not show.

Clearly this is an understatement. Lipoproteins are heterogeneous. They are heterogeneous in density, size, electrophoretic mobility, composition, functions, and binding affinity.

We get other heterogeneity from just the delipidation cascade will briefly look at the schematic for it.

But here is the thing: we make assumptions about the existence of discrete subject populations, and we call them subfractions and subclasses and subspecies. And we do that because there is no other practical way to approach it. But let's look at real evidence that there are really such things as subspecies for example.

Here's the issue: when you use one physico-chemical property to separate and define a subclass, the correlation with the other properties is lost. You start

losing that correlation.

So this depiction that you see would have you suggest that there is a clear relationship between density and size, and there definitely is a correlation there.

But actually it's a continuum, and the idea that we have of discrete populations within these I think is an issue that has to do with people looking at gels, looking at fractionation processes. And you see bands and peaks.

So from a practical point of view, a working point of view, we have to somehow define them as subfractions or subclasses.

You'd think that in the terminology the term subfraction should have a connotation that you are fractionating, you are separating it. And that is what happening, you're doing it based on a principle, such as buoyant density or size. You can fraction it based on size.

Subclass is more general. You can just name your criterion. You are creating classes.

But the term subspecies, and I'm using this on some of my slides because I've borrowed them, and that's what's on the slide, it means that there are discrete defined species, and I think that is probably not -- we'd have to put in probably hundreds of thousands of subspecies.

The interactions are incredibly complicated as we look at the same design for a globular structure that has the triglyceride and the cholesterol ester inside, and the outside changing composition.

We have different Apos that are providing an organizational structure that these Apos are changing conformation and giving different views from the outside as it carries out its true functions, only part of which we understand. So it makes

analysis based on any of these properties quite a challenge.

Again, all of the different Apos that help identify.

Compositionwise, if we look at these trig, cholesterol, ester, protein, phospholipid free cholesterol, huge range in properties.

And the other thing is, when you are detecting it -- see the reason we detect used cholesterol measurements if because it's easier than measuring the Apos. It's just a more practical thing to do.

And when we are going to measure it in a gel, for example, or anything that's a stain, the composition affects the same properties, the staining stochiometry, staining efficiency, and I'm going to give you relative different concentrations depending on the composition, unless you overcome that in some way.

Again just to point out you can

have genotypic variations in all of these proteins that are involved within all of the myriad proteins within our lipoproteins, and that is a real source of heterogeneity.

Let's talk about how you standardize size. The studies, have a Pattern A, Pattern B, large for A, small for B, and this is one definition that is used. You have some methodology, very sophisticated, a lot of these, to analyze where the mean, or the weighted mean of this peak diameter is, and you have a criterion, say it's going to be tight if it's less than 25-1/2 nanometers, and greater than 25 -- and the question is, this could be standardized, may need some standardization, but why have, for something that is so weak as a phenotype to qualitatively describe it seems like its pointless to spend a lot of effort standardizing that, especially since there are standards, and some effort, could be done for that.

Definitely within these we need some way to say, within a separation scheme, what the sizes are. I think standards exist where this can be done. The Donner lab I think is the source of this data that has assigned sizes to density ranges. You won't see the same sizes published for the correlation between the radiant -- this is the density range in terms of buoyant density separations, and particle dimer by independent methods.

They can be standardized. If we looked across different methods, and this had some of the methods presented today, gradient gel electrophoresis, the naming system is quite different, so there is a nomenclature problem in relating this is very approximate to try to line them up and say, what is the fraction for one method compared to the fraction for another method.

This is -- like for example, these densities and diameters don't agree

with the other table. That's because we're looking at properties that don't correlate perfectly.

Clearly composition affects LDL size. We note triglyceride is a strong factor in the size of the various lipoproteins, but all of the components are changing as this study shows. Big compositional changes.

And if you have a method that depends on staining, and you are trying to get ratios of the small ones and the large ones, then you have to have an accurate staining efficiency, or the same staining efficiency that leaves the same scale for the large and the small to get a ratio or to say which is predominant, or what should be done to measure concentration, in order to be proportionate to the concentration or the particle.

In general we know that as triglyceride concentration goes up, the size

of the particle goes down, and that pretty much sums up that LDL size is predominantly a reflection of the triglyceride level, and it's an inverse relationship.

Okay, for the HDL subspecies it's even more complicated, incredibly complicated. There are 14 bands by gradient gel electrophoresis, and these density, the changing components for Apo, A-1, A-2 and E.

I won't go through all these, but all the metabolic conversions that are taking place, that are changing these constantly, that are making them in vivo anyway a -- each person very -- quite different.

And these factors are all affected by of course by the therapy, by diet, by all the TCE TLC therapeutic lifestyle changes.

So if I'm painting a very complex picture, in the first place, to summarize that, it's difficult to define subclasses.

Measuring subclasses are invaluable though, and have been used for studying interactions, responses, to all of these -- the mutations and genotypes, lifestyle, nutrition and drug therapy.

Let's look at the different methods now. Ultracentrifugation could be considered, and some may consider it, the reference method. There are other variations of density gradient centrifugation where you have iso-picnic where you do sequential changes and isolate fractions.

You can do gradients where you isolate separating based on density.

There have been reports though that ultracentrifugation changes the lipoprotein so that you have different size populations.

Polychrome and gel eletrophoresis and gradient gel eletrophoresis, again, are faster, more practical methods, compared to

ultracentrifugation, based simply on size, very fast.

HDL c, purely a size capillary separation, and tends to keep some of their, you can argue, in their native state. Capillary isotachophoresis, purely a charge-based separation. And it's a technique that will separate for example the HDEL component as a fraction that is associated, and has the CETP and LCAT (phonetic) activities.

So there are many bases for separation. And these have been used.

Let's go through the methods briefly and just hit them. Quantum metrics method, and a lot of this has been described very well today, so this makes it a lot easier.

I'm going to focus on the analytic part of these methods, and the principle.

Separation is based on charge and size by this method. They give a

cholesterol concentration in each subfraction by using a separate source of total cholesterol.

Like all the methods, there is a mathematical deconvolution of areas under scans, and that's because with all the methods, you don't resolve into subfractions. You make an assumption about how many are there, and you have a mathematical calculation to report it as individual subfractions.

I think with the -- the key thing to that is, there's a computer here, and that's what has changed and allowed us to do quantification in gels and allowed a system to make it very practical and fast.

Something that used to be semiquantitative, QuantiMetrix was the first one that took this and made gel eletrophoresis quantification approach from semiquantiative to quantitative. But to point out that there are not -- you do not

have subspecies. You have fractionation, and you define what the fractions are. Since you don't resolve them, you mathematically resolve them.

And this method does compare very well for accuracy, for HDL cholesterol, for the total. So in terms of staining, they can stain the whole collection of them quite well. Whether they can relate this back to the fractions with depends on how uniform the staining is across those subfractions. I don't know if that, for the subfraction, if that would work for HDL and LDL both. That isn't the case.

The Atherotech VAP method. It's a fractionation based on density gradient ultracentrifugation. My point is that again you don't get resolution so you have proprietary software for deconvolution of the profile. Though like this, the algorithm is based on purified fractions, so there is a basis for it.

Again, this is from one of their slides from Athertech. It says the profile, you don't actually get complete resolution, and you have within these bands, within these zones, you are doing mathematical deconvolution to come up with the subfractions.

The method does correlate very well, when they have done studies, and these studies have been done with CDC reference method laboratory network, laboratories, good correlations for the basic components and for subfractions.

Again, for reproducibility, as far as something capable of being standardized, it is quite reproducible. They provide an interpretation then of this depiction of size versus density, and like all other -- all of the companies, we have a risk connotation that is small dense is more risky, and it's desirable to get the more buoyant LDL, and that the HDL-2, 3 the HDL-2

being more desirable. All that is assumed within their profile.

