at

DR. NAKHASI: I have a question. What would you say about the sensitivity and specificity if you compare NAT versus the serological because we know serological tests, they have a lot of problem with specificity.

DR. LEIBY: I think, in this case, the sensitivity and the specificity in this is quite good. Because of the sequences, you can identify with the kinetoplasts so it really can be quite specific as well as I showed you sensitive.

There are problems with serologic tests with some cross-reactivity with other agents and, certainly, the false-positive level plays into that. But we have seen enough cases where individuals are clearly serologic positive but PCR negative, NAT negative. So if you just had a NAT test alone, you would miss those individuals.

DR. BUSCH: Any work with leukoreduction? Does that remove those organisms, the leukocyte filters that are being "universally used?"

DR. LEIBY: To my knowledge, there is one published study that looked at that. While it knocked the numbers down, certainly decreased it, it didn't eliminate all the parasites. Now we going to go back to the same story. If one gets through, that is one too many. It is certainly that as well as pathogen activation is another

at

issue that some people have raised as a potential for eliminating T. cruzi.

DR. NAKHASI: Thanks, David.

I think the last talk is detection of possible bioterrorism agents in the blood, Steve Kerby. I think you better be sure and finish in twelve minutes.

DR. KERBY: No trouble.

Detection of Possible Bioterrorism Agents in the Blood

DR. KERBY: When I was first asked if I could do this talk, I said sure. I said, "What do I have, an hour?" And they said, "No; you have fifteen minutes." So I guess I have five. But no worry. I am one of the first people in the world that can probably give an hour talk in five minutes.

[Slide.]

Basically, what I want to do is tell you about detection of possible agents, not necessarily all-inclusive as has been done here before. What I basically want to say is most of my work was done at the Diagnostics Systems of USAMRAD at Fort Detrick. I have only been at FDA for a short while. Also, I was invited to give a talk here to the FDA not long ago when I worked there and so, basically, if you have heard this before, if you are in the pangs of

at

major caffeine and carbohydrate deprivation, you can probably go ahead and leave while my back is turned.

[Slide.]

Basically, USAMRAD is looking at all kinds of an integrative approach for diagnostics, all kinds of classic microbiologicals. It is not necessarily equal because my preference was in these two areas, maybe not much with sampling but definitely within rapid diagnostics.

At USAMRAD, we did both what they call floored deployment which is a rapid diagnostic test and we did confirmation which was a more sensitive or may take longer to develop.

[Slide.]

How we would use it here is basically maybe the diagnostic tests that are developed at USAMRAD could be used in donor screening whether it be blood or even saliva or a quick screening of the donor. Maybe the confirmatory test would be definitely probably within the range of the high specificity. Again, this is probably not mutually exclusive. It probably could be done both ways.

[Slide.]

My first comic relief is probably this little sign here; which way do we want to go with rapid diagnostics? We are probably pretty good at being scientists that we have a point we want to get to so we

at

plot out our strategy. But there are actually a lot of nice scenic routes out there that maybe don't look at one little area. Think about expanding. Look into the bioterrorism agents.

[Slide.]

The quick existing challenge that we had before was basically most of the stuff that was in the old days, it would take about two to four hours to do specimen prep. Amplification was generally one to two hours and detection was one to two hours also.

Obviously, we want to change that. So, basically, we have a quick specimen and we are going to do it in less than five minutes and maybe do a hand-held device. The Spot McCoy at StarTrek recorder would be a great idea.

[Slide.]

What we have now is actually what we are trying to look at, process, amplification, detection all in one as a floored deployment. When this cartoon was made almost a year ago, it was a prototype thinking about it. As it turns out, in the last couple of months, USAMRAD has actually been working on this device. It is as third-generation MAT-C from Lawrence Livermore National Lab.

[Slide.]

at

Also, they found they had lots of problems with them, as in this case, what they could do for specimen processing. The idea is that they could go ahead and take whole blood and put it into separate--a laboratory in a box, if you will.

[Slide.]

Typical specimen-processing options we generally have are like the automated work station people have seen before. Everybody has got one. This is a Kaigen CY 600. There are other ones with a portable device. Hohawks was bought out. Extrana or Visible Genetics or it doesn't matter. It was somebody. And we have paper-based message which are S&S isocards, isoreaders.

[Slide.]

Obviously, the gene-amplification section option, foremost was the Perkins-Elmer BioSystem which you have heard about. It has been commercially available for the last six years. USAMRAD had it about ten years ago. They, at first, didn't like it because it was too slow, too heavy, too large, too expensive. They promised that it would be a real-time amplification and, ten years later, they still didn't do it. I have heard now they basically have a new machine out. I think it is 9700, 6700, which actually does do real-time amplification.

[Slide.]

at

The typical chemistry, which you all know about, is the two-primer system with a quencher and reporter. As you amplify this, the reporter gets clipped away from the quencher so you have a light emission. Last night, somebody said they had eight. I thought there were only five. There are only five I know about. I would like to know if there are eight.

[Slide.]

Typical of real-time amplification, you would have what they call a cycle threshold. You would run this for a number of cycles. Then, when you start seeing a decrease away from the baseline and this is what would be called a threshold cycle where you would actually determine it being positive. The trouble before is that you had to run 34, 40 or 50 cycles and come back and re analyze it, so it wasn't real-time. They are doing it now.

[Slide.]

So what we have is we actually we have other machines. Here is a Cepheid Smart Cycler, about the size of a battery. It is a real-time diagnostic tool. It is a 16-chamber unit. This is what the Army has been using as their field device. It is called the XC. Again, the same thing, your 16-chamber unit for doing PCR.

[Slide.]

at

This is Idaho Technology, which, if I am not mistaken, Roche bought out recently. Again, it doesn't matter. This is what the Army has been using for actually point detection. It is a typical light cycler which used to be the Idaho Technology's LC50 which put to recordize a chamber.

[Slide.]

Again, you could probably get this off their internet since it is pretty much their characterizations of Cepheid of Idaho Technology. The TaqMan was a typical 96 well. They are talking about changing to a 384 format. Cepheid is pretty much 16 cycles but can be piggybacked on up to four more units onto one computer. Idaho Technology is 32 sites on one computer, again more characteristics.

[Slide.]

Again, USAMRAD's idea of possible disease threats; it is quite a variation. There are lots of infectious diseases. There are lots of biological threats which you have all heard about. I'd like to let you know there are 58 more that USAMRAD has actually been looking at.

[Slide.]

This is CDC's Category A which is the three categories. The only reason I highlighted it is this is where most of the emphasis is being put on because it is

at

either a--because it is Category A, it is probably a very lethal one. It is highly infectious. Two or three of them which are highly contagious. But I don't want to necessarily harp on this, the point being is would you really want to do a lot of work on something that the patient may be dead in two days, anyhow. So would you rather have a transfusion-transmitted disease? I don't know.

But, again this is probably what people are playing with the most so the idea is you probably would have to look at it.

[Slide.]

Rapid-identification approach at USAMRAD. I just want to basically show you that there are all kinds of stuff they have been working on for all kinds of diseases. Basically, looking ahead, we have got primers for everything, and even things that haven't been decided yet.

[Slide.]

So the trouble is we are looking at it, and we are trying to avoid technological surprises. So, biomarkers, most people, you could look in the literature and at USAMRAD, also. A lot of the work has been done on the specific virulence markers. A little bit more, maybe, on genus and species. But we are just basically scratching

at

the surface. We want to go deeper into and get more diversity and depth into it.

Common pathogenic markers would be a good one. Host response would be a great one, too. Typical things, you talk about, well, you talk bacteremia, you talk viremia. All these are the result of some sort of agent or infectious disease but, as soon as it gets to that stage, pretty much the body is responding the same way. So it might be a good way to look at markers that actually not necessarily say what the disease is but say something is going on and then just look further.

[Slide.]

Again, my second comic relief is the idea is just to avoid technological surprises.

[Slide.]

Basically, what I want to do today is hit three different areas. We took a spore-former bacteria and a virus basically just to look at our proof of concept of taking the typical gel-based thydiumbromide stain and taking it to a TaqMan machine and then later on into a Cepheid light or a light cycler.

[Slide.]

Again, if you have read the newspapers and watched CNN, you are an expert now on anthrax. So you don't need to know this. Pretty much what I wanted to do

at

is just basically tell you the targets we are looking at. Pretty much, we are like everybody else. We are looking at the virulence factors first using them as a tool.

[Slide.]

