing one or the other, two pieces of information, to gain more information about vascular risk.

And then of course remnant lipoproteins got some podium time as well.

And then the last group is the American Association for Clinical Endocrinologists, and this is basically the guidelines for endocrinologists. And the version that I am referring to is the 2002

amended version.

And again, it lists the following subclasses as risk factors for CAD. Small LDL subclasses with reference to insulin resistance; and then of course Lp(a) should be considered in patients with future coronary vascular risk.

I appreciate your time. Thank you. Mr. Muniz.

MR. MUNIZ: My name is Nehemias Muniz. I'm with Quantimetrix Corporation. I'm an employee of Quantimetrix Corporation. We have worked on the development of diagnostic tests for measuring LDL subfractions, and we are also interested in measurement of HDL subfractions, and we would like to have a test that can do that, provided that it shows that it's safe and effective.

I am not going to talk about the LDL subfractions at this time, but since our current interest is in HDL subfractions, I

will give you a slide presentation of some of the findings we have discovered in the testing of HDL subfractions.

And we have looked at two different populations, one of normolipidemic versus dyslipidemic population.

We all know that HDL is heterogeneous, and differs in composition and function and has organic potential. There have been different methods that have been employed to measure these subfractions, among them some that have already been discussed, are NMR, gradient gel electrophoresis, ultracentrifugation, precipitation, and the method that we employ, which is linear polyacrylamide gel electrophoresis.

As we know traditionally HDL has been divided into two major subclasses, which is HDL2 and HDL3. And depending on the method of separation employed, as many as 10, 12, 13 subfractions have been

identified.

Using the linear polyacrylamide gel method, we identified about 10 different subfractions, and we grouped them, just for the sake of simplification, into three major categories, which we call large HDL, intermediate HDL, and small HDL.

Most of the changes in HDL seem to be of genetic origin. However environmental factors, such as diet and other things, may contribute to the distribution of this HDL subfractions. And we found that the subfraction that usually has the most change is the large HDL subfraction. That's where most of the change occurs, based on diet or genetics or whatever, seems to be the subfraction that has the biggest change.

While intermediate density lipoprotein seems to be more consistent, not to shift as much, while the smaller HDLs seem to be controlled, possibly genetically,

and seem to be different from the other two subfractions.

There have been studies that have questioned -- and that's why we're here today -- to discuss whether this is really applicable and beneficial.

And if we look at the literature, there are lots and lots of studies that show the importance of large HDL, but there are other studies that have shown not so good a relationship between the HDL subfractions and disease state.

The technique that we use, the method that we use, as I indicated is a linear polyacrylamide gel. It consists of a separating gel, a stacking gel, and a loading gel which contains a lipid lipophilic that binds the particles.

Then by measuring the area under the curve after scanning the gels, we can calculate the are under the curve, and make an estimation of the cholesterol in the

various subfractions.

As you can see on the right there, three different patients in duplicate that show the differences of the distribution that can be observed from the gel.

In this next slide we can show what we see, the type of profile that we see in a normal population. We can see that, since the subfractions are separated in size, starting from left to right are the larger particles, in the green; the intermediate is in the yellow; and the small particles are in the red on the right-hand side.

And so in a typical normal profile, this is what we observe. In none -- normal population this is more likely the profile that we observe. And you can see that the large HDL is totally diminished. The intermediate HDL remains relatively constant. And on the right side you can see

that the red, small dense particles, can extend significantly, and their number can increase, based on the quantification, not this.

We also did some comparison by looking at the various subclasses, that is, the large HDL, the intermediate HDL, and the small HDL. And we did correlations with other known risk factors. And the ones that have the little square on the left side are some of the more important ones. For instance, you can see that the large HDL in the first line correlates very highly with the total HDL, as you can see by the length of the bar.

However, when you look at the total cholesterol, there is no correlation, or very tiny small correlation, really, with total cholesterol.

We also compare it to particle size of the LDL. And you can see also there is a relatively strong correlation with the

particle size, but a strong negative correlation with LDL cholesterol, and very strong negative correlation with triglycerides. This is for the large HDL.