A very report that is consistent with NCEP ATP III on many counts, you can measure where -- a good point is they can measure without triglyceride interference. They have add-on tests for homocysteine, and high sensory CRP.

They list all the components of LDL as defined by NCEP actually has all these, and LDL, they differentiate them.

All in all, they have a risk stratification for HDL-2 and VLDL-3 within their report.

So in summary their test and report is constant with the ATP -- NCEP ATP III, in terms of merging risk factors and metabolic syndrome. They are traceable, their calibrator is traceable to CDC reference method through CRMLN.

They've used comparison studies with our CRMLN laboratories to evaluate and

monitor that, and the subfractions are evaluated as well, not standardized, but they've done things to show the relationship to CDC's standard.

Okay, NRM, this is the same slide, and I wish Jim had explained how this works. We can go through it briefly. There are bases here. But the test, NMR, quantifies subclasses without fractionation, and it provides lipoprotein subclass particle concentration numbers. So it's particle concentration number, as well as a size is provided.

The key to their quantification particle number is that they have a library of more than 30 level signals representing every spectra-distinct subclass likely to be encountered. In other words, within this envelope they have a library of 30 that as long as every sample they measure, if it contains something that is equivalent to one of these things, or similar to one of these,

then you will get a solution, this is what they're actually measuring, that library of 30, if it's robust enough, then every sample they measure will give you this type of a fractionation, and it's broken down from all these into small, medium and large, all the way across the lined, combining adjacent signal envelopes.

The result in terms of reproducibility, let's look at LDL. The CV for any of the components is going to be higher, or more uncertain for these components very small, medium, small, large LDL, high LDL. But for the total particle concentration of LDL they just add those up and you get a very reproducible, very precise measure of particle concentration.

It does -- this particle concentration, this is a small study, it's very controlled, it has the highest correlation. That's from their publication, so you would expect that they'd report their

best data. But all the correlations with Apo B are -- show good correlations. There is a particle number that correlates with Apo B.

The size is calibrated by electron microscopy or size standards by gradient gel electrophoresis, the standards that I referred to earlier.

And I guess the good size correlation, and this was LDL is smaller than gradient gel because there is a different basis for assigning size. I mean there are 30 -- they reference 30 envelopes have size determination by electron microscopy or by grade in gel electrophoresis. In the LDL range apparently it was done by electron microscopy, which gives small numbers.

What's happened with this test is, it's such a small sample size, with no pre-treatment, and it's fast, that there are a huge number of publications, perhaps as a

result now they're dominating the database of clinical studies about measurement of lipoprotein subclasses.

Berkeley HeartLab Method is a method that is based on the really segmented nonlinear gradient gel eletrophoresis. It's based on the research gradient gel electrophoresis that was done most of the earlier gradient gel studies; very close. It separates based on size and charge. A mathematical deconvolution is done to give percent area for each subfraction, and there is some new -- apparently new stains that they'll be doing cholesterol concentration in each subfraction, and even relating it to I understand to Apo B particle concentration, using a total Apo B.

Where all the gel-type methods make the assumption that you have a total for everything under the scan, let's look at one, these are what the gels look like. Then you have a scan.

So you can make an assumption about what's the total under the scan, in terms of LDL, Apo B, and you have that direct measurement. Then you have the relative areas that you can then assign concentrations to, the subfraction.

And like all the methods, this very flat profile does not look like something that is actually separating subspecies. It is fractionating and giving a -- some type of proprietary deconvolution that relates, for example, this area, to a particle concentration, or let's say a subfraction, subspecies, subclass concentration.

Their report then does give you a -- does give a risk assessment based on relative area, and concentration. Here is the relative area. I'm having trouble seeing it. The relative area. Then there is a risk that is associated with it in their report.

Same for the HDL in reference to HDL 2b. It shows the desired treatment direction. They provide progress summaries. This report shows that there are many -- this subfraction analysis is just a small part of all the risk factors that are analyzed. It's not like it's a stand-alone test.

I think one of the unique things about the Berkeley HeartLab Approach is that they use a 4myheart.com database that allows patients to go to see their progress over time. And they have advice on diet, exercise and medication.

And diet is one thing that we know changes the size of the particles, and changes fractions. It's not something I'm addressing today, but that's one of the unknown things, that diet and lifestyles, in terms of therapeutic lifestyle changes, the good diet, and exercise and all that, produce better patterns and less heart

disease.

But the link then to that is in the actual way we assess risk has not been established clearly.

A study was done to compare LDL subclass methods, and essentially in this study four methods were run simultaneously and evaluated for particle size and LDL phenotype.

That's the only thing that could be actually compared, because the tests don't give concentrations that can be compared. The bottom line is that if you are talking about the phenotype, only three of 40 subjects gave the same phenotype in this comparison study.

Look at the difference for particle size. The NMR method, by definition, for LDL is going to give smaller particle size. But in terms fo coming up with the same phenotype, which has to do with small particles versus large particles,

they are apparently not measuring the same thing, the same concentrations, to get this type of -- this agreement.

It's not a perfect study or comparison study, and I was curious about how they could have a LDL cholesterol reported for the NMR method, since they don't actually report LDL cholesterol. They report particle concentration. So that was part of this, I'm saying, this is not a perfect comparison study. But it's worth noting.

You look at the distribution of phenotypes, there was a couple of methods agreed pretty well. This is the gradient gel, and for the B pattern, gradient gel, it's the VAP NMR method.

That's fairly good agreement on type B, but all of the methods, out of all of the samples, as you would expect, during the develop NMR method, I think there were comparisons of the gradient gel. So I would

expect a better agreement there with the VAP method, and it's staying right with them.

So the conclusion that this author made was that subclass measurement is not standardized, and we definitely agree with that.

But predicting pattern A or B can be done as reliably using triglyceride cut point of greater or less than 150; it's hard to argue with that given the data that was presented.

So the conclusion is, these methods, you get method-dependent results, and it's very difficult to compare among studies.

I think each method is probably defining a different subpopulation of lipoproteins. They take a different slice of a continuum of properties that just don't correlate with each other. That's what it amounts to.

The choice of the best reference

method to standardize them, then, is simply not really obvious. We're familiar with it, at CDC, with the density gradient method, and things based on density. But if you did that type of standardization, then you would have to have the other methods make arbitrary modifications that might not be appropriate for just the sake of being, quote, standardized.

What we need is really to get a direct comparison among these methods, and identify then the commonly defined subfractions, and the ones that we think are associated with the rest.

And these should be then characterized. You've got to find materials, common materials, that are characterized.

What's enough to characterize small dense? Is small size and density, or do we need to go with electrophoretic behavior and composition?

We need the material characterized. It's possible you can't harmonize all the methods here, but the goal definitely should be, some kind of standardization is needed of defined subpopulations of the atherogenic and anti-atherogenic lipoprotein particle.

And so their concentrations, standardization of their concentrations should be the goal.

So thank you, and thank all the wealth of people that gave me all this eclectic mixture of slides.

DR. STEELE: Thank you, Dr. Waymack.

I just want to make one comment here. I'd like to remind the public observers at this meeting that while this meeting is open for public observation, public attendees may not participate except at the specific request of the chair.

QUESTIONS AND ANSWERS

DR. STEELE: And the chair asks, does anyone on the panel have any questions for Dr. Waymack? Dr. Tsai.

DR. TSAI: How difficult is it to standardize these? Are you giving us fairly optimistic or --

DR. WAYMACK: I don't know if this is optimistic. The problem, to standardize, you need a reference method, and you need reference materials.

DR. TSAI: And that's size dependent.

DR. WAYMACK: But the problem we're having to standardize is, what is it that you are standardizing? How are you defining this analyte that is a target of standardization?