Again, plague. The only thing I want to point out here is Yersinia pestis which is plague-associated, there is a unique plasmid which has lots of different nice little genes. It is used for a tool. Yersiniosis, which we are not as much concerned about.

[Slide.]

And then the virus we are looking at are Ebola and Marburg. We are looking at--I want to backtrack. The idea is that a lot of work was done on small pox and I didn't want to present it because it wasn't my data and I always like presenting my data.

[Slide.]

So we were going to hit a little bit of the viruses. Again, a typical genomic amplification. We are looking at targets that were either in the biochemistry aspect of it or different protein profiles.

[Slide.]

Nobody cares about goals. So, basically what I want to do is we will go on a little faster. You may or may not be able to match it. It all depends on how much money you have.

at

[Slide.]

Basically, the typical design is we want to take those primers that are already in existence for the gel-based approach and go ahead and put it into the TaqMan. This is pretty much your rule of thumb of what is available out there.

[Slide.]

A typical profile again as you look at the different concentrations. Let me back up--okay; different concentrations. What I did here is just to show you I lied when I said all these slides. Basically the idea is this is a summary slide. We took all those things and first did a primer protodesign, ran it and then we backtracked and said, okay; can we optimize this more fully, develop a CT faster, a threshold or even more robust.

We did a lot of work of changing primer design, primer concentration, primer probe, magnesium chloride, Taq polymerase. You name it. Major process. Major amount of work. For all that work, we virtually have nothing to show for it.

So, basically, what is happening, the threshold cycle is the same regardless. If you look at robustness, there wasn't much difference between the two concentrations pre-optimization versus post-optimization. So this

at

Bacillus anthraxis one was a complete disaster for us. But, again, it still worked.

[Slide.]

Nobody cares about gels, either. Just the idea is that we have fluorescence and the idea is that it was an amplifiable product that can be seen on a gel.

[Slide.]

Then, again; sorry it is kind of dark. This is just a summary of what I said before. If you look, the CT values, the threshold cycle, is pretty much the same regardless of if it is optimized or after optimization. You look at fluorescence. Really, not much changes. Maybe a little bit slightly so. A lot of work for nothing.

[Slide.]

Going into Yersinia pestis, on the other hand, was a major difference. We didn't know this beforehand, but the point is if you look at the difference between what we call the preoptimization versus the postoptimization--here is the pre, there is the post.

[Slide.]

Again, looking at it, the CT didn't change much but the robustness of the assay did. So, in that case, all that work we did to it, the technicians didn't go up and quit on us.

[Slide.]

at

Again, the gel. Nobody cares.

[Slide.]

And a summary of the page is pretty much we, again, we saw the CT is the same. But, again, if you look at the actual readings of the fluorescence, major increase, a major robust assay difference.

[Slide.]

Yes; again, I hat to say this is the way we do it, but the point being is that even at USAMRAD, nobody likes to give us material. Even within the same division, even in each department, you have to fight, plead, beg, steal, whatever the case may be to get some of their material. So basically, we had to do what I call proof of concept, obviously.

So what we did was, even though I hate it, you go ahead, we spiked blood to show what happened. We spiked some serum. Again, good assay.

[Slide.]

And we spiked the plasma, getting good assay, no changes.

[Slide.]

Future strategic plans. Again, this is probably badly worded, but the point being is I wanted to say is that even though USAMRAD has a lot of stuff, CDC has a lot of stuff, some of it doesn't get out into the public

at

information. What we would like to do is see, actually evaluate the risks. Back in my pre-FDA days, I used to laugh about, it would be great to have some human trials done especially with the lethal agents, maybe volunteer some lawyers to do it. The trouble now that I am with FDA, I find out that we probably will have to go and challenge that as being really a human trial.

Then evaluate existing NAT detection and BTA agents. We want to do more of that. What is out there already? Can we adapt it to it? Can we take the non-blood, as we say? A lot of the stuff is done with soil, water, air, food, fully adapted into a blood system and then the biggest point of this is what can we do now? What is out there?

We talked a lot of stuff about HIV, HCV. We are looking at more emerging pathogens. Let's go ahead and look outside the box, as they say, or, as my cartoon showed, maybe not take the same pathway, do a different one.

The final thing, which I lost, is basically, as has been said before, we have three outbreaks every time there is an outbreak. There is an outbreak of the disease. There is an outbreak of fear. And there is an outbreak of meetings, which, apparently, this is, like, one of them. That is why they invited me here.

at

But I wanted to use this opportunity, actually, to sort of plead with you. I am not sure FDA wants to be the hub of a wheel but we definitely don't mind being the spokes of something, the spoke between the wheel. So the idea is that if there is something out there, if somebody would like to take the lead and actually develop more symposia, getting most of these people, getting USAMRAD to maybe come and divulge some of this information that we are not supposed to do--or, I'm sorry; they are not supposed to do.

The idea is that this is basically what it is, sort of a plea to get out there and start getting this coordinated.

That's it.

DR. NAKHASI: Thank you, Steve, for a very hilarious and comical experience. I think everybody woke up from sleep. I think that is very nice, a very nice, really, presentation. Any questions, now, on the serious side?

DR. BIANCO: I liked very much this session, but I would like very much to hear what is your feeling in terms of bioterrorism and blood. What are the issues? I think that we are kind of secondary in this chain because of the way we work because our lots of product are so small.

at

But what is your feeling as you discuss those things?

DR. KERBY: You are probably right. What I tried to hit on is, if you look at the three different categories, I hate to say not to look at it, but I would really like to see more people look at Category C, if you look at the CDC. So the hanta viruses, Denghi fever, stuff that does have a large window period to the point that it actually may be used.

The idea of CDC is--well, USAMRAD's also--is that the first category is probably the most dangerous because it is lethal, but the point being is how it is going to effect us is probably not as big. I don't want to say not look at it because you can actually use this since this is where maybe most of the money is at USAMRAD and maybe elsewhere. You are going to have to follow the money trail.

But, while you are looking at one organism, why not look at something that is related to it, another organism. There are a lot of surrogate markers out there. Go ahead and show proof of concept with the surrogate markers first and develop it more.

So, to answer your question, I would say I would not eliminate thinking about it, obviously, because the bioterrorism, perhaps, with two or three days, you may not

at

worry about it for a blood transfusion. But I would definitely, as has been pointed out before, the major ones in Category C and maybe some in B are major, may be major, problems. And these may be as likely because, again, my paid political announcement is the idea that if we had to have an outbreak, I was glad it was anthrax. I am sorry for the eleven people who died, but eleven deaths in a risk analysis is not very good and the amount of benefit we may be from the actual outbreak is going to really exceed what we could have gotten otherwise.

Again, if you read the paper, it used to be there are 470,000 different people or individuals had anthrax spores. And then you read the paper later, it is, like, okay, it is the Ames strain now and now we are pretty much down to, like, 10 organizations and now we are pretty sure USAMRAD is the one that disseminated this.

Well, USAMRAD did disseminate a lot of stuff but it was pretty much laboratories that knew what they were doing but the point being, and what the paper doesn't say, is the Ames strain wasn't the Ames strain. It was something else before 1985, so the point being is somebody may have something--I am not saying they are not going to track it down. So I am probably getting off on another tangent, but the point being is you really don't know what is out there until you start getting more material. Don't

at

look at one organism. Look at bunch of organisms. Try to find surrogates.

Present data and maybe put some pressure on--I hate to say it, USAMRAD was pretty much a closed fist for quite a while. They started opening up before this last terrorist attack and they have gotten very busy. So they are probably not going to be very informative with us for the next couple of months. But I think, after it gets over with, they might be more relaxed. So to get CDC, USAMRAD and other organizations out there, get GenProbe, get Roche and people involved because there are machines out there that could be used, could be adapted, to other organizations.

I am not sure if I can answer your question, so I got to spend five more minutes. So thank you.

DR. NAKHASI: Short answer, Celso, to you is that I think we want to be prepared in case there is a catastrophe bioterrorism attack and the agents which have a long incubation period, so they don't go out and keep on donating blood. I think we need to be prepared for that.

Any more questions? If not, I think we still made it. Let's eat. It is 12:35, so we are only five minutes late, and be back at 1:30, please, because we want to get the next session started.

at

[Whereupon, at 12:35 p.m., the proceedings were recessed to be resumed at 1:30 p.m.]

at

A F T E R N O O N P R O C E E D I N G S

[1:35 p.m.]

VIII. New Technologies (Duncan/Kaplan)

DR. DUNCAN: Welcome to this afternoon's session. As we moved from the existing pathogens like HIV and HCV into emerging pathogens, now we are going to begin to move from the existing technologies, like the NAT test, into emerging technologies.