Now if you look at the intermediate HDL you can see pretty similar relationship, except now instead of having a negative correlation with total cholesterol, it has a slightly positive correlation with total cholesterol. But really it doesn't differ very very much from the large HDL.

Now when we look at the small HDL, you can see that the small HDL does not have the same strength of correlation than the -- to HDL cholesterol --

DR. STEELE: Could you wrap this up, please?

MR. MUNIZ: Now it has a positive correlation with triglycerides.

When we look at the means of the two populations we can see the means of the large HDL and the small HDL are different in

the two populations, and this is graphically how they are represented.

One more second? So in conclusion we found that not all HDL subfractions are the same. They have different correlations with different risk factors, and especially, the greatest difference is between the large HDL and the small HDL.

So based on this we conclude that all HDLs should not be considered the same. They are very different and have different influences.

Thank you.

DR. STEELE: Thank you.

Dr. Mora.

DR. MORA: Good morning, thank you for inviting me -- or for listening to me this morning.

My name is Samia Mora, and I work at the Brigham Women's Hospital in the division of preventive medicine. And I'm

also a cardiologist, so I work in cardiovascular medicine.

These are the financial relationships, travel and lodging for this trip were paid by LipoScience. No other financial relationships.

So many studies have shown that patients with smaller LDL size have greater CHD risk. So the question is, is this increased risk due to LDL particle size, or is it due to particle number?

Shown in this slide is two scenarios, actually, one here on the left where for the same LDL cholesterol, which is 130 milligram per deciliter, you have fewer LDL particles, but they are larger size.

And on the right here, the same LDL cholesterol, 130 milligram per deciliter, and you have a larger number of particles, but they are smaller.

As you can see here, the smaller LDL particles are also associated with

higher particle number. So the question for CHD risk, is it the particle size or is it the particle number?

So we asked this question in the MESA study. And the question we asked, is the relationship of LDL size with CHD confounded by LDL particle number?

And a confounder as shown here is associated with the risk factor, and also causally associated the outcome.

So the question we had, was the LDL particle number, LDL-P, which is associated, as I just showed you, with LDL size, is that confounding association of LDL size with CHD?

And the other question, is small LDL particles, are they confounding the association of large LDL particles with CHD?

I'm basically summarizing our results which were published in Atherosclerosis. They are online, not out in print yet, but the reference is up there

for you.

So the MESA study is an NHLBI sponsored study. We recruited patients from six different sites across the United States, and we had about 5,500 participants. They come from four different ethnic racial backgrounds, as shown here, and half of them are women. The mean age was 61.

And first we looked at the individual chemical lipid measures. So the standard LDL cholesterol, HDL cholesterol, triglycerides.

Now what we did was, each linear regression model, looked at the association of each of the lipid measure with carotid intima-media thickness. And shown here is first-handed (phonetic) deviation increment in that lipid measure. So for example, one standard deviation increment in LDL cholesterol was associated with 37 micron higher INT. And that was statistically significant.

And similarly we found for HDL cholesterol was inversely associated with carotid IMT, as we would expect. And triglycerides were positively associated.

And each of these models was examined separately, so each variable at the time was in the model. And we adjusted for the other risk factors -- age, sex, race, smoking, and hypertension.

Now this is for the LDL particle associations with carotid IMT. Shown here again is each lipoprotein variable, but one separately in each model. For example, LDL size, one standard deviation increment was associated inversely with carotid IMT.

Total LDL particle number was positively associated with carotid IMT. As you can see here, one standard deviation was associated with 14 micron higher IMT. And remember, for LDL cholesterol it was 37 micron higher IMT. Also highly significant.

Now, then, we asked for large

versus small HDL, and we put again each one separately in the model. And we found large LDL was not associated with IMT and put separately in the model, whereas small LDL was associated with carotid IMT.

Now there are potential sources of confounding. So as you note here, large LDL and small LDL are negatively inversely correlated, with a negative correlation coefficient of minus point six.

Note that small LDL and large LDL have differing associations with LDL size. And small LDL is inversely associated with LDL size, and large LDL positively associated with LDL size.