In terms of, we can -- we've already had problems with like LDL cholesterol. We have defined it as a mixture, and we're having manufacturers come up with tests to get the cholesterol and a

mixture of different types of particles, because that's what the risk, the database, epidemiological database was based on that mix; that's one of the first slides I gave.

So it comes down to, is there data that associates the database, or associating the risk factor with the specific particle. That just doesn't exist.

DR. TSAI: Can you not standardize them just according to particle size?

DR. WAYMACK: Yes, you can. You can do it according to density. You can do it according to particle size. You could come in and do that. And the consequence that methods based on other principles would have to make some type of adjustment to fit that standardization box.

But the real issue is, or should be, how does what you are measuring relate to risk, and how you incorporate that into the treatment guidelines.

DR. THAI: Can I have one other

question? Just very few things are really truly standardized, very few things that we measure in the clinical lab, which is less than ideal.

On the other hand, each -- some of these methods, they don't correlate with each other, have each in itself shown clinical promise, and given that, is standardization absolutely necessary?

DR. WAYMACK: Some kind of standardization is necessary. The question is whether each one might be standardized separately if it had a database related to risk and a way to apply it you could standardize it separately.

But to group them together, and put them all to the same standard is going to require you to come to a common nomenclature, and a common definition of the particle whatever the defined analyte.

DR. STEELE: Dr. Zhang was next here.

DR. ZHANG: I have a quick question. And I realize it's difficult to standardize all the methods. But based on your knowledge, your best knowledge, what are you suggesting in terms of the biology behind this method and correlating epidemiology treatment and the clinical bigger picture? What do you think in terms of -- I don't want to understand the whole methodology, but whether or not there is a biology behind this in lipoprotein, and which one should be a top priority, based on the particle, based on the quantity, based on --

DR. WAYMACK: Well, I think the answer is that we know the LDL particle is the source of the problem. It's that LDL particle concentration, where it's distributed across the different LDLs. Where it's distributed, that is the key thing that we need to be looking at. So any type of standardization of some fraction

should go to LDL particle concentration.

DR. ZHANG: Thank you.

DR. STEELE: Dr. Remaley, please.

DR. REMALEY: Yes. I'd just like to agree with what Dr. Tsai has said, and I guess you agree as well. And I think it's, probably at the outset, it'd be very difficult to standardize all these methods. But they each may have inherent value, and perhaps standardization program aimed at each of the major methods would be worthwhile.

But could you also mention the utility of proficiency tests, and how you would imagine a proficiency test program would be created for such assays.

DR. WAYMACK: That's a good question. I think again that each one I guess would be separate method, for each one, each peer group.

DR. STEELE: Dr. Levinson, please.

DR. LEVINSON: Thank you. I want

to thank you for a wonderful presentation, and I wish I had some of your slides to teach to our fellows and residents.

The only point I wanted to make here is, you mentioned a recent paper in clinical chemistry which I saw regarding differences between methods. And there were a few other studies regarding that, and one that I have here was actually in the Journal of Clinical Lipid Research, you probably saw that one, in 2004, by D.R. Witt and associates. And they found very similar that the -- comparing NMR with gradient gel electrophoresis got less than 50 percent of people classified as pattern B was also pattern B on the other.

So there are -- I just want to mention that there are a number of other studies.

DR. WAYMACK: And really, what is the value of the pattern A, pattern B, the phenotype would be my reply.

DR. STEELE: Dr. Marcovina.

DR. MARCOVINA: Could you go a little bit further on your statement that LDL cholesterol methods are well standardized. Can you define the limit of the standardization?

DR. WAYMACK: Okay. Would you repeat that?

DR. MARCOVINA: Yeah. You made the statement that LDL cholesterol methods are well standardized.

DR. WAYMACK: I don't think they were well standardized. There are standardization efforts through the CDC, as you are well aware, through the network, we work with the manufacturers to have methods going out the door that are traceable to our database, through the network laboratories.

We do not have an LDL -- that as a form of standardization. We don't have a lipid standardization program. CDC does not have an LDL standardization.

We don't actually standardize LDL cholesterol through our lipid standardization program like we do the HDL.

DR. MARCOVINA: Well, if the LDL cholesterol measurements -- I'm not talking about particles, number, size, just the simple definition of LDL cholesterol in plasma, the reference made to separation by ultracentrifugation, the determination of cholesterol by being demarcated. Nobody discussed its accuracy measuring cholesterol.

But you made an interesting statement that ultracentrifugation separation alters the lipoprotein composition. So we are trying to standardize --

DR. WAYMACK: No, HDL cholesterol is the one that's usually cited for that problem, the biggest changes.

DR. REMALEY: So LDL is not? LDL particles are not?

DR. WAYMACK: Are we going to just parse the words here?

DR. REMALEY: No, I'm trying to understand -- I would like to know your opinion on the term, standardization. Standardization means an individual, independent of the method used to determine your cholesterol, is correctly classified. That is standardization.

Do you believe are we at that point for cholesterol?

DR. WAYMACK: Well, the routine methods are not as well standardized as they could be. You look at the results from the CAP which is not material itself, they are not commutable, whatever. And free of matrix effects. But you do see different tests giving a lot different results. You do see a number of tests that agree very closely with our target value.

DR. STEELE: Dr. Winter, yours will be the last question. There will be

another opportunity to ask questions later this afternoon with Dr. Waymack.

But go ahead, Dr. Winter.

DR. WINTER: One of the papers that you reviewed showed poor correlation between A and B phenotype in the four different methods that have been discussed today.

I'd like to ask, is there any data about head to head comparisons among the four study -- or four methods, as to whether one predicts risk better than any other? Because in my mind the public good is not served if there are four different methods, as opposed to two or even one method that would be the best method to subfractionate LDL or HDL and predict risk.

DR. WAYMACK: I think that is the only study that has compared the four head to head. Like has been mentioned by Dr. Levinson, there are some other one-on-one type studies.

DR. STEELE: Okay, with that we will conclude the questions for Dr. Waymack at this moment.

(Whereupon at 10:36 a.m. the proceeding in the above-entitled matter went off the record to return on the record at 10:49 a.m.)

DR. STEELE: All right, we will now hear from the FDA. Scientific reviewer Douglas Wood will be presenting.

FDA PRESENTATION

MR. WOOD: Good morning.

My name is Doug Wood. I'm a reviewer in the division of Chemistry and Toxicology for the Office of In Vitro Diagnostics for the Center for Devices and Radiological Health.

And before I begin my talk, I just want to point out that a number of these slides will seem frighteningly

familiar.

My talk today will cover the subjects of the identification of lipid fractions; the cholesterol pathway; effects of cholesterol; public health concerns; lipid subfractions; pertinent research; and subfraction recommendations as well as conclusions.

All the information provided for this presentation was derived from literature. Information was taken only from peer reviewed articles or texts.

The search criteria for the literature was as follows. All searches were conducted on PUBMED and MedLine. Key words used for searches were used independently and in combination and included lipoprotein, lipoprotein fractions, lipoprotein subfractions, LDL, HDL, cholesterol and electrophoresis.

Some of the articles selection for use were cited in other references that

were found on PUBMED and MedLine.

A complete list of the references I used is available in the executive summary.

Cholesterol: that's why we're here. Cholesterol is a waxy substance that is found in the body -- found in the bloodstream and the cells of the body. Cholesterol is supplied to the body by dietary intake and by synthesis in the liver.

Cholesterol is crucial for normal body function, and is utilized to form cell membranes, produce hormones, and other functions.

Because cholesterol is not soluble in water, it's transported throughout the body via specialized proteins as lipoproteins.

Lipoproteins are spherical particles containing nonpolar lipids such as triglycerides and cholesterol esters in

their core, and more polar lipids, such as phopholipids and free cholesterol, near the surface of the particle.