In introducing this session, I would like to bring your attention to the NAT Workshop that was held in 1994 at which many people left feeling that there was really no practical application for the nucleic-acid test. And here we are in 2001, seven years later, where this workshop has essentially been a showcase of the success of the application of the NAT test to blood safety.

So that sort of begs the question what technologies that are merely in the research lab today are going to be in clinical application seven years from now.

So, to start this session off, we have invited Dr. David Peterson from the Advanced Technology Center of the National Cancer Institute who is going to give us an overview of new technologies especially based on his experience with the microarray.

Overview of Microarray Technology

at

MR. PETERSON: Good afternoon.

[Slide.]

I thank you guys for the invitation. Hopefully, you had a nice lunch and now that all your tummies are filled, I will try not to put you all back to sleep.

[Slide.]

I am going to give a basic overview of microarray technology and try to touch on some of the topics. What we do at the NCI is mostly expression arrays but, hopefully, you will able to project how that will apply to other technologies. I am the Production Manager of the NCI Microarray Facility. We have been making microarrays for the past three years and giving them to the NCI community for work in their experiments. We also provide training.

[Slide.]

So when we say microarray technology, what we are talking about is DNA that has been immobilized on a solid support in an ordered array in such a way that you can then take your sample which, for expression arrays, is usually RNA that has been labeled and then detect thousands of genes in a single experiment.

This is what we mean when we talk about gene expression. I am sure you have all heard the buzz word "functional genomics." That is one of the applications of this technology.

at

[Slide.]

The DNA that is put on the arrays is either oligos or it is cDNAs. The oligonucleotides can be synthesized in situ with photolithography and I believe there will be an Affymetrix person speaking about that later. There is also a synthesis process using ink jet which uses a phosphoramidite chemistry on the solid support. You can simply have the oligos presynthesized and spotted using a robot.

[Slide.]

So the oligos are anywhere from 24 to 80 bases in length. Obviously, they have to be designed. So they are selected from the database, whereas cDNA arrays are typically from clone libraries that people have collected and they are PCR amplified, purified. Once you have the purified cDNA in your microtiter plate, you can spot it using a robot.

[Slide.]

So the Affymetrix gene-chip process is using photolithography. The first step is you deprotect the substrate allowing you to add a base and then do the synthesis. This process is controlled by a photolithography mask and the density of the array is determined by the size of the holes in the mask that allows the light to pass through. And there is the basic

at

limitation of the actual wavelength of light that you are using.

[Slide.]

That is what one of the cartridges will look like. [Slide.]

After you put your sample on, you will get--this is your data. So what you are seeing is each gene has about twelve oligos, twelve with a perfect match and twelve with one mismatch. You compare the signal from the perfect match to the mismatch. The idea is that if you see mostly the perfect match lighting up and very few of the mismatched not lighting up, then that gene is being expressed.

[Slide.]

What we do at the NCI is we are actually printing our arrays. What we are printing right now are cDNA that we have PCR amplified. In order to print, you need to have DNA of a sufficiently high concentration in order to get enough DNA on the array to be able to hybridize later.

The glass that you are printing on has to have an even coat that allows the DNA to bind to it. Then, your printing pens take the DNA and spot it on the glass slide. In a typical run, each pen needs to be able to reliably print 50,000 times just to create one batch of arrays which is, in our hands, about 100 slides.

at

After you have the array, then you need to post-process it to make sure that all the reactive sites that are left on the coated slides are blocked so that you don't get any background hybridization.

[Slide.]

So this is kind of just a schematic. To start out, this is the PCR reaction. Over here, we are showing you the coating glass. Right now, we are coating the glass with polylysine. Then we use the robot to take our DNA, put it onto the polylysine-coated slides. Then we do the post-processing and now we are ready to actually do a real experiment with the array.

The trickiest part is the labeling. You have to have high-purity RNA. You do your reverse transcription to create your cDNA and then you label with your CY3 and your CY5 which are the floors that are commonly used in array technology. You put them on your array. You hybridize. You wash. And then you scan it and, hopefully, you will get some red and green spots so you can analyze.

[Slide.]

This is the Biorobotics Arrayer. It is a very popular arrayer because it has a relatively small footprint or a high throughput. They achieve that by having four trays, each holding 27 slides apiece. That way, they minimize the amount of space it takes up on the bench.

at

Over here, you have your automatic plate handler which allows you to load 24 plates at a time. Technically, you can load it up and walk away and it should do the run all by itself.

[Slide.]

I am using a GeneMachine arrayer. It is a little more primitive device. It has only got three moving parts. It is based on the Brown Lab Arrayer. On the X axis, the slides are being held on a platter and those are moved in the vertical. You also have your microtiter plates where the DNA is. On the Y axis, it moves the pins back and forth horizontally. Then the X axis controls the pens, moving them up and down.

[Slide.]

Here is a closeup of the pens printing an actual array. What you will notice there is you have each of the individual slides, standard microscope slides, that have been coated with polylysine. Then there are 32 pens. The pens are spaced at 4.5 millimeters apart so they fit into a microtiter plate perfectly.

[Slide.]

Here is a closeup of the pens.

[Slide.]

What looks like a shiny nail is actually a very complicated device.

at

[Slide.]

This is the business end of the tip. It is called a quill-pen tip because basically it just draws up the DNA using capillary action. It takes about a quarter of a microliter and it deposits less than half a nanoliter per each spot. Depending on what you are printing with, your spots should be about 100 microns in diameter. This is called contact printing. That is what the majority of labs that are printing arrays are using, some variation of either telechen pins of quill-type pens.

[Slide.]

There is new technology, noncontact printing. You may have heard of inkjet printing. This is a slight variation of the theme. It is using a piezoelectric collar around a capillary tube. As it is activated, it propels a small droplet of water out of the tip and you can spot on the array without ever having it touch the actual slide.

[Slide.]

That is what the array looks like after you finish printing, what you are seeing there. This is the salt from the printing buffer that has dried on the array. And those are 32 blocks. Each block corresponds to one pen. If you look at the blowup here, each spot would represent an individual gene and each spot is about 100 microns.

at

The typical array that we are printing now has about 10,000 features. But, without too much trouble, we can print 20,000 features using 32 pens.

[Slide.]

So now that we have the array, we need something to do with it. We are using expression arrays so we are looking at RNA. We need to purify the RNA. Now you need, using reverse transcription, to incorporate a nucleotide that has a label on it. As I said, right now the convention in the industry is CY3 and CY5 dyes. So, once you have your RNA that has been reverse-transcribed and labeled, you can hybridize it onto your array.

You wash it. Then, in the scanner--you put the actual slide in the scanner and you have two colors of lasers that will excite the individual floors. When you collect the data, you are actually collecting two individual files. The advantage of this, or the whole point of this, is that now we can look at the ratio.

So it doesn't matter the actual concentration of DNA that is on the feature. What is important is the ratio which will give you how much is--which sample is being expressed above the reference or below the reference. People will commonly say overexpressed or underexpressed. What they are referring to is the ratio which, just by convention, is CY5 divided by CY3. If it is expressed in

at

the CY5, it is called overexpressed. If it is brighter on the C3, it will be underexpressed.

When you see all these pictures of red and green and yellow spots, what you are looking at is actually a combination of two images.

[Slide.]

That is a scanner that we use, the Axon scanner. It is nice. It is compact. It is simple to use, very reliable.

[Slide.]

That is what the computer interface looks like. So you use the computer interface to control the scanner. You control where you are scanning and you can control the PMTs. PMTs is the photomultiplier tube. What that simply is is a device that electronically amplifies the signal that you are collecting from the excited floor.

As the photomultiplier voltage is increased, you are going to collect more of a signal. However, you need to be careful because you are also going to collect more signal from the background. So if you have a very dim array and you need to increase your photomultiplyer tube, you are going to increase your background.

The way to tell a good array is you want to have high signal and low background.

[Slide.]

at

If everything went perfectly, you have a really pretty picture. It is displayed as red, yellow and green spots which is really just for our convenience. The yellow spots indicate a ratio of 1. So that would mean that the RNA in both samples was equivalent. Again, the red means that the sample of the CY5 was higher and, in the green, the CY3 would be higher.

[Slide.]

Just to prove to myself that this works, I like to see these kinds of experiments done where you do reciprocal labeling. You take your two samples of RNA. You put them on two separate arrays. On the one array, you are labeling Sample 1 with CY3. In the second assay, you take Sample 1 labeled with CY5.