So this becomes very important when we do the next models. Total LDL particle number was inversely associated with LDL size.

When we looked at LDL size, as I showed you earlier, put it in the model separately, adjusted only for these risk

factors, but not for LDL particle number, that was the negative association shown here.

Now when we adjust LDL size for LDL particle number, so we put the two together in the model and adjust for these risk factors, we found that the P value becomes nonsignificant, and actually the direction of the association is reversed. But again this is nonsignificant.

Here are the individual subclasses. So large LDL-P particle number, when put separately in the model as shown before, was not associated with IMT.

Now when we adjust for small LDL size, so large LDL-P adjusted for the number of small LDL particles, we found now that large LDL particle number was associated with IMT, highly significant, and small LDL particle number, when we adjust for large LDL, is also highly significantly associated with carotid IMT, and note that the change

in IMT is similar between large and small.

Once you take into account the particle number of the other subclass.

So adjusting for the small LDL particle number and mass, the true association of large LDL with IMT. And again shown here on the left side is when we don't adjust for small LDL. So these are increasing quintiles of large LDL, and you see there is no association with carotid IMT.

Now when we adjust for small LDL-P, all of a sudden we see that highly significant relationship of large LDL-P with carotid IMT.

And these findings from MESA showing the negative correlation between large and small LDL --

DR. STEELE: Could you wrap that up, please?

DR. MORA: Yep. Were also confirmed in the VP hit, where when they

adjusted for large and small LDL-P they also found both were associated with events.

So our summary is that without adjusting for small LDL particle number, we found large LDL particle number was only weakly associated with IMT, which is consistent with the prior studies.

However, when both the small and the large LDL particles were examined jointly together in the model, both were highly significantly associated with carotid IMT, even after adjustment for the traditional risk factors.

And LDL particle size, as I showed, contributed little after accounting for LDL particle number.

Thank you very much for your attention.

DR. STEELE: Thank you.

Dr. Otvos?

DR. OTVOS: Thank you.

I am happy to say a few words

about the other technology used to provide information about lipoprotein subclasses, NMR spectroscopy. And I do have a relationship with Lipo Science. I am an employee and a stockholder of Lipo Science.

Just a quick background. We've been in this business about 10 years, have a CLIA-certified laboratory that is CAP certified; have analyzed over 2 million NMR lipoprotein tests. And in 2006 the AMA issued a CPT code specific to quantification of lipoprotein particle numbers by NMR.

Now the topic of this meeting is going to be to address the meeting of whether the so-called quality of LDL and HDL, the subclass distributions or subclass concentrations, are clinically relevant.

And as you all know, the quantity of LDL and HDL are already well established as important risk factors for cardiovascular disease, and the way that these are quantified is to measure the cholesterol in

LDL and HDL. So I just want to distinguish between the quantity of LDL and HDL well established, and the question about whether subclasses are the quality add to that.

But I also want to raise the point that there are alternative measures of LDL and HDL, alternative ways to quantify these particles. Apo B is one of them. Apo B measures the protein constituent on LDL and VLVL and gives you a pretty good approximation of LDL particle number.

So now along comes NMR spectroscopy which not only enables or gives visibility to the concentrations of various subclasses, but is also an alternative way, alternate way, of quantifying LDL and HDL. According to the number of particles.

So the method measures the particles themselves, not just the cholesterol constituent, and it has a number of attractive analytic characteristics.

It's rapid, automated, reproducible, and it doesn't require physical separation of the particles.

How does it work? I can't go into this in detail obviously. But it basically takes advantage of a natural phenomenon, which is that different lipoprotein subclasses, for natural physical-chemical reasons broadcast characteristically different NMR signals, and by measuring how big those signals are in a patient's plasma, the amplitude of the signals, you get direct information about the number of particles contributing to that signal.

So the signal shows up in an NMR spectrum as shown here, proton NMR spectrum blood plasma that just takes a few seconds to acquire. When you blow up that signal, you can see certain fine structure, and with good preknowledge about what the signals look like from each of the different size

VLVL, LDL and HDL subclasses, one can spectrally deconvolute the signal to get the amplitudes of the individual subclasses. That's a process that occurs with a computer, takes less than a second to accomplish.