They also contain one or apolipoproteins on their surface.

Lipoproteins are divided in two basic groups: low density lipoproteins, and high density lipoproteins. Low density lipoproteins, or LDL, contain apolipoprotein B attached to their surface. LDL consists of a trio of particles, and they are separated by size and density.

One particle is pictured here is LDL. In addition low density lipoproteins include very low density lipoproteins and intermediate density lipoproteins.

The other group of lipoproteins are called high density lipoproteins. These lipoproteins have a greater density than LDL, and they have apolipoprotein A-1 attached to their surface.

Please note that on all of my

slides on which you see these graphs or these pictures you will see an A or a B. Those are my artist's renditions of apolip protein A and B. VLDL you will see apolip protein E, apolip protein E C-2 and B.

In individuals with normal cholesterol levels, cholesterol is absorbed, manufactured by the liver, and down into low density lipoproteins or bad cholesterol and released into the bloodstream.

I'd like to point out that although LDL is identified as bad cholesterol, that's not entirely correct. LDL is essential for the transport of cholesterol from the liver to the cells.

Excess cholesterol or unused cholesterol is removed from the cells and transferred back to the liver via the HDL cholesterol or good cholesterol. That's how it works normally. And this is very simplified, with just an LDL particle.

However, when LDL and cholesterol

levels are elevated, the scenario becomes much more complicated. As cholesterol goes up, particles of VLDL, IDL, and LDL become more prevalent. The cholesterol pool -- sorry, my water is deflecting the slide -- my cholesterol pool becomes greater. The cell becomes an arterial wall macrophage, and HDL cholesterol does not transport to the liver to be removed from the body.

If this condition is allowed to continue, excess cholesterol will build up along the arteries in the brain, the heart, and peripheral vasculature, and together with other subsets, can cause plaques in these arterial walls. This is known as artherosclerosis.

If allowed to continue this condition will eventually lead to a completely occluded artery as seen here, which may lead to a heart attack if it occurs in the heart; a stroke in the brain; or chronic vascular occlusion throughout

other small arteries in the body.

According to a report by the World Health Organization cardiovascular disease has been a leading cause of death for years in developed countries. In fact in the United States according to statistics provided by the American Heart Association cardiovascular disease remains the leading cause of death in recent years despite a significant reduction in mortality.

Because of this frightening statistic public health initiatives have focused on an increased effort in the early indication, prevention, and treatment of heart attack and stroke, as well as in the prevention of recurrent cardiovascular events.

Efforts by a number of organizations have led to guidelines available to identify people who are asymptomatic of cardiovascular disease, but who are at a high risk for heart attack or

stroke.

These guidelines include the American Heart Association prevention conferences; the National Cholesterol Education Program, adult treatment panel three; and the National Academy of Clinical Biochemistry from the ACCC which is still in draft.

In addition to guidelines, risk prediction algorithms such as the Framingham risk score are also used to assess global risk of cardiovascular disease. While global risk factors play a key role in the assess of cardiovascular disease and cardiac risk, there are recommended methods to assess cardiac risk that include the measurement of specific risk factors such as total cholesterol, low density lipoprotein cholesterol, or LDL, and high density lipoprotein cholesterol, or HDL.

Because of the prevalence of cardiovascular disease, despite the

significant reduction in mortality, the discovery of new biomarkers to detect cardiovascular disease in patients who could benefit from medical intervention is critical.

Recently a number of candidate biomarkers have been introduced that may emerge as new risk factors for cardiovascular disease. These biomarkers could potentially reduce the risk of cardiovascular disease in apparently healthy individuals.

This slide represents some of these new candidate biomarkers. Of interest to this meeting are two of these biomarkers, obviously: LDL and HDL subfractions.

Recently the FDA has received a number of queries concerning assays used for the determination of lipid subfractions. The purpose of this panel meeting is to obtain input and expert recommendations of the analytical and clinical validity of

lipid subfraction assays.

One of the things that have been brought up repeatedly in the talks this morning is pattern A and pattern B. Pattern A and pattern B came about due to early studies of cholesterol and lipid profiles by Austin et al., in Ron Krauss' lab in the 1980s. They determined two distinct lipid profiles, and they denoted them profile A and profile B, and they've also been called pattern A and pattern B.

And just a basic definition of the two patterns is, pattern A has a lower risk for cardiovascular disease, and pattern B has a greater risk for cardiovascular disease.

Granted, that definition took a lot of research, and put it into very short terms, but that is the basics behind it.

In the course of these studies, profile B individuals were found to have an increased amount of non-HDL apolip protein B

containing particles and elevated triglycerides.

These particles, later to be identified as atherogenic, are composed of very low density lipoprotein, low density lipoprotein, and intermediate density lipoprotein. All of the lipoproteins that transfer from the liver to the cells.

As mentioned earlier, lipoproteins are spherical particles containing nonpolar lipids in their core, bound loosely with protein and more polar lipids near the lipoprotein surface.

Later studies helped establish the presence of a variety of HDL and LDL particles, due partly to the nature of these loosely bound core lipids.

And we've seen this slide a couple of times, so I won't explain it a lot. But as a result each of the micro-proteins can be further divided into a series of subfractions. A variety of

technologies has been developed to separate and measure these lipid subfractions.

For example samples may be fractionated and quantified for density, particle size, molecular weight and/or particle number.

Some investigators have identified significant differences in interpretations of the different technologies used for lipid subfraction testing.

In one recent study, and we've seen this slide before too, Bays and McGovern provided a table comparing terminology of subfractions based upon method.

As you can see, because each technology is different, each technology identifies the subclasses differently, including different nomenclature, and different number of subclass particles.

And as you can see, depending on

the method, we have different names for the particles, different numbers of particles, and even different fractions within the same similar numbers of particles.

When reviewing the simplified terminology of lipoprotein subclasses, the marked differences between the nomenclature of these subclasses is readily apparent. This slide helps show the striking difference in the number and types of particles found depending on the assay method.

This is basically the same slide with a pictograph showing the differences in the particles as identified by these three methods.

In a separate study, Ensign, et al, compared LDL subfractions by four commercially available methods. They took samples from 40 apparently healthy persons, 30 of whom were male, and they ranged in age from 23 to 61 years.

Samples were processed and shipped, as directed, to different facilities for lipoprotein subfraction testing. Each facility used a different method for testing. The four methods used were gradient gel electrophoresis, density gradient ultracentrifugation, nuclear magnetic resonance, and tube gel electrophoresence.

In their comparison, Ensign et al identified a number of differences between the four methods. These differences included differences in nomenclature, differences in expected values, differences in the total number of subfractions was determined to be very method dependent, and Ensign et al identified a substantial heterogeneity of interpretations that existed with only eight percent of the samples in complete agreement.

The first method that we'll take a look at is gradient gel electrophoresis.

In their comparison Ensign et al observed that gradient gel electrophoresis separates LDL into seven LDL subfractions based upon size and shape, as pictured here. The LDL is separated into three patterns. The first pattern is pattern A, with a size range of 26.35 to 28.5 nanometers.

Pattern AB or indeterminate risk of 25.75 to 26.34 nanometers, and small pattern LDL, pattern B, with a size of 22 to 25.74 nanometers.

LDL subfractions with gradient gel electrophoresis are reported as percentages based on the area under the curve for each subfraction. With this method the small LDL particles correspond to LDL IIIa and IIIb.

The findings of Ensign et al suggest that these subfractions, IIIa and IIIb, based on gradient gel electrophoresis are indicators of the severity of the artherogenic profile.

The second method, density gradient ultracentrifugation, Ensign et al observed that with this methodology the LDL is separated into six subclasses based on absorbence curves. And the subclasses are identified as LDL-1 through LDL-6, with class one the most buoyant and class six the most dense.