So the green spot here, if you do the reverse, shows up red. What that means to me is that the sample is really expressing on that one gene that you are seeing. Some of these spots are very dim. It is too limited an amount of signal to really decide whether or not they are actually working.

[Slide.]

Her is an experiment from a collaborator of ours using a mouse model. These are double-spotted so each gene is spotted twice, right next to each other. It was a mouse knockout overexpressing a beta catenin. The CO is the

at

wild-type mouse. This is the self-hybridization so everything is yellow. That is good. That means you have equal labeling in both RNA samples.

This was actually done accidentally but it just shows a really nice result of how good the system, how robust it actually can be. Here you see these genes are expressed in the beta catenin and they are almost absent in the wild type.

Over here, you see the same thing. It is not expressed in the knockout mouse and it is expressed in the wild type so that when you do the actual ratios, it actually confirms what you suspected. It is being expressed as beta catenin and it is being expressed higher in the wild type.

[Slide.]

As I mentioned, the spots are just conveniences for us to visualized. It is actually just numbers in the computer. So this is the graphical representation. Each spot is plotting along the CY5 and the CY3, if I am doing it right. CY5 and CY3. Everything is lining up at a 45-degree angle which is a ratio of 1.

When you are doing the experiments, if one of the channels is a little bit higher or lower than the other, what happens is you automatically need to normalize the sample so you take an average of all of the red signal and

at

all the green signal and you multiply it by ratio so you still get the constant value of 1.

Over here, you will see differentially expressed genes. This is being expressed over here in the CY3 and up here in the CY5.

[Slide.]

Just another example with a little picture of the self-hybridization. Everything is yellow. And then two different samples, you see red and green. What that would be on this chart--this is in log scale, by the way--is everything above this line is overexpressed. Everything below this line is underexpressed.

[Slide.]

This is why microarrays are still in the research lab and are not ready for clinical application just yet. They are not a perfect technology yet. There are still problems and errors that occur. There are background problems. There are hybridization problems. Most common is low detection which usually means you have bad RNA or you had a poor labeling reaction.

[Slide.]

But assuming you get a good array, you can upload it to a database. So now you have 10,000 genes for a single experiment and you want to analyze that data. You

at

need to have some sort of the database management tool in order to interpret what you have.

At the NCI, we actually have an in-house database which provides the majority of functions and search tools. There are a lot of commercial companies that are trying to promote their particular database system. Luckily, the CIT group has already developed this. It gives you all the tools. It gives you the hierarchical clustering algorithms, the multidimensional scaling.

If you have read any of the expression papers, you probably have seen people, particularly the tree views where you are trying to compare many different arrays all on a single experiment.

[Slide.]

What is the future of microarrays? Microarray doesn't need to be on a solid two-dimensional platform. It can be on a bead. So all you need to do is somehow have a bead that you can identify and give it a unique identity. Then you have your DNA on the bead. Now you can do a hybridization with your DNA on your uniquely identified bead. Then you can do a typical hybridization, a liquid hybridization.

Theoretically, the liquid hybridizations have faster kinetics and are more reliable. Because we are doing it on beads instead of a solid substrate, you can mix

at

and match the beads depending on the assay that you would want to do. There is also some new technology for direct detection of hybridized probe where you don't need to actually label your probe. You can directly detect a hybridization event.

[Slide.]

Here are quantum dots which have been used and probably will be used further in giving the beads a unique bar code, if you will. Plus, it is a pretty picture. So you have all these different color dots. What does that mean? All that is is each dot has a unique signature of wave lengths. So when you are doing your analysis, you know that this particular microbead has the identity that has been determined by the quantum dots that you have put into the microbead.

Now you know what it is so when you label it with your DNA, you should be able to know what the DNA molecule was that you put on the bead.

[Slide.]

This is basically how the test would--this is a schematic. In this point, you have three different IDs for the bead and each individual bead will have a different DNA molecule on there. Technically, by combining the different wavelengths and the different intensities, you should be able to come up with thousands, perhaps even a million,

at

different unique identifiers for each bead which, in the array business, we are always trying to get more genes on a single test.

So this technology isn't quite there but it will be there shortly.

[Slide.]

Here is a mouthful; surface plasmon resonance. Don't worry about the word so much. I am not going to claim to understand all of the various physics and optics that are going on here but the take-home message is that you can actually detect the change in reflectivity when there is a hybridization event.

[Slide.]

This is a proof of concept. This is an array that has your DNA that is actually on a gold film. You can take your sample. You just hybridize it to your array. Then you put it in the magic machine and it will tell you whether or not there has been a hybridization event on the spot or there hasn't been.

Right now, I think the test that I have just done is using oligos, but it is not very hard to figure out that you can use any kind of sample, the advantage being you don't have to do any kind of a labeling reaction. You can reuse the chip over and over again.

[Slide.]

at

That is briefly some of the things I think that are going on in the array field right now. I would like to thank my lab and take any questions that you might have.

DR. KAPLAN: Thank you very much for a very interesting talk.

There is a change in the order of the talks. The next speaker will be Dr. Holger Ottleben who will talk about non-nucleic-acid arrays and will give us an overview of what his company is doing which is really a very different technology and very exciting technology.

Graffinity

DR. OTTLEBEN: Good afternoon.

[Slide.]

Thanks to the organizers for giving me the opportunity to give you some information of our technology which is designed to allow screening of chemical microarrays.

[Slide.]

We have developed this technology basically for drug-discovery purposes. I will walk with you through the technology and point to certain aspects that are particularly important for small organic molecules. I am happy that the speaker before me explained most of the basic principles of microarrays so I have not to go into very much detail in every point.

at

Basically, some words in advance about our company. You are probably not familiar with it but it is already four years old. We founded it four years ago. We have currently eighty people and have substantial lab space and are located in Heidelberg.

[Slide.]

We did a second round of finance which made us, for the first time, international because we have had the opportunity to convince also investors from the United States to invest in our company.

[Slide.]

We have started exploiting this technology already in a series of corporations with the pharmaceutical industry. Taken together, we are already one of the biggest nonpublic biotechs in Germany.

[Slide.]

So some words on the approach. Our approach is to have chemical microarrays of small molecules. When talking about microarrays of very small organic compounds, certain features come into play that are not so important as if you deal with DNA, for example. The surface properties are absolutely critical because proteins tend to adhere nonspecifically to proteins.

Also if you have washing steps involved, as you have it in hybridization protocols for DNA arrays, you

at

might wash away most of the weak binders to small molecules. You cannot have an array of a small organic drug-like molecules, you cannot expect that they have the same or similar binding affinities like a hybridized oligonucleotide. So you have to have a different readout system.

For that we developed a system that allows us to do label-free imaging in a highly parallel manner.

[Slide.]

So the technology, as I said, comprises surface chemistry. We also produce libraries in-house to be applied and immobilized on the assays. It involves lots of production processes for the chips, itself, as well as for the microarrays.

[Slide.]

So surface really matters, as I said, if you go to small molecules. We are basing our surfaces as self-assembled monolayers on gold. The gold is necessary for the SPR detection. The self-assembling monolayers allows us to create a surface that is highly resistant to unspecific protein binding. So when there is no ligand present, or no ligand bound, virtually no protein is bound and we have checked that with a hugh series of different proteins.

at

Of course, the optimization of the presentation of ligands is of similar importance since the ligands should reach into the active site of proteins, for example, and should not stick just on the outside. As I mentioned, label-free detection becomes a crucial issue and this is here indicated by a wavelength shift.

So when the protein binds, it creates a mass change on the surface of the chip. The mass change is upheld by the surface plasmon resonance so it creates a wavelength change and an angle change of the light that was reflected onto the chip.

[Slide.]

So, first some words on the diversity they put on the chip. We start with conventional resins and then we attach to all the resins the same linker molecule. Then we synthesize in a parallel fashion the organic compounds so that they are all different but are similar in respect to the linker.

Of course, that requires substantial protocol development. I don't go into details for that, but it allows you, then, finally to produce compounds in 10,000-compound batches in a miniaturized synthesis. We did some substantial development also in that area and this enables us to produce our in-house library which you can spot on the arrays.

at

So, once the compounds are synthesized, they are cleaved from the resins and then they are--with a tag in the mother plates. This allows us to do subsequentially quality control before we put something on the chips. Again, this is extremely crucial for small molecules because once you have something on the chip, you are really unable to analyze it anymore. It is just too little material. So you have to make sure that the compound was really there and was there in the right--was synthesized in the appropriate way.