So right now we have a number of NMR spectrometers that we have tried to turn into clinical analyzers in our laboratory in Raleigh, North Carolina. And I just wanted to show this to indicate that what we've discovered is that one can get very good agreement between the information produced on the different machines.

So standardization of this is not going to be difficult. It will actually give very good inter-machine and inter-laboratory relations, we believe.

So we are now using, as I said, NMR spectrometers that are essentially off the shelf mated with sample handling equipment, off the shelf, and we have turned

these into clinical analyzers in our laboratory.

But we believe the future of this is that these machines can be integrated, and this is a machine in the final stages of development, where any laboratory in the world will now be able to automatically produce this information very efficiently.

So again what the assay actually produces initially are the concentrations of the individual subclasses, but currently, we are reporting for clinical use only three pieces of information: the total LDL particle number, LDL-P. And from the particle information, we also can calculate HDL cholesterol and triglyceride information that is very highly correlated, essentially clinically equivalent to chemically measured HDL cholesterol and triglyceride.

We also report all the individual subclass information and particle sizes, but these are reported for informational

research uses; no clinical claims being made for this at the current time.

So the assay is well validated analytically. Just a quick couple plots showing the relations of chemical and NMR triglyceride and HDL cholesterol. The closest thing that LDL particle number is related to is LDL Apo B. This shows the relationship is very good between those two measures.

The size information or the subclass information also agrees well with other methods; gradient gel electrophoresis in particular is what we've used to characterize these relationships. These are all information that was published recently.

The assay is also well validated clinically. We've actually gone out of our way over the past five or six years to try to learn what good is this information? What relationships does this information

have to clinical outcomes.

So there have been over 600 studies completed so far; 180 studies are in progress, about 10 new studies a month. This assay is being used by lots of pharmaceutical companies to characterize various agents that have affects on lipoprotein metabolism. Many of these have conducted audits since 2002, because of the intended use of this information to support FDA submissions, 125 publications to date, mostly since 2003.

And among the outcome studies, there is I think been eight to date showing prospectively showing that LDL particle number has a stronger relationship to incident cardiovascular disease that LDL cholesterol.

You've heard results from the MESA study. Many other studies have been conducted in the same way in which frozen samples at baseline have been used to learn

about the associations.

Lots of different cardiovascular endpoints, hard outcomes as well as subclinical outcomes. I'm not going to go through these in any detail obviously.

Also, the assay as I've mentioned has been used by many pharmaceutical companies to look at many different types of therapeutic interventions. You see a list of those for which published information is now available.

So finally just to conclude this assay has been in use now for almost 10 years. It's well validated analytically and clinically.

We very much believe that any claims about clinical utility should be evidence based. And there is a lot of evidence that we have generated, and broader utilization will now be enabled by decentralization of the assay.

Thank you.

DR. STEELE: Thank you.

At this time, does the panel have any questions for the open public hearing presenters?

Questions? Oh, excuse me, Dr. Gronowski.

DR. GRONOWSKI: My question is for Dr. Otvos. Have you or anyone else looked at the effects of freezing and storage on particle number, particle size, these kinds of things? In particular, temperature of storage, length of storage, and repeated freeze-thaw?

DR. OTVOS: Right. Virtually all those studies that I referred to involved samples frozen at minus 70 degrees for long periods of time; some studies up to 30 years. Mr. Fit (phonetic) was an example of that.

Under control conditions where you measure it fresh, freeze it, thaw it, measure it again. Very good associations.

Only issue is in the highly -- triglyceride rich samples in which freezing does affect some of the large triglyceride rich particles.

But no affect on LDL or HDL information. Freezing at minus 20 degrees for more than a couple of months -- sorry, for more than a couple of weeks -- starts to cause changes, so that's not an acceptable storage condition.

So yes, we do have a lot of information on that.

DR. STEELE: Thank you.

Question for Dr. Watson?

DR. WATSON: This question is actually for anyone, the companies that do subclass distribution.

A lot of these clinically are not well studied, so we clinicians use LDL/HDL as you've mentioned. But we are starting to use the measure, non-HDL cholesterol, sort of as a poor man's way of approximation Apo

B or total particle number.