In this methodology LDL-1 and 2 comprised pattern type A; LDL-3 and 4 comprised pattern type B.

Nuclear magnetic resonance, or NMR. Ensign et al observed that with NMR three LDL subclasses are generated. No references are provided for the basis of the risks for these categories.

Method four, tube gel electrophoresis. This final method Ensign reviewed produced seven possible LDL subclasses, and they are identified as LDL-1 through 7.

In this methodology the

lipoproteins are separated to yield a score. Specific range of scores correspond to different LDL patterns, with normal being less than 5.5, or pattern A, intermediate 5.58 to 8.5 or pattern AB, and atherogenic is greater than 8.5.

Tube gel electrophoresis does not measure LDL particle size directly, but estimates the size by comparing electrophoretic mobility to the mobility of particles of known sizes.

As reported earlier, one of the major differences observed in Ensign's research is the number of subfractions detected as illustrated here.

As you can see each method gives distinctly different results for the LDL subclasses.

In this histogram, Ensign et al describe the distribution of LDL phenotypes. Among the 40 persons they tested for each method shown. The 40 samples were divided

among the type A profile for low cardiac risk; the type B profile for increased cardiac risk; and the type AB profile for indeterminate risk.

As can be seen by the charts, results vary considerably between the methods. Tube gel electrophoresis classified 30 of 38 patients as profile A, seen here, for low risk. Density gradient ultracentrifugation only classified three. The density gradient ultracentrifugation and NMR method classified 21 persons respectively for profile B, or at risk, and the tube gel electrophoresis method only identified two.

Tube gel electrophoresis and gradient gel electrophoresis identified six and five persons respectively as having intermediate pattern, while density gradient ultracentrifugation identified 15.

The lab performing the NMR testing did not report an AB pattern.

Although the methods used for total LDL concentration was significantly different within each assay, within subject LDL concentration was relatively consistent. These findings indicate a degree of bias within each method.

In conclusion Ensign et al observe that the variation between the four methodologies does not allow for data derived from the different methodologies to be readily comparable. As a result this prevents any clearcut conclusions regarding patient results that are not assay specific.

The NCEP ATP III guidelines have established a link between LDL levels and cardiovascular disease. They have also identified the combination of elevated triglycerides and low HDL cholesterol as an associated risk of cardiovascular disease.

The guidelines recommend treatment of individuals at high risk based on LDL cholesterol values and triglyceride

values.

Other study findings have shown that as LDL increases, small density subfractions increase also. This has been establish by a number of investigators that are listed here.

They have also found that as HDL decreases, there is a marked decrease in larger HDL particles. These particles are the ones identified as most protective in the HDL species.

Based upon these findings it has been suggested that elevated LDL, elevated small dense LDL subfractions, low HDL, and low HDL subfractions, are predictive for cardiovascular disease.

Although there is evidence that lipid subfraction profiles differ between individuals with established cardiovascular disease, and normal lipidemic individuals, it is unclear to the FDA whether meaningful and reproducible diagnostic cutoffs for

particle size, density and/or number can be established.

Some investigators have observed that lipid subfraction reference ranges for patient risks for cardiovascular disease as defined by the NCEP versus normal lipidemic patients have considerable overlap.

An example of what the FDA believes to be typical performance of these assays appears in a published study by Morais et al. The considerable overlap observed between the normal lipidemic population and the dyslipidemic population suggests that the concentration of lipid subfractions may not be predictably different between normal and at risk populations.

The FDA is concerned that this type of data could be submitted to support the use of these biomarkers to predict an individual's cardiovascular disease risk, or to determine lipid lowering therapy.

With lipid lowering therapy in individuals identified as normal lipidemic, with values identified as dyslipoproteinemic, as seen in this chart, result in a greater risk to patient's health than the benefit provided by beginning therapy.

Notice on this chart the HDL large range is eight to 43 for normal lipidemic patients, while it's two to 90 for dyslipidemic patients.

Similarly the intermediate size is 18 to 44, versus 13 to 53. Small size is zero to 12, versus 119.

These values have considerable overlap as I mentioned earlier.

The NCEP ATP III guidelines recognize that small LDL particles have been identified as components of atherogenic dyslipodemia, and that some studies have suggested that some HDL fractions may make important contributions to cardiovascular

disease risk assessment.

The guidelines state that LDL particles are formed in a large part as a response to elevated triglyceride. However, while these guidelines assert that LDL subfractions plus elevated triglyceride is associated with cardiovascular disease, they also note that the ability of LDL subfractions to predict cardiovascular disease independently of other risk factors is not well defined.

The guideline also points out that the clinical performance of HDL subfractions has not been established. As a result of this and a ready availability of standard methodologies, the ATP III does not recommend the measurement of small lipid particles in routine practice.

In addition the NACB recently proposed new guidelines for the use of several biomarkers for the assessment of cardiovascular disease risk. These

guidelines are still in draft.

The NACB proposed the following three recommendations concerning lipid subclasses. Recommendation one: lipid subclasses, especially the number or concentration of small dense LDL particles, have been shown to be related to the development of initial coronary heart disease events, but the data analysis of existing studies are generally not adequate to show added benefit over standard risk assessment.

The classification of weight of evidence for this recommendation is, the committee found that there is evidence and/or general agreement that measurement of lipid subfractions is not useful, and in some cases might be harmful based on data obtained from multiple randomized clinical trials that involved large number of patients.

Recommendation two: there is

insufficient data that measurement of lipid subclasses over time is useful to evaluate the effects of treatments. The classification or weight of evidence: the committee found that there is conflicting evidence and/or divergence of opinion about the usefulness or efficacy of these assays, with the usefulness and the efficacy of the tests being less well established.

This conclusion was based on a consensus of opinion of experts in the field.

Recommendation three: several methods are available to assess lipoprotein subclasses. Standardization is needed for this technology.

Again the committee found that there is conflicting evidence and/or divergence of opinion about the usefulness and efficacy of standardization. With the weight of evidence or opinion being in favor of standardization.

This conclusion was based on a consensus of opinion of experts in the field.

The proposed recommendation cited above, and the published reports provide insight regarding the current understanding of the clinical usefulness of these types of assays, and the strengths and weaknesses of these potential biomarkers.

However, FDA's task when evaluating whether a novel assay should be cleared or approved, it's determined whether the assay can be found substantially equivalent to existing assays, or is reasonably safe and effective for its intended use.

For that purpose we focus on the analytical and clinical validity of the assay based on the specific claim or claims that are made when promoting and labeling the device.

The FDA seeks the advice of this

panel regarding whether the clinical use of these devices pose a risk to the public health. The FDA also requests that the panel discuss the effectiveness of these devices to measure and diagnose lipid disorders and atherosclerosis.

Thank you.

DR. STEELE: Thank you.

We have 15 minutes for the panel to ask the FDA questions. These questions should be mostly clarification questions. However, we will have further opportunity to address questions to the FDA immediately before and after lunch if needed.

Any questions?

Dr. Grines.

QUESTIONS AND ANSWERS

DR. GRINES: Since the FDA is responsible for making sure these tests are not harmful, and one of these NACB draft recommendations it was commented that measurement of subfractions are not helpful,

and in some cases might be harmful. Can you clarify that statement? How would it be harmful?

MR. WOOD: The NACB's guideline?

DR. GRINES: Right.

MR. WOOD: I believe the interpretation of the NACB guideline was the proposal of using these subclasses in normal lipodemic patients for lipid lowering therapy, and the possible side effects of lipid lowering therapy on patients that may not need it.

DR. GRINES: But has it really been proven to be harmful?

MR. WOOD: No. As I said, I believe that's what their definition is. I do not have a clear understanding of what their definition is.

DR. STEELE: Dr. Zhang.