Finally, the compounds get spotted onto the microarrays. Each sensor field, each SPR sensor field, contains a limited number of binding sites. By applying an excess of the small molecule, we can guarantee that, in a covalent reaction, on each of these sensor fields, there is the same amount of the different compounds. In that way, that is extremely important also to quantify the binding data afterwards.

[Slide.]

The microstructure that allows for having highly parallel SPI imaging is produced in clean-room facilities, and I don't go into the details there also. The array production, itself, is done using spotting robots, which were mentioned by the speaker before me, and we have adapted that and customized for small organic compounds.

at

The main difference here is while DNA samples, even different DNA sequences, they are rather a homogenous set of solutions. If you have a combinatory library, of course, features like viscosity differ very much throughout the library and you have to have a spotting routine that is able to handle all the different sample viscosities, et cetera, and, nevertheless, produce a highly reliable array.

[Slide.]

The array production currently involves a microtiter plate footprint. This is important for robotic handling, simply. We use, despite there is gold on them, them as disposables because we can mass-produce them from the mother plates since we are penning and not synthesizing on the arrays. They are really ready for mass production.

[Slide.]

The array readout is done, then, with a surface-plasmon resonance instrument. It is the first really parallel surface plasmon resonance imaging instrument. Once you have the array with the different compounds ready, you can apply the protein solution in a biochemical buffer on the chips and everywhere there, where the protein has bound, a wavelength shift is monitored.

An important thing is it is a truly function-blind assay. So, virtually, you don't have to know anything about the protein that you have put on the array

at

except that it is soluble. Therefore, you can avoid all the assay development and you can derive these binding maps of proteins right away with even fairly uncharacterized protein samples.

[Slide.]

The imaging instrument is currently working with different numbers of spots or sensor fields. The highest density is 4,608 spots at the moment and this is routinely employed in all our projects. The binding takes place under equilibrium condition and we get an immediate rank order of affinities from the binding experiment.

[Slide.]

The imaging device I mentioned, we are looking for the wavelength-dependent change of the plasmon resonance effect. The important thing is here, since we are looking for the wavelength change and not for the angular change, no moving parts are necessary in the instrument. This allows for a very robust setup of the technology.

[Slide.]

This is some real data. This is an array that is 5,306 tethered small argatrobanlike fragments which is a known inhibitor for thrombin. We have taken only fragments of these inhibitors, immobilized them on the chip. The important thing is that blue is background. There is

at

virtually no background binding. It is all in the order of 0.5 nanometer wavelength shifts.

But, on the other hand, if the protein binds somewhere, then you can get, in this software, a different color code and this allows you, if you recheck this, this is related to the binding affinity and you can monitor binding even of rather weak binders, the weak but specific binders, which can be--if you have a drug-fragment-guided diversity on the array, this can be an important starting point for the first optimization of the drug.

[Slide.]

I should mention that the CV values for such arrays are rather good. They are in the order of about 5 percent and this is for array experiments rather good.

[Slide.]

The reproducibility is even more striking. This is an array fingerprint of the protein against an array which contained 4,608 different organic compounds. You will hardly notice the difference in the sensitivity pattern from two different experiments from this data. However, if you do the correlation of the two datas, you see that there is some variation. But the correlation, altogether, is really excellent.

[Slide.]

at

For an important application, of course, is now to use this library since we are able to mass-produce these arrays and to fingerprint them against a different set of proteins; for example, taking all kinases of a certain class or taking all interaction domains of a certain class, and bringing them in contact with the same diversities.

This allows you, of course, to get some information of potential specific binders right away from the primary screening.

[Slide.]

Just as another example, this is a protein tested against 4,000 different compounds. I have restricted myself to 4,000 compounds because otherwise the resolution of the screen would have been limited. But you can do really large numbers in a rather short time with virtually no assay development.

[Slide.]

Of course, you need to have some software and some database to analyze it, the binding data. We have an in-house development. It is called JArray which is used to analyze the software. Some of our libraries are built in a binary fashion. It allows you to look for the building blocks and fragments that were used. For example, in the binary system, since you use long rows and columns, you can

at

get a rather quick analysis of whole compounds and building blocks.

[Slide.]

And you can import data from the imager in that software. The software also allows you to click on each sensor field. It is popping up the compound that was on the sensor field. As we did a parallel synthesis, everything is caught and coded by the X/Y code. They are kept throughout the process so that you know always what compound is behind which sensor field.

In addition, you can get histograms of the screens and you can do similarity searches. Based on the similarity of the organic compounds, you can rearrange your data and just derive some SAR analysis from that data.

[Slide.]

The software is then coupled also to some other state-of-the-art chemical information management systems like IsisBase or can even be used to dock the compounds quickly into the structure using TriPath software

[Slide.]

So the whole process, as we have employed it here, is starting with small organic compounds that are put onto arrays which are constituted by different sensor fields. The way we usually proceed is that we can derive some information already from the primary screen and then

at

we produce libraries that are guided by the information from the initial screens and put these libraries on the arrays.

In the third step, we mostly use the information that those compounds we found to be active carry a spacer here, carry a tag here, and we use the information the combinatorily expand the library around that site of the molecule. This is shown here schematically right here. These compounds are then used in solution assays. They are then no longer tested on the arrays.

We went through that process with a series of proteins. In the one case, it was thrombin which is a rather well-studied enzyme. We discovered a new chemotype which was kind of a surprise since, for thrombin, this molecule is tested in research for the last twenty years.

We could show that it has promising pharmacokinetic features. We, meanwhile, cocrystalized this structure and have patented the structure. Nevertheless, I am not able to show it right now. We have shown, with this example, what we can really discover by using small organic fragments novel compounds as inhibitors of proteins.

The whole process, as we see it now, is that we have a very quick and efficient process to come up with novel leads. Since we are synthesizing these compounds

at

that carry the tag produce a lot of a series of daughter arrays and we can use them in label-free imaging, avoiding assay development. Then all the data from all the different proteins is stored in a database and, of course, is then subject to inspection by the trained eye of the medicinal chemists.

We produce, then, free active compounds and do the follow-up assays which is then kind of conventional secondary assays. We can, then, start to do some medicinal chemistry based on the wells of information from the fingerprint data that is stored in the database for the molecules.

[Slide.]

With this slide, I would like to end. I think I am almost on time.

DR. KAPLAN: Any questions?

DR. NAKHASI: I have a couple of questions. I think this basically understanding the rationale and how do you choose these compounds, first of all, based on what information and, second, can this technology be also used in DNA and RNA instead of just a protein?

DR. OTTLEBEN: The first question, we are following the design principles that we call leadlike. So the initial compounds on the arrays are usually smaller like the druglike compounds that follow the Lipinsky rules.

at

They are, on average, have a molecular byte below 350 Daltons. So the most important thing is that there is room for improvement. So you can shape these molecules. The problem with other HGS compounds was always that the medicinal chemist has had to cut away certain parts of the molecule, lost some of the activity and has had to rebuild that.

So our philosophy is to have more leadlike diversity for small organic compounds on the chips, so, fragment-guided and also guided by the knowledge from known drugs.

The second question regarding what else can you put on the arrays. Basically, you can put anything on the arrays. You can put DNA on the arrays. We did that. You can put peptides on the arrays. We did that. You can also put proteins on the arrays. We had to focus ourselves to certain things, and, therefore, the small-molecule arrays.

The challenges, of course, were in a way, from the technology point of view, the highest for small-molecule arrays because there you have usually weak affinity binders and you have the problem, the potential problem, with the unspecific binding of proteins.

So, since we had overcome these problems, we could then also use that for other kind of biomolecules.

at

DR. KAPLAN: Do you think that this technology can be used for screening, for instance, of antigens or to just describe the antibody repertoire? Can you speculate a little bit on that?

DR. OTTLEBEN: I guess you could use it for that. The advantage is that it is a label-free technology so you wouldn't have to have a secondary antibody to do the assay. Also, if you could start with peptides that have rather weak affinity. Probably even if you would like to describe the antibody repertoire of an individual, it is unlikely that you have for each antigen the best binding molecule on the chip. So that means that there is also a requirement to discover some weak affinities. This, of course, then is helpful if you have no washing steps involved in the process.

The critical thing will be to work with crude mixtures and to see whether there is too much competition ongoing between the different molecules in the crude sample. So that is something one would have had to figure out.

DR. CHIEN: David Chien, Emeryville. What is your sensitivity of this assay in your small molecule array. One of the slides you showed, your detection is 1 micromolar?

at

DR. OTTLEBEN: Sensitivity with respect to what we can detect as binders goes up to binding constants of 10 to the power of -4, so rather weak binders. In terms of the sensitivity of protein concentration you need to detect that, it varies from molecule to molecule, from protein to protein. It starts from a few nanomolar.