And I guess I didn't get a sense about how your assays correlate with non-HDL cholesterol, and how is there added benefit above measuring the non-HDL cholesterol, which is already done in every clinical lab.

MR. FRENCH: We actually at Atherotech are using the vertical profile technique, are able to calculate a Apo B 100 value that is right now correlating greater than 95 percent to using -- but of course you have to use information beyond just non-HDL. The best work I've seen so far, by several people, Grundy (phonetic) being one of them, is around the 827.92 range. So the fact that we can get a better correlation with that Apo B, using the non-HDL and subclasses of LDL, that tightens up that correlation much much better. So you can use non-HDL or Apo B interchangeably, but you've got to be careful of the techniques that are being used. And all of the

techniques listed here are much more sensitive at determining that information.

Did that help?

DR. WATSON: So the correlation to non-HDL that you are seeing --

MR. FRENCH: With the technique that was used.

DR. WATSON: Is .87 is what you are saying?

MR. FRENCH: No, ours is greater than .95. That would be the vertical profile technique. But if you look at traditional total cholesterol minus HDL, that method of non-HDL, then what you see is a lower correlation of Apo B direct measure too.

Did that answer your question?

DR. WATSON: Yes.

DR. OTVOS: Let me just add something to that. The use of non-HDL cholesterol has been promoted as having efficacy because it includes particles

besides LDL, VLVL particles.

The reality, though, is that I think non-HDL cholesterol has stronger relationships with outcomes than LDL cholesterol because it is a surrogate marker for LDL particle number. And we have a lot of data that speaks to that.

So then the question is, is there any advantage of measuring LDL particle number over non-HDL cholesterol? There was a paper that was published just this week actually in AJC that looks at discrepancies between categories or non-HDL cholesterol and NMR measured particle number that shows that yes, in hyper-triglyceremic patients, non-HDL cholesterol gets you closer than LDL cholesterol to LDL particle number, but there are still lots of discrepant situations.

So it is better than LDL cholesterol, but not the same as LDL particle number.

DR. STEELE: Thank you.

DR. SUPERKO: Hi, I'm from the Fuqua Heart Center. I want to make two quick comments.

I was in and developing this field for the past 20 years, 10 years at Stanford, Peter Wood, Ron Krauss, 10 years at U.C. Berkeley, John Gofman, Frank Lingren, tons of NIH research.

Two quick points I'd like to make. Number one, a lot of these issues can be resolved with standard measures of triclycerides and HDL cholesterol. Strong correlation in 1999 in the Medicare Bulletin we got Medicare to pay for these tests.

However, in that bulletin it also said that they are not useful, excuse me, when triglycerides are over 250 or less than 70. So number one, measuring true Apo B, LDL Apo B, or B 100, you can eliminate the need for a lot of these tests. So that goes to your point.

Number two, this field is totally nonregulated. What you really need to think about is, do we need standardization for any of these techniques, such as ultracentrifugation, density gradient, ANUC. So please consider those two points.

DR. STEELE: Thank you. I have to apologize. This is only open to the presenters.

There is another question here from Dr. Marcovina?

DR. MARCOVINA: Yes.

One is for Russell Warnick, please. Russell, do you have a correlation standard between the determination of HDL 283 by differential precipitation technique in the gradient gel electrophoresis?

MR. WARNICK: No.

DR. MARCOVINA: And one is for James Otvos. Do you have a data on a correlation between LDL particle number and the total Apo B?

And also you presented some small correlation between LDL Apo B and elevated particle number. How was that LDL Apo B measured?

DR. OTVOS: It was measured nephelometrically with the --

DR. MARCOVINA: Yeah, but with LDL, so how was LDL particularized?

DR. OTVOS: The LDL was separated by preparable ultracentrifugation. Well, no, so all that was done was that the VLDL was removed, so it was one spin, and then bottom fraction Apo B measurement, to give LDL Apo B.

And yes, the correlations are essentially equivalent between plasma Apo B and LDL particle number and LDL Apo B, because 95 percent of the Apo B is on LDL particles typically.