DR. ZHANG: When FDA review this on a couple of occasions, have you or your associates looked into actual studies,

reports, not just reviews, association recommendations? Whether or not to really look at -- for example one of the method used for more than 100 publications. So whether or not this FDA review, the group of reviews, would look at these publications.

MR. WOOD: Yes, we did. A number of the publications are actually cited in the executive summary that we reviewed, but there were many many more besides the ones that were listed for this presentation.

DR. STEELE: Dr. Marcovina.

DR. MARCOVINA: You cited several times, doctor, the consensus was based -- I mean the statement was based on consensus of experts in the field.

MR. WOOD: Yes.

DR. MARCOVINA: That means those experts participating in the panel, not experts in the field in general. Shouldn't that be the premise?

MR. WOOD: Yes, I believe it

should be defined that way, yes.

DR. MARCOVINA: Because it could be a huge disagreeance among experts in the field. So it should be noted that there this is limited to the experts that were sitting on that panel.

MR. WOOD: I'm not sure who their paneled for their group of experts, but I'm sure it wasn't everybody in the field.

DR. STEELE: Dr. Tsai.

DR. TSIA: Can I follow up with the same, do we have specific papers, literature, citing the harmful effect of doing these tests? A specific paper?

DR. GUTIERREZ: Can I just interject on that, the statement that says, what it's based on, is a general statement. And there was no specific -- it's a general statement for any recommendation they make. So it's whether they are harmful, eventually could be, but they weren't specifically addressed with respect to lipid

subfractions.

DR. GRINES: But that statement, I mean maybe it's just semantics, but it sounds like a warning. I would interpret the way it's worded.

DR. GUTIERREZ: But it is for general, and it is again the cost. So it could have been -- you have to see whether they found any reason to think that there was any specific reasons for that or not.

DR. GRINES: But as far as we know it's just speculation that's common, true speculation.

DR. GUTIERREZ: When they do these draft guidelines, they have three different classes that they consider us to be, the recommendation and the weight of the evidence. And when they give the people who considered these things, they essentially give them points that are general. This is what you must consider. And among them are whether things are harmful or not.

So it may not be -- it may not be relevant to this specific recommendation or not.

DR. GRINES: You don't know the level of evidence for postulating that it might be harmful?

MR. WOOD: We do not know the level of evidence for that. My opinion was an estimate of what I thought. That's not definitive. It was not defined where their evidence came from by saying it might be harmful.

DR. TSAI: So this whole issue of harmfulness as cited by this particular NACB or recommendation for all practical purposes is somewhat irrelevant to our discussion because you don't have a specific paper, a specific instance, that proves the harmfulness, right? It could be used loosely as in less than cost effective, something of that nature, which is of no concern to us.

MR. WOOD: Well, it could be, yes. However, that's the NACB guideline as it was stated, and I did not feel I should paraphrase the guideline.

DR. STEELE: Dr. Remaley, please.

DR. REMALEY: I would just like to point out, Doug, I know you are aware of it, but the NACB guidelines are now about five years old. And a lot of these studies of course were done since that time, so we should take that into consideration in whatever decision we make.

And also, the NACB guidelines are only draft. There's actually a small number of people on that panel, and I remove those carefully. And overall I thought they were very well developed. But on the subfractions in particular, there were only one or two pages. It's not clear to me how they came up with those conclusions.

And there's literally one sentence on each subfraction.

So I think we have to be careful because they are draft, and because, in my opinion, they are not very well developed, and I'm not sure on what basis they made their conclusions.

MR. WOOD: And that's why I specified they were in draft when I started the discussion of it.

DR. STEELE: Dr. Watson.

DR. WATSON: I'd like to agree with Dr. Remaley and say that I also have reviewed the draft, and they are still accepting revisions, and it's stated that the weight of evidence of this is little c, which means consensus of quote unquote experts in the field, and that's the weakest level of evidence that we have.

DR. STEELE: Dr. Levinson.

DR. LEVINSON: Yes, I'd just like to add to the question about clarification.

So it seems to me from what you said that it really wouldn't make a lot of

difference if a lot of these methods agree or disagree to one extent or another. But the real question is whether or not they agree with, let's say, total cholesterol or HDL cholesterol in terms of classifying patients, because those are the reference markers; is that correct?

MR. WOOD: That's one of the things we are trying to determine, is whether these subfractions can correlate to establish biomarkers, or whether they are safe and effective based on their own use. That's part of why we're here.

DR. LEVINSON: Well, the safe and effective is sort of a different question.

MR. WOOD: Right.

DR. LEVINSON: I'm just talking about, the agreement needs to be with something that is routinely used and generally accepted, so that would be either for it seems to me for LDL subtypes would be either total cholesterol or LDL cholesterol,

or maybe in the future non-HDL cholesterol, but I don't think we've gotten there yet.

And then for HDL subtypes, it seems to me the agreement would be with that total HDL cholesterol.

MR. WOOD: Again, that is what we're asking the panel. Are there methodologies -- or methods to compare these with established biomarkers as substantial -- that's part of what we are asking.

DR. STEELE: Dr. Winter.

DR. WINTER: Is there data in the literature on individuals that don't have the metabolic syndrome, non-metabolic syndrome patients, normal triglyceridemic, normal HDL, normal LDL, as to how common the A versus the B phenotype is?

MR. WOOD: Yes, there are examples of that in the literature.

DR. WINTER: And what are the relative frequencies of the B phenotype in the control population versus the population

that would have heart disease? Because we've seen at least in the comparison again shown earlier between the four methods, there's not consensus even in the control population.

MR. WOOD: I unfortunately don't have any of that with me, and I can't speak off of my head about what they are. But there are papers available.

DR. STEELE: Dr. Zhang.

DR. ZHANG: I have a question related to the subclass or subpopulation, what do you call, has anything to do with treatment.

There is one recommendation or comment on the slides that show this subclass had little to do with treatment over time. My question is, what kind of evidence or population study or clinical study or published peer review of paper make up the study.

MR. WOOD: Those have been

established in -- I'm sorry I don't have the exact number of papers -- but there have been studies showing that in several papers.

DR. ZHANG: Fairly big clinical study, or just a small analysis type of paper?

MR. WOOD: They are not small studies.

DR. STEELE: Dr. Loew.

DR. LOEW: Two questions about the slide you showed with the HDL fractions comparison. You had the normal lipidemic group and the dyslipidemic group, and the various measures of HDL concentration.

MR. WOOD: Yes.

DR. LOEW: The judgments about normal versus dyslipidemic were made how?

MR. WOOD: They were made by the author, and I'm not sure how he made those judgments. This was a poster that was presented at the American Association of Clinical Chemistry. I'm trying to find it

right now, this one. That's the one you mean?

DR. LOEW: Yes, sir. Do you know whether a similar type of comparison has been made for LDL fractions?

MR. WOOD: Not to my knowledge.

DR. LOEW: Thank you.

DR. STEELE: Are there any further questions of the FDA representative here?

No? Okay. Excuse me, sir, do you know the answer to the question that was just raised?

Yes, you may, please come to the mike.

MR. MUNIZ: I'm Nehemias Muniz with Quantimetrix, and this is the slide that was provided by us, Dr. Mora and myself.

The question was, how do you differentiate between these two populations.

It was a very simple clinical distinction. We took the criteria of ATP-3

NACB ATP-III guideline, and we only separated the two populations based on whether they were within the ATP-III for any single parameter -- it could have been triglycerides, could have been cholesterol, could have been HDL, could have been LDL, could have been anything. So it was whether they were within the ATP-III or outside.

So therefore the overlap is obvious. We didn't discriminate for whether -- but if you look at the means of the two populations, you can see that they are very different. But we didn't exclude based on anything else; only whether they were within or outside of that NCAB.

DR. STEELE: Just a moment, Dr. Remaley, please.