DR. KAPLAN: Thank you very much. A very interesting presentation.

The next speaker is Dr. Konstantin Chumakov from the Food and Drug Administration. He will talk about applications of microarray on pathogen detection.

Application of Microarray on Pathogen Detection

DR. CHUMAKOV: Thank you.

[Slide.]

As you can see, I am from another of the Center for Biologics. But the topic of my presentation, pathogen detection, certainly transcends the boundaries between different kinds of biologics and is, in fact, I think, one of the central issues in medicine in general and recently has become something that even the Postal Service is involved with. So it is really a very general thing.

So today I will talk about how the new emerging technology, microarray hybridization, can be used for effectively doing this.

[Slide.]

at

What, actually, are microarrays? In fact, this is a highly multiplex format for performing a lot of different things. Hybridization is only one part of what can be done on this platform. You can also do enzymatic reactions like DNA-ligase reaction on the chip, DNA-polymerase reaction and even PCR can be done on a microarray.

[Slide.]

So what is so special about this format? Of course, the first thing is that it contains numerous individual elements. The results that you obtain usually are composed of sets of hundreds and even thousands of individual measurements. So it is a very highly multiplex result.

Small size allows you to control reaction conditions and, therefore, you can adjust to perform a fine adjustment and that you have balance of sensitivity and specificity. Of course, the large number of spots that you can have on one array allows you obtain highly redundant results improving its robustness.

[Slide.]

Now let me go into more technical justification and rationale of the platform that we have chosen. I think that this conference provides a good testimony to the shift in focus in patient detection from biological assays that

at

were originally the only choice that people had through analysis of proteins, immunological assays, which are still being used widely to nucleic-acid detection.

In fact, nucleic-acid detection, nucleic-acid-based techniques, are multiple. The state of the art at this moment is, of course, PCR because it provides ultimate sensitivity, potentially being able to detect even a single molecule of DNA.

[Slide.]

So microarrays come in a variety of different shapes and forms. I am very glad that Dave Peterson had a talk before me and he described some of the variants. So, of course, I will talking about nucleic-acid microarrays so they can be either DNA, long DNA, or short oligonucleotides. It can be the cloned or PCR-amplified materials or it can also differ by the physical nature of the substrate. It can be either a glass surface or maybe a plastic surface, or it can be a three-dimensional gel matrix.

It can be synthesized in situ. For instance, like Affymetrix technology does it. Or it can be done by spotting or spraying and it can be used for hybridization or enzymatic reaction. It can be used for gene-expression analysis like Dave showed you in his first talk, or for nucleic-acid identification, genotypic and single-

at

nucleotide polymerism studies. So this is what I will be focussing on most.

[Slide.]

So here is the robot that basically spots oligonucleotides. In our case, we work with short oligonucleotides, around 20-base long, that are modified so that they can be easily immobilized on the treated-glass surface. As a result, we have microarrays that contain spots of immobilized short oligonucleotides.

The slides that are prepared this way are hybridized with a single-stranded DNA probe that is labeled with fluorescent dyes. So we do it by PCR amplification with one primer labeled or maybe with triphosphates labeled with fluorescent dyes. A second primer is attached to biotins. So, after PCR amplification, you can easily separate strands and have one complementary strand that is fluorescently labeled with either CY5 or CY3 or any other fluorescent dye, for this matter.

[Slide.]

So here is the overall flow chart of the technique that we use. So we start with DNA or RNA, in this case. It is a reverse-transcriptase reaction. Then we PCR-amplify it and, at this point, it can be fluorescently labeled with-- either you can do strand separation by biotin, as beads, or you can potentially do

at

PCR amplification and get single-stranded DNA directly in this step, hybridize with microarrays, scan it on a commercially available instrument and then analyze pattern and get the results.

So it is a relatively easy scheme and it was fine-tuned by Vladimir Chizhikov who is present in this audience. So now it works beautifully. The amount that it takes from this point to this point is usually less than one day. Hybridization takes less than an hour. The main time-limiting step is PCR.

[Slide.]

Of course, the main question is what do we put on our chip to make it really efficient for discrimination of virus. Here I just showed a simple example. In this case, it is a small set of orthopox viruses. You can see, for instance, that these mutations, they all distinguish vaccinia from other orthopox viruses. So if we synthesize oligo in this region, it will bind vaccinia but it will not bind variola. It will not bind monkey pox or other viruses.

So if you have a simple case like this, it is easy. You just eye-ball the multiple sequence alignment and identify the appropriate positions. But, of course, in reality, we work with genes sometimes over 1,000 bases long

at

and it can be a set of more than 100 sequences, so it is becoming a tough thing.

[Slide.]

So we had to develop our in-house custom software that allows us to algorithmically select appropriate sites for making both PCR primers and oligonucleotides for discrimination.

Here is the diversity chart. This is a GP7 genome for rotaviruses. The places where the curve dips means that there is no variability. So this is an ideal place to put PCR amplification primers. Where the curve soars, it is, of course, an ideal place to look for discriminating primers, meaning that in this position, this is the maximum heterogeneity among different kinds of rotaviruses.

[Slide.]

I am sorry for this garbled slide. It was prepared on MacIntosh and it didn't translate right. So you see this cluster of viruses. It looks like a phylogenetic tree but, in fact, it has not been constructed by counting the number of nucleotide mismatches but, rather, by the number of oligonucleotides of the same heterodynamic parameter that these viruses share, meaning that all these red--this is actually variola. These are all small-pox viruses.

at

You can see this really. You can distinguish them because they share the maximum number of the same oligonucleotides. So we can ask to list all the oligos that are unique for this group, that all of them share and none of the other viruses do have.

[Slide.]

So you can have something like this. You see that this is one example. All small-pox viruses have the same identical region while all other orthopox viruses have multiple mismatches here. So you can select the number of oligos in this region. In fact, we have a very big number of such, very convenient sites for putting this combinating oligos.

[Slide.]

As a result, it is relatively easy to identify oligos that will distinguish any particular group. On the first line, there are oligos that are actually common to all orthopox viruses. So you see that all of these viruses are orthopox viruses.

On the second line, these are monkey-pox specific oligos. Third line, small-pox specific oligonucleotides. These are cow-pox specific. This is elephant pox. And this is vaccinia. So this is relatively easy. Within a few minutes, you can unambiguously what virus do you have.

at

Even if you have some spots missing, it is still quite clear that this is monkey pox. Even presence or absence of certain spots gives you more detailed information down to the level of isolates. So, for each particular isolate of a particular virus, you have a sort of profile which is a portrait of this particular strain.

[Slide.]

So the same works pretty well for rotavirus genotyping. So this is a panel for G1 genotype, G3, G4, G9. There is some crossover here but it is just because they are--G9 and G3 are similar in this region. You can even detect mixtures of both. This sample was a mixture of more than on genotype.

So, again, there is no question about the result. But, in some cases, when you use multiple samples of the same genotype, you can see some spots missing which gives you an additional dimension in your analysis.

[Slide.]

It can also be used for quantitative analysis. In this case, it is analysis of a mumps vaccine which is a mixture of two strains. So you can determine the relative content of two such strains. In this case, it is ranging from 100 percent of GL1 to 100 percent of GL2, all intermediate mixtures. So you can use this technology for

at

quantitative analysis of different viruses present in the mixture.

[Slide.]

Now this is a nice example of the power of this analysis for fine discrimination between the viruses. This is a case of polio recombinants. You know that in many--not in many cases, but occasionally, polio virus vaccine causes vaccine-associated paralysis. The majority of the isolates are recombinants between different types of polio virus vaccine.

So, in this case, we worked with two recombinant strains which were composed of two parts of Type 1 virus and one piece of Type 2 genome.

[Slide.]

Here you see. The first line contains oligo-specific for Type 1. This is the Type-2-specific oligos and Type 3. You can see a physical map. It starts with Type 1. It switches to Type 2. And then back to Type 1. So it is one of the first slides. Now the quality of the picture improved. We improved it very much. We don't have missing spots anymore. So you can instantly obtain a genetic map of a virus including the exact position of crossover points.

To do this before, it would take complete sequencing which is like about two or three months of work.

at

So this can be done on one glass slide. You can put up to five or ten different strains and do it within five hours.

[Slide.]

This is the second part. I think I will skip this, I mean go over it very fast, because this is basically very similar to what Dave Peterson described.