So that's typically what we find our correlations on .95.

DR. MARCOVINA: Between LDL

particle number and Apo B?

DR. OTVOS: Between LDL particle number and plasma Apo B, .9 to .95.

DR. MARCOVINA: Thank you.

DR. STEELE: Okay, and the last question will be from Dr. Levinson.

DR. LEVINSON: Thank you.

I want to say that I'm impressed by the presenters, Dr. Otvos and Russ Warner and others who have been in this field for many many years.

Nevertheless, and I would like to address a question to several people that spoke. They present a lot of data, some of which is in press, I guess, so I haven't had a chance to see it.

But I have a few papers here that I brought with me. And one of these is the first author's Gardner, and the last author is Krauss. And according to this paper the conclusions, and this is what I'd like a response to is these conclusions: However,

talking about small density LDL, however, when added to physiological parameters above, the total cholesterol of HDL-C cholesterol was found to be a strong independent predictor of coronary artery disease status.

That was in JAMA in 1996. And here I have -- I don't have the original paper, but this is a letter referring to a paper by Dr. Campos, and it's Dr. Krauss who is referring, and Dr. Campos apparently found in his studies that bouyant LDL was a better marker actually than small dense LDL.

Then another paper here, Ernest Schaefer is the last author, and they say the data indicated that small LDL particle size is not an independent discriminator for coronary artery disease after conventional risk factors and lipoprotein parameters such as LDL and HDL cholesterol are taken into account.

And again, this doesn't include,

as was mentioned, Apo B, and also, non-HDL cholesterol, which several studies have shown, at least statistically, very similar to Apo B.

And let's -- yeah, okay. So those are the three. And so it seems to me that adds a lot of question as to whether small dense LDL for example are as important as some people have suggested. So I'd be glad if any of the speakers would respond to that.

DR. STEELE: Is there any response from the speakers? Dr. Moore?

DR. MORA: Yes, I just want to bring up one point again, which is that in the MESA what we found was that because the small and the large were negatively correlated, moderate correlation, minus point six, I think that's explaining a lot of some of the confusion in the field about LDL size.

As I showed, two people can have

the same LDL cholesterol, but some may have more particles if they have the small ones, compared with fewer particles of the large.

So when you just look at small LDL size, for example in MESA, alone, that was associated with atherosclerosis, but then when you take into account particle number, it turns out it's actually the particle number, not the size. So both the large and the small.

And I think some of that -- some of the findings from the prior literature can be explained by this. Different populations have different proportions of people with small versus large LDL; for example, people with familiar hypocholesteremia have more of the large LDL. That's why their cholesterol is higher. And people with metabolic syndrome, as we heard, we know have more of the small LDL particles.

So different populations have

different mixtures, and if you don't take into account particle number, and you just look at particle size, then you are going to miss that association.

And that's why I think there is differing results in the literature before. Because as we demonstrated clearly, when you just look at LDL size, without taking into account particle number, it seems there is an association. But then when you take into account particle number, the association goes away, and both large and small were actually associated with atherosclerosis and the carotids.

DR. STEELE: Thank you. We are running out of time. You were up ready to go, why don't you go, Mr. French. Or if you want to defer to Mr. Warner. Please, we are running behind, and we need to -- if you have a real brief statement, Mr. Warnick.

MR. FRENCH: Dr. Livingstone, do you mind just repeating that question one

more time for me please.

DR. STEELE: I don't know if we have time for that.

MR. FRENCH: Well, I tell you what, if I understand his question, and I've seen all three of those papers, the overwhelming body of evidence is what we are kind of looking at. But one of the key things you want to keep in mind is how these points are defined. At some of these clinical trials they are very very different. So offline I'd love to have that discussion with you.

But that's what we're really looking at here in some cases is how you define what's pattern A and pattern B.

Thank you.

DR. STEELE: Yes, please, just very brief, please.