DR. REMALEY: The data as it is shown is very difficult to interpret with just the range. As you said you point out the mean is perhaps more meaningful.

It would be of course more

worthwhile to look at the distributions, or better yet, the area under the curve if you've done ROC curves.

And in fact, if you look at, as you know, LDL cholesterol or total cholesterol there's tremendous overlap between the disease and the non-disease population.

So I think the range could be misleading, and what you really need to look at is the ROC area curves.

MR. MUNIZ: Well, that is why I pointed out when I gave my presentation within this slide also.

DR. STEELE: Okay, thank you, sir.

Any further questions for the FDA representative here?

No? At this point we have time for the panel to have an open discussion. We can have general thoughts and comments from the panel. And we will have time later for specific FDA questions, we can address

those later.

But any comments, questions or thoughts at this point?

Yes, Dr. Grines.

PANEL DISCUSSION

DR. GRINES: I guess I'm a little confused, because we have a lot of the presentations talked about how important particle size mattered, particularly if your cholesterol profile was normal; that was one of the things that was predictive of atherosclerosis, and yet other speakers are saying that it's directly correlated with total cholesterol and non-HDL cholesterol and it's not that important.

And I guess I'd like to ask the panel members, how do I -- being a clinician who doesn't specialize in lipids -- how am I supposed to sort this out?

DR. STEELE: Dr. Watson.

DR. WATSON: Being a clinician that does specialize in lipids, I can tell

you, it's just as confusing for me.

And the two things that I really think are missing from this field are, one, population-based studies where you prospectively follow a group of normal healthy people to see how does this really predict disease.

And the second thing is, intervention studies showing you that if you treat people with this certain phenotype, with this therapeutic intervention, you make a difference.

And I don't think we have either of those, which makes it very difficult to interpret all of this.

DR. STEELE: Dr. Zhang?

DR. ZHANG: These presentations are great, but there are two major questions I have, or general comments.

First is the need for subclass. It seems to me you have at the least have three bases. First, you have a biology

behind it. So in other words, you find a subclass or group of subclasses of lipoproteins. They have a baiter molecular mechanism or clinical mechanism, that carbon use the markers.

Second, to follow up on Dr. Watson's comments, it seems to me there is no well designed study use a method -- I don't want to say a standard method across the board -- use a method to do a multiple center study to demonstrate one subclass or group of subclass of lipid proteins have better indication in clinical diagnosis or treatment.

Number three, several comparisons presented here today either with very limited number of studies, for method of comparison, I'm surprised, only have 40 individuals -- 40 individuals -- four zero. It's very, very small number, around 40. You only have eight percent in agreement. Such numbers go to public. Patients were

confused.

So to my understanding, why there is a general recommendation, say maybe harmful, sticking to my opinion as a toxicologist, if you have data or something such markers that go to public, cause confusion. Forty-individual study, only 80 percent are in agreement. And then such interpretation maybe cause patients with borderline, whether or not he or she should go for treatment, most of the drug will have side effects.

If an individual do not need such a treatment, you go for such a treatment, or even don't have a marker we use for followup, whether or not it can be used for monitoring, treating effectiveness or side effect, such marker can be harmful.

As a toxicologist, this is my interpretation; not to the panel men, whatever. Strictly personal as a toxicologist.

This is why I think the three points just in general, I'm not going to mention this method.

DR. STEELE: Dr. Levinson.

DR. LEVINSON: Yes. Well, I would say that over the years, following these stories, I've come very much to similar conclusions as Dr. Wade seems to be indicating in his presentation.

But the question here is not -- it seems to me -- is not whether or not -- and I would say that when clinicians come to me, and they do sometimes in the lab, I tell them they will get no more useful information out of these tests.

Nevertheless, this doesn't seem to be the question. The question seems to be whether or not these tests agree with maybe total cholesterol, and give that same kind of information, or HDL cholesterol, and give that same kind of information.

And I must say that's not

something I've really paid a lot of attention to, but I'm not sure that that's not what we have to focus on.

DR. STEELE: Dr. Shamburek.

DR. SHAMBUREK: I think there are quite a number of issues coming up. From a clinician's point of view, it is very confusing, just trying to interpret these different methods.

And we've heard today that their different properties, and it's often trying to lump them altogether as one pattern A or pattern B.

We say with this study looking at different methodologies we are not going to get that.

You can look at an individual study and there are several questions. One, will it predict in different studies, say a clinical study, the same result over different populations?

Or the other question is, are

there other risk factors, HDL, triglyceride, non-HDL, that will predict is just as well?

One instance could be with the pattern A/pattern B and there are quite a number of clinical studies that suggest the small dense LDL is predictive, and change with statin treatment.

Then you can look at another study, another large study like the CARE trial, which saw a 24 percent reduction in coronary events with a statin, but there, the small dense particle, the pattern B, was not predictive.

So you have several trials where you are not showing it. So the question is, is there enough evidence to suggest you should be using it, one, as a diagnostic thing, one as a way of following it.

And that gets into the issue of risk. Are we going to be able to allow physicians, allow patients, to treat based on this? Are we going to abandon the LDL

levels? We saw one of the slides here with the Ensign study that was shown where, and you can argue about the validity of it, they tried to show with three of them the LDL concentrations, and the mean concentrations in those of 130 versus 180, and again, you could argue whether the NMR was a valid way to do that.

But if you say, in the mean values for these same 40 patients, you are varying from 130 to 180, that's going to be quite different in our already defined, well established criteria for treating patients. At 180 you may certainly consider considerable treatment in that patient versus 130, depending on other risk factors.

But I think there is confusing data with studies. And I also think we have to really consider whether or not our currently ones we have, non-HDL triglyceride, HDL, is enough for clinicians at this point.

DR. STEELE: Dr. Winter.

DR. WINTER: I just want to make a comment that obviously atherosclerosis is multigenic, polygenic, there are lots of factors that cause atherosclerosis. And I wouldn't predict any one test of lipids is going to be 100 percent predictive.

I think people can be normal lipidemic, probably not have any abnormalities in their lipids, and get atherosclerosis because of hypertension in the family history. And I don't think we need to look for markers that are 100 percent predictive or correct.

But if we can find better markers at present, then who should they be applied to? Again, from a public health point of view, if people just looked at the NCEP guidelines, that would probably be a great improvement, looking also at weight, diabetes, prevention of hypertension.

DR. STEELE: Dr. Remaley.

DR. REMALEY: I think it's worth repeating that our current tests are inadequate. You know half the patients at risk aren't diagnosed with our current tests.

So I think, obviously this is an important unmet need. I'm not sure that this is the full solution of course.

But I also think we have to make a distinction that my view is we are not here to make guidelines in terms of use. We are supposed to help guide the FDA in terms of whether these tests will be available.

And I think that is an important distinction, because as Dr. Winter said, this is a very complex disease, and there may be a subset of patients where the small dense LDL is very valuable, and this may be obscured by the fact that when you do these large trials you lump them all together.

And also I think we have to make a distinction whether we're -- again, I

don't think it's our role here or whether tests -- I don't think anyone here is advocating it as a screening test, but whether it should be used as the NCP recommends, which I think is the existing recommendation for at least the small dense LDLs, and an ancillary test for people who are past that intermediate risk, and about 40 percent of the population is at intermediate risk.

I think people who have two or more risk factors, they don't need any extra test. They should be treated. And those who are low risk may need this test. But clearly people who are intermediate risk, and that's a large part of the population, we have a dilemma here. Should they all go on statins?

And I think that's where these ancillary tests could be useful, and it's a very complicated question because it's a complicated disease. And I think it's up to

the connoisseurs or the experts to decide whether they want to order the tests and whether it's available. And I think that's the role of the FDA, whether to make these tests available, not creating guidelines.