This is another aspect of microarray technology, gene-expression analysis, that can also be used for pathogen detection because basically it can detect the difference in gene-expression patterns in mRNA profiles of biological systems that are normal in disease, in the general sense. It can be either normal in tumor. It can be normal in infected.

So this scheme analysis of gene-expression differences can be used, for instance, as a biosensor for detection of the presence of something that you don't even know. For instance, if you have a culture that you treat with a sample and then you see a change in the profile of gene expression, you can predict by comparing the specifics of this change profile to something that you already know about the behavior of a certain virus group or bacterial group that induces some response in a biological system.

[Slide.]

This is actually an image of microchip that is produced in Dave Peterson's lab and the same chart,

at

basically, when you are looking for points that are off this diagonal line. By selecting and analyzing patterns of spots that are off the diagonal line, you can create profiles and then do cluster analysis and identify common patterns of gene-expression profile and then compare it with known pathogens and, as a result, predict the presence of some pathogens in your system.

[Slide.]

So, of course, these pictures reflect a unique pattern of genomic activity in cells. It is specific for cell type and it reveals different types of cellular pathology. But, of course, it also reflects a response to different biological substances, cytokines, viruses, drugs. In fact, this is a very powerful tool that can be used for search and analysis of all these substances that are of interest to people working with blood.

It is a very sophisticated tool both technically and intellectually. It is not easy to interpret the patterns that you are getting but I think it is worth exploring and developing. We are starting one project that we hope will help us use it as a tool for analysis of cell substrates used for vaccine production.

[Slide.]

So, in conclusion, of course, this microarray technology is only in its infancy. It can be tailored to

at

achieve maximum sensitivity and specificity. It can be also tailored into biosensors, just like I indicated in the last part of my talk.

[Slide.]

Finally, I have to acknowledge all these people. Vladimir actually he is our microchip guru. He was behind all these developments. All these people also worked on some parts of what I have showed you today.

Thank you.

DR. KAPLAN: Thank you for a very interesting presentation.

Do you have any questions?

DR. NAKHASI: Konstantin, how much complexity can you build in your system? What I heard was two or three or four, you can see the thing. How much complexity can you build in your system and still see the differences?

DR. CHUMAKOV: Complexity; do you mean in terms of differences between different objects?

DR. NAKHASI: Not only that--yes. Let's say if you have a biological sample, you have many of these components in there. This is okay when you know what is in there. Let's say, in an unknown sample, you want to put so many other primers to see whether that hybridizes to it, whether that agent is in there, how would you use that in that situation?

at

DR. CHUMAKOV: I think that it is just like with any other method. There is always a balance or some kind of tradeoff between your sensitivity and specificity. It is always a dilemma that you face. But, in this case, I sort of feel that, at this point, it is the best combination of both specificity and sensitivity because, for instance, if you take PCR, everybody knows that if you increase stringency, you can dramatically increase specificity, but you lose sensitivity.

If you relax your conditions, allow it to amplify other things, then you will get a picture that will not be possible to interpret. You will have all the smears on the gel. So that is why the kind of part of PCR analysis, the second phase of it, gel analysis, or it can be TaqMan technology, that validates that what you have amplified is what you really wanted.

So, in this case, when we use PCR in combination with microarray hybridization, we can separate it completely. We don't care about specificity on the first phase. We only focus on amplifying material. We create primers that are targeting the most conservative regions so it will be the most robust amplification you can achieve.

So it will be very sensitive, very robust and you don't care that your material that you amplified is garbage

at

because, on the second phase, you hybridize with specific oligos and then you see the picture.

So, at first you focus--you have to separate things. In no other method is there such a clear separation between these two aspects. For instance, TaqMan; it is a very good technology but it is done in one phase, so you both amplify and you validate your material. And you only target just one, or maybe two, probes.

In this case, it can be thousands. So, basically, I think that the place of this technology is not an alternative to PCR but it is a supplement to PRC. It is a replacement of a gel electrophoresis.

DR. HEWLETT: Konstantin, can you comment about the relative sensitivity of PCR on a chip versus the standard PCR, you know, in a gel or whatever other format that is currently being used? For example, for your rotavirus system, have you compare that and can you tell us what the relative sensitivities are?

DR. CHUMAKOV: It is better than regular PCR. It is. It really is. The rotavirus example is a very good example for proving this because there are currently a PCR-based genotyping methods for rotaviruses. They use a genotype-specific primers for amplification because of inherent variability of viruses, high mutation rates.

at

There are about 10 percent of isolates that cannot be genotyped by PCR method just because they won't amplify because there was a mutation that would happen to be in these primer-binding areas. Since we select our primers in the conserved regions, there is a much lower chance that there will be an incidental mutation that will kill this primer.

So that is why amplification is more robust than would be in the specific PCR case. So the specificity can be the best in PCR. We did not perform formal analysis of sensitivity which we should and will, but I feel that it will be superior to conventional PCR methods.

DR. RIOS: Maria Rios. Are you planning on using the methodology here to study what species and treatment for HIV and analyze some drug effectiveness in HIV treatment, for instance?

DR. CHUMAKOV: Yes. I mean, we just started a project with Indira on HIV so, hopefully, it will go well.

PARTICIPANT: The testing will be quantitated?

DR. CHUMAKOV: Do you mean the signal?

PARTICIPANT: Yes.

DR. CHUMAKOV: It can be quantitated for different purposes, mostly because it provides you more information than you can see with your naked eye because you have a pattern. In some cases, this pattern has

at

additional significance in distinguishing within-genotype differences, isolate-specific differences. So it is not easy when you deal with hundreds of spots to do it--so you better analyze quantitatively and then let the computer do all the pattern recognition.

So it certainly is a strength of this technique that it also provides you quantitative information. But, on the other hand, you can ignore it because the patterns are so clear that you don't need anything. You just see it. You can even write "small pox" with these dots. So it can be a really visually very attractive output that anybody in the field, for instance, can see what is there.

DR. KAPLAN: Thank you very much.

The last talk of the session will be given by Dr. Hurt from Affymetrix.

Affymetrix

DR. HURT: Thank you.

[Slide.]

You have heard a little bit about GeneChip technology already today. Hopefully, I am going to be able to just flesh that picture out for you a little bit and talk a little bit about the work we have done in the pathogen-detection area.

[Slide.]

at

The GeneChip Probe Array is, as you have been told, an oligo array. It is housed in a plastic cartridge, as such, and the array, itself, is the window here. A hybridization chamber is formed by the plastic cartridge. The oligos are actually on the inside of that array and access to that hybridization chamber is through two self-sealing ports on the back of the array.

It is a very robust package. I have dropped them on the floor before and gotten away with it. That is not part of the standard protocol but it is quite easy to handle in the lab and that is one of the great features about it.

[Slide.]

Of course, we supply an integrated system that allows us to utilize those arrays. The GeneChip Array really is the heart of Affymetrix Technology. We have a fluidics station scanner analysis software, et cetera. It is a complete package, Bioinformatics software at the back end.

[Slide.]

Kind of how it works, you start out with your array. You hybridize a labeled sample to that array, process the array, washing and staining steps, under the automated control of the Microarray Suite software on our fluidics station, acquire the image through the Agilin TNA

at

scanner. Image capture is done by the Microarray Suite software on the computer work station and image analysis is done on that same software package.

We also provide a suite of the Bioinformatics tools to analyze the data. The data can be quite complex.

[Slide.]

As you can imagine, there are a lot of different things we can do with these high-density oligo arrays. We have heard a lot about these today. Our experience is summarized in these various categories where you can analyze the genome, the genomic DNA, or you can analyze the expressed RNA. So gene expression is really our best selling, our most common, application right now but we also have used it in limited commercial release and mostly in the research lab for variant detection or resequencing application--that is, assessing difference from a known sequence.

Deletion analysis; I am going to talk to you a little bit about that in terms of pathogen detection and typing. And genotyping applications.

[Slide.]

The arrays, themselves, are built by using a photolithographic process that you have heard a little bit about today. I am going to try and fill in the blanks. We start with glass wafers. It is about a five-inch, round,

at

silicon quartz glass. We start using a machine that looks like this and derivatize the surface with the sylene linker. That linker is capped with a photolabile protective group.

Hitting that protective group with the U.V. light photodeprotects the reactive end exposing the hydroxyl to chemistry in the synthesis cycles. The photolithographic step is portrayed here. The checkerboard pattern that you see back there is actually a photolithographic mask. This is a technology that is commonly used in the semiconductor industry and Silicon Valley and we borrowed that to make these arrays.