MR. WARNICK: Measurements of the lipoproteins and subclass are very difficult, very challenging. The methods

have evolved over the years. I know gradient gel electrophoresis best, and we've found that by adjusting the gradient we can improve first the separation of subclasses. We've found that the early absorbence dyes, oil red O, and Sudan black, are non-stoichiometric; that is, they underestimate the dominant particles. So studies, all of the early studies done with the absorbent dyes are compromised by that fact.

Also we find that by quantitating (phonetic) particles, rather than reporting relative percent we can eliminate the variability of the inner influence of the various particles on the quantitation. So by absolute quantitation, we can eliminate some of the noise.

So I think these studies are compromised by the particular use of the techniques and by the lack of refinement of the techniques in the early studies.

DR. STEELE: Thank you.

Dr. Zhang had a question. Can it wait? Okay, go ahead.

DR. ZHANG: I have a very quick question. Any of the presenters can answer this one.

It's not clear to me in the general -- in your opinion, you were like to have a panel of lipoproteins as future assay, or you think or you believe one of them or two of them should be independent assay, as a general strategy, I would like to know.

Because in the clinical practices right now, at least we have three as a panel to look at.

And I heard some of -- I'm not going to repeat an individual indicator, sounds like when you emphasize one over others, I'd like to know your general thinking about a strategy. You want a panel 5, 10 today you can get the 10 through 19, whatever. You have several parameters in

the panel.

Whether or not you believe one -- I'm not going to point out specifically -- you believe one is more important than the other.

Anyone can answer my question as general thinking.

DR. STEELE: Seeing no responder, and this can be brought up again, and probably will be brought up again this afternoon, I now say that the open public hearing session is now concluded.

GUEST PRESENTATION - DR. PARVIN WAYMACK

DR. WAYMACK: Okay, I'm Parvin Waymack, Centers for Disease Control, research chemist. For 17 years I was chief of the lipid reference library.

We standardize HDL and LDL cholesterol, and for many years, beginning in `95, there was an ATP -- CDC is a partner with NCPP, and standardizing risk factors for cardiovascular disease.

The first -- CDC follows the recommendations of working groups like the 1995 working group, follows recommendations of the NCPP adult treatment panel working with them as a partner. We standardized LDL cholesterol through a cholesterol reference method laboratory network. And we did this on the basis of a recommendation that we should use our HDL reference method, extend it, because the database indicated that the risk factors were LDL, IDL, and Lp(a). And our method included those risk factors.

This is a definition of LDL cholesterol that is actually within the database. It's more than just LDL cholesterol.

And we've found in standardizing HDL and LDL cholesterol that the existence of these subfractions are making the practical assays, the routine assays, are causing problems with standardization. So that's how our interest -- clearly, small

dense LDL and subfractions are risk factors within the LDL cholesterol.

LDL cholesterol is the cornerstone for the ATP treatment panel. Lowering LDL cholesterol is the cornerstone. And this recent update shows that taking all the way down to 40 milligrams per deciliter was recommended, and of course this is the first thing you have to realize is that this is a result of population studies. And yet it has to be translated into recommendations for individuals.

So there is a large up and down uncertainty around what would be for individual patients.

Small dense LDL then is within this population of LDL cholesterol. It's very effective for treatment and management.

And the issue really is, within this, we have small dense LDL, and you are going to see some slides here you've seen before. Because I've borrowed a lot of

slides. It's a very eclectic set of slides.

You can measure -- let me go back to that -- the key thing here that's been successful is if you lower the LDL cholesterol concentration one percent, you lower the risk one percent.

If you measure Apo B as a surrogate for the LDL particle you would see a 1.1 percent lowering for every one percent lowering.

So the issue is the LDL particle concentration as the thing that is causing -- is the true risk factor. At any concentration, equal iso LDL concentration, a small dense LDL is going to have more particles, and that can be a confounding factor then in using -- I don't know how often it really affects the -- effective as a treatment. But once you take it to a low enough level you have effective treatment, like taking LDL particles down.

One study showed that in 222

patients that the -- had no prior history of cardiovascular disease that 70 percent according to the ATP 3 didn't qualify for pharmacotherapy.

And if you look at the general population, the general risk category, using these same criteria, which involves a Framingham risk score, 35 percent at low risk, 40 percent at intermediate risk.