DR. STEELE: Dr. Marcovina.

DR. MARCOVINA: Based on the fact that the standard lipid profile, that takes only -- practically less than 50 percent of individuals that had a risk of coronary artery disease. I believe that we really should be open to see what are the tests, can be out there, that we are not discussing replacing I guess at this point in time. We don't have the body of evidence that we have of lipids that we have collected throughout the years.

But what tests can be added? What are the requisites for these tests? How do we judge these tests? Just simply based on the simplicity of a make-up or because they are cost effective in all --

certainly based it on the fact that they can be potentially harmful to patients.

How they can be potentially harmful is already harmful for those 50 percent of patients that we are not recognizing by using the lipid profile. So we are harming our patients anyway by not recognizing them.

So I believe that we should at least be open and evaluate each method independently and look at the clinical evidence.

DR. STEELE: Dr. Levinson.

DR. LEVINSON: I didn't raise my hand, but I did have a question, and I was going to -- or a statement, I should say.

And that is, you know, the question is, maybe, but I don't think that's the question we're here to answer, and I agree with just about everything that was just said, that is, do we have something here that is substantially better than we

had before.

And the area under the ROC curve, usually if you take non-HDL cholesterol or LDL cholesterol and HDL cholesterol and all these other risk factors, so-called metabolic syndrome, lumped together, if you want to go that way, then the area under the ROC curve is usually about point eight, that's usually about the best you can get.

So do we have something that can get our area substantially higher? Because when that is translated into actual Bayesian prediction values, it doesn't come out to be very high.

And you know I would say probably this is not the way to get that at this point, much higher, and I don't think anybody has been able to show that, that it does that.

Now I know in some of his articles Alan Tall talked about what we need to measure in terms of HDL is the flux of

HDL out of the macrophage, okay, but surely that is not something we're in a position to be able to do even if it's true.

So unless somebody could show that something is really a much better predicter, and could get a much better area than that, even along with other factors, then I don't think we have something that is going to add substantially to what we can already do.

But I'm not sure that's the question we're here to answer today. So.

DR. STEELE: Dr. Grines.

DR. GRINES: My comment was going to be that I do perceive that there is a need to try to get more information. Just having these devices available in clinical settings will allow groups of physicians to analyze the data and look at their own patient population.

But I do a lot of acetomy (phonetic) research, and I can tell you that

there are an enormous number of people who come in with infarcs who have met the guidelines with regard to their cholesterol.

The average person coming in with their infarc has an LDL cholesterol in the 120s. And so one fair diagnosed with an NLI (phonetic); of course that is no longer acceptable. But they meet the guidelines up until the minute they have their infarct. And we're still seeing a lot of those patients.

Furthermore, there is a lot of evidence on regression of atherosclerosis where patients who meet the guidelines are continuing to progress rather than regress, and maybe the guidelines aren't strict enough in that regard.

But I have to think that additional knowledge with these subfractions might allow us to figure out who those progressors may be.

DR. STEELE: Dr. Watson.

DR. WATSON: I agree with all these points that currently there are patients still at risk because of -- with normal lipids and that are meeting guidelines.

But I also very strongly believe that there could be harm. And it's not just harm in using this data to overtreat patients. But the big thing I see -- I get tons of referrals for this -- is people use them as an excuse to undertreat. So someone sees an LDL of 180, but they get the subclass distribution; it's all type A; and they say, oh good, I don't have to do anything.

I think the problem is that clinicians are very confused by all of these different assays, what they mean, how to use them. And so I think -- I do agree that these tests should be available, but I think there has to be some way of standardizing it so that people understand what they are;

that they are not to be used instead of guidelines, but maybe for additional risk assessment and that type fo thing.

DR. STEELE: Dr. Tsai.

DR. TSAI: I again agree with all of you, and I -- so far I'm just going to address Dr. Watson's point to begin with. Because I think -- as you know one of the papers that is presented is the MESA paper that does address the fact that just because you have large LDL does not mean it's not harmful. It's a message that is not totally well understood. And there is a lot of confusion.

Nevertheless, going back to Dr. Grines, and Dr. Marcovina and Dr. Remaley, is that currently when you run the risk fo underdiagnosing the patients, and as you yourself just mentioned.

So I think balancing the two, I see the risk of having too many tests that are not standardized available. On the

other hand I also see in the current situation the largest, the most important risk, is that we are not capturing most of the patients.

When we see our neighbor dying of MI just like that because his cholesterol may be normal.

So balancing the two, I would rather see more tests. Would I like to see less standards? Of course. Would I like to see more research in comparison and regulate? Of course.

But I really think at this point, yeah, the fact that clinicians do not understand the utility of this test falls -- the burden should fall on the pathologist who introduced these tests to educate the clinicians.

But we are going beyond that. So I think in sum there are some usefulness of these tests, and we should let these tests sort of run its course, and see how useful

they are.

DR. STEELE: Any further comments or questions? Or, excuse me.

DR. LEVINSON: Yes, I was just going to make one comment also.

I think there is a big distinction. I think from the research aspect, clinical trials diagnosing patients with abnormal profiles, these different tests have added immensely to our understanding.

I think the process of what's going on with treatment is a dynamic one. And I think we are all pondering over how do we move from a research aspect where there are probably teasing out where it's important to a clinical aspect without totally confusing people.

And I think we have some who would say, well, we have a study with 40 individuals. What if we had 400 individuals? Well, I think we would get the

same result. I thin as someone said earlier, we are looking at properties that don't correlate.

So perhaps we need to be looking at the individual tests and seeing how they have clinical utility, and this is something I don't think we have.

Maybe we can't be saying everyone should be able to put a pattern A and a pattern B. Maybe they have their own defined criteria.

But I'm not sure we're yet to jump in the clinical realm, as we're hearing from a number of individuals.

DR. STEELE: Dr. Levinson.

DR. LEVINSON: Yes, I'd just make one other comment, and that is, when Austin and associates identified the subclass B, the atherogenic phenotype, and that was defined as being genetically a true phenotype, but now it's become more and more apparent that people who are insulin

resistant and overweight, that this phenotype can also be environmentally produced, not only kinetically produced. And perhaps now more people are appearing with this phenotype that are actually environmentally produced.

So the real thing from a clinical point of view is to deal with the questions of overweightness and insulin resistance since we are getting into that, as opposed to looking at this subtype or that subtype.

DR. STEELE: Any further comments, points?

Well, we managed to get a little ahead of schedule, and we will break for lunch.

I will remind the panel members not to discuss the topic at lunch today. And we will meet sharply at 1:00 o'clock.

Oh, correction. We'll make that 12:45 so we make sure that some people I know have to get out, and we'll try to get a

little early: 12:45.

And I might add, please leave the room as expeditiously as possible. It will be secured by FDA staff during this break. Please take any personal belongings you may want with you at this time, and you may not be allowed back in the room until we reconvene.

(Whereupon at 11:51 a.m. the proceeding in the above-entitled matter went off the record to return on the record at 12:53 p.m.)

DR. STEELE: If we can start a little early, we'll be able to finish maybe on time.

I would like to call this meeting back to order, and at this point we still have general discussion.

Anybody on the panel would like to make any further comments? Bring up any

new issues?

Yes, Dr. Winter?

DR. WINTER: I'd like to find out what the reproducibility is and long term stability of the various assays and what they use as reference materials.

Maybe this is a question I should have addressed to the FDA speaker.

MR. WOOD: Actually, in reference to that question, the actual industries themselves will have to tell you what the reproducibility of these studies are. We don't have that data, so if you want to bring them up.

DR. STEELE: Is there anybody from industry that would like to make a comment to that question or answer that question briefly?

And please identify yourself and say where you're from.

MR. FRENCH: Kenneth French with Atherotech, Birmingham, Alabama, the