And this is not an indictment of the treatment guidelines for ATP3, it just says there is another population there that has other risk factors that are important. It's not just LDL cholesterol and the lipids that are causing this.

We have a complicated disease process. And the emerging risk factors, the lipoprotein subfractions are among those, and their relations with metabolic syndrome. So we have a complicated process with all the initiation progression, and all the factors that lead to different endpoints, we

have a lot of studies that have different endpoints that we could possibly get apparently different results.

The metabolic factor then includes what we are talking about here today, small dense LDL. Remnants lowered level of HDL or small HDL particles. There are two types of risk factors, then. There are positive factors, and there are markers. So you have to keep it clear when you are talking about what kind of a risk factor, there is a process for determining that.

This schematic represents the smoking elevated H-LDL blood pressure directly caused it, but the lipoprotein subfraction then, experts have pretty much said, these are markers clearly associated with and predictive but not direct causes.

ATP3 emphasizes that we must have standardized tests, and that's what I'm talking about, standardization of the prospect. There is no standardization for

subfractions. We have the LDL cholesterol standardized within our network, plus or minus two percent. The CDC network has LDL plus or minus one milligram per deciliter that interacts with the manufacturers. But there is no standardization of any kind for subfractions.

Again, characteristics of use, a marker must have, right on top of the list, it must be able to be standardized.

Okay, guidelines, let's talk about guidelines a minute, just briefly, the purpose of guidelines, and how they're developed.

Their purpose is to allow the latest scientific evidence to be applied to clinical practice. And there is a process for this, where we have -- it's useful, not useful, or there's conflicting evidence or a divergence of opinion.

And that pretty much describes the situation with small dense LDL, and the

lipoprotein subfractions.

You can categorize where you have evidence if it's just a single study, multiple studies, all the way down to just the opinion, consensus opinion of the experts.

NACB did have a meeting recently when lipoprotein classes, empirical size, were considered. The draft recommendation was that risk assessment is the first step, and second was lipoprotein subclass determination is not recommended.

But let's look at this. That's for initial; that's for primary prevention. It is based on highest A level of evidence in three, then, is the strongest meaning it's just not useful.

Third recommendation, there is insufficient data that measurement over time is useful. Again, this comes from experts' consensus. There is a controversy here, disagreement, against the -- so this is the

process.

But the third thing where it comes to the standardization issue, clearly it's saying that you need standardization of the technology.

What is interesting to me is that there is a divergence of opinion even on this issue in favor of recommending it. But that has to do with some people are saying, don't standardize it. It's not even worth standardizing if it's not useful.

Go back to 2001, the recommendation for small LDL particles, was not recommended because of three reasons. It's not an independent risk factor, it's not standardized methodology, and there's not inexpensive methodologies available.

Of course the third one I think, the inexpensive objection stated, there are methodologies now. But still we're not standardized.

What is the role of the practice

guidelines? The role clearly is to implement state of the art cardiovascular prevention. And the central role then of the physician in this is to translate these guidelines from population studies into advice to an individual, and to exercise clinical judgment in the process.

So if you look at ATP3, the term clinical judgment is used 27 times. That's the spirit of how it's done.

So that's fine there being merit measured now. Is there -- definitely there is an association with risk, and a metabolic syndrome, and a clinical judgment of physicians. A measurement is finding a better way to characterize risk, and they think there is more information for managing treatment.

At the same time this does go beyond the guidelines.

To put it another way, Hawkenson (phonetic) in the Handbook of Lipoprotein

Testing says, intervention studies have shown that small dense LDL predicts the enzographic (phonetic) changes in response to lipid lowering therapy, and converting small dense LDL to buoyant LDL is associated with CHD regression.

So again conflicting studies and conflicting opinion.

Let's go to the standardization. What are we standardizing here? We are standardizing a type of particle that is very heterogeneous. These are -- we have a core that contains the triglycerides and cholesterol esters. We have -- you couldn't number the number of different possible fatty acids involved in all these esters in terms of the chemical composition so that's too difficult to consider, we just assume that's not a factor.

On the outside though then you have the free cholesterol and the phospholipids that this X-ray depiction does