The way photolithic lithography works is you essentially have a mask that is a mirror and you etch the mirror finish off of that mask at defined points allowing selective illumination of the derivatized wafer here, activating controlled portions of that substrate for chemistry in the next round.

Many layers of different mask patterns allow us to build a tremendous amount of complexity on the array surface, in situ. So we are building the oligos up in place. The checkerboard pattern you see here are actually the individual array-sized pieces that are all being built in a batch in parallel synthesis steps.

at

Once those steps are finished, we dice that array up into its individual array components and package that into the plastic cartridge that you have seen. We do individual pressure testing on each cartridge assembly to make sure that that cartridge if of high integrity and it is going to hold on to your precious sample.

Here is a view of the manufacturing lab in action. These canisters actually contain mask sets and arrays that are in the process of being made.

[Slide.]

Graphically, photolithography just allows us to control the exposure of the derivatized substrate to light, Here is the photolithographic mask, activating only selected portions.

[Slide.]

We have pictured here the sylene linker. The orange boxes portray the photolabile protective group. The mask portrayed here, illuminated with U.V. light. This is done in ambient light. It is a U.V.-drive process so it is very convenient. You don't have to do it in a dark room, which is nice.

Illumination selectively deprotects the exposed area, exposing the reactive chemical group. We add the phosphoramidite, also photo capped as well, and specific chemistry takes place at the activated region. By addition

at

of different mask patterns, we activate different areas for additional synthesis steps.

There are a lot of things about this process that make it really amenable to high-throughput, high-quality, screening of genetic samples. For one thing, it is a highly parallel process. The T-layer in these two positions is laid down in this cycle but so is every T in that first layer. So, if one probe was made correctly, all probes are made correctly for that layer. If one probe was made incorrectly, then the entire array will show that as well. So it is highly parallel.

It is a batch method. That means, in these parallel steps, we are making many, many arrays at the same time in the range of 50 to 400 unique arrays and there is a great deal of economy there. It is a combinatorial process and that means that, for any given layer, you have one of the four bases. So it can take maximally four cycles of synthesis to complete that layer.

So if you are making 25 oligos, as we do for our expression arrays, then it takes a maximum of 100 steps to synthesize, for example, 50,000 oligos. However, if we wanted to make 500,000 oligos, which is the content of our soon-to-be-released human genome array, it takes exactly that same number of steps. So there is a great deal of economy. It is just as easy or challenging to make 500,000

at

oligos or any number of oligos as it is to make a small number.

The photolithic process is also amenable to miniaturization. We are working right now with 18 micron squares containing each unique oligo sequence in our upcoming release. This has come down from about 100 micron as little as four years ago and we are continuing to push that limit so we can place more and more genetic information encoded on that array.

Essentially, it becomes an information-storage medium in the form of genetic information. We do have prototype data from our Research and Development lab with 2 and 5 micron square features. That would give us the capability in the same size array that we are using today to array many millions of probes, all in the same small, about 1 centimeter area.

[Slide.]

The array, itself, is divided up. Here is the original wafer divided up into 49 to 400 chips or arrays. Each array is comprised of a number of 20. We are moving to 18 very soon--micron square features containing millions of identical probe molecules. That means, in a 1.3 centimeter standard size array, we now, at 18 micron, are going to be able to array more than 500,000 unique oligos in that array.

at

[Slide.]

As I said, the feature size, being so small, allows us to get a great deal of complexity in a small area. So, for monitoring the human genome, a very ambitious task, a very complex task, we use relatively large-size arrays that contain that 500,000 probe set. We are coming out with a two-array set to monitor the known human genome early next year.

On the other hand, if you have a more directed task, smaller genome, a less complex question that you would like to ask, you can use that same economy of photolithographic synthesis to make up to 400 chips per wafer gaining even more manufacturing leverage. So the format is flexible. The capacity is flexible and growing. And the feature size is going down allowing us to grow that capacity.

[Slide.]

Expressed in terms of genes, this is in terms of expression data, genes per chip, back when we started this effort in 1994, we had about 250 genes per chip. Early next year, we are going to have about 21,000 gene expression levels monitored per chip, per array. Going forward, the complexity of the transcriptome is yet to be fully assessed but we hope to be able to give you detectors that will allow you to assess a very large proportion of

at

that in the near future as that information becomes available.

[Slide.]

Back in 1994, it cost about a buck a gene to do those 250 genes. But now, out here in 2002, we are down here in the pennies range and we absolutely expect that to keep going down as we gain more and more leverage from that economical manufacturing strategy.

[Slide.]

I want to talk to you a little bit about some of the applications that we have both been providing commercially for many years and also some research applications that we have been working on behind the scenes. The first is actually the product that brought me to the company. I spent a year of my life helping to develop our HIV PRT array. PRT stands for protease reverse transcriptase.

This is a variant detection array or a resequencing array. We are not discovering new unknown sequence with this. One of the properties of designing these arrays is we really need to have a good handle on the sequence we are working with in order to design that array. This is not a fishing expedition. We are using information that we already know looking for confirmation or changes from that information.

at

So we are asking the question, is a certain HIV sample or isolate different from a wild-type or an average sequence, if you will.

[Slide.]

This was our first commercial product. It was launched back in '96. It screens about 1500 bases comprising of Clade B virus, comprising the complete protease gene and out to codon 400 of the reverse-transcriptase gene. It analyses sense and antisense strands on a single probe array. For each position, that is done minimally four times, two antisense assays and two sense. The data is combined.

In areas of hot spots of mutation, we add additional redundancy so there are as many as 50 or so levels of redundancy looking for areas of anticipated sequence change. The throughput is about four samples per hour so you can get this sequence information about four samples per hour.

[Slide.]

The array, itself, looks a bit like this. I am kind of disappointed because the previous speaker was speaking of writing things with the arrays. Actually, our more recent releases have a little hybridization label up there that will tell you exactly what array it is. That is kind of neat but, unfortunately, we don't have that here.

at

But what we are seeing here is essentially four probes for each base of the sequence of interest. Those four probes, they happen to be twenty-mers on this particular array. At either the ninth or the fifteenth position, we substitute the four bases. The rest of that oligo is perfectly complementary to the wild type Clade B sequence.

So one of those four bases should light up and we get a sequence. You can actually read it somewhat like a sequencing gel since the top row is the A, G, C and T, like that. So you can actually just walk down there.

on this particular array, there are around 50,000 oligo probes. Fortunately, with computers today, we don't have to do that by eye. We can use the analysis algorithms that are contained in the Microarray Suite software to do that for us.

At the top here, you can see there is kind of a different character in this region of the array than there is down here. It is a little more spotty down here than it is up here. It is sort of a wave-looking intensity as you go across the sequence. Essentially, we are reading down the sequence when we go across these streets.

What that is, up at the top here, we have what we call our standard tiling. Tiling just means how we chose the probe arrays. So that is where we have a set of probes

at

for each position and just simply march down the sequence one base at a time, moving that construct.

But, in areas where there are known hot beds of mutation, we are going to go in and we are going to actually provide--we are going to assume that there are different sequences that we might encounter and we are going to put on what we call alternative tilings. The alternative is alternative sequences that we might expect to see.

So that is what is going on down here. In many cases, if there is a wild-type sequence, if there is the reference sequence that is encountered, then these will not be weighted at all in the analysis. We will simply use the primary, the information from up here, to make the call.

However, when there is a mutation, it can make it more difficult to call the next base and the next base and the previous base and the previous base because of that change from the wild-type backbone. That is where we would look to those alternative tilings to help us make a better call, a more specific call.

[Slide.]

Another thing we have done, and this really comes out of our research lab, work done by Tom Gingeras who couldn't make it today but sends his apologies for that. He has been working on some mycobacterium work for quite

at

some time. What they did was they started out thinking, let's do some expression analysis. Let's make a mycobacterium expression array.

[Slide.]

Sure enough, they did. And here it is. The mycobacterium genome comprises about 4.5 megabases. The array interrogates 4706 genomic loci or genes and intergenic regions. That is about 4000 ORFs and about 700 intergenic regions

Here is what that array looks like hybridized to mycobacterium RNA.

[Slide.]

What sort of came out of this was yes, this is a great tool to look at gene expression. We can extract the message. We can label that up. We can hybridize that to the array and ask the question, what genes are on, what genes are off, get this complex data and start going to town analyzing that data. It is very complex data, though and sometimes it is so complex that to come to a firm conclusion is very difficult. Gene expression data is very complex.

But what they found was--one of the ways that they do quality testing on these probes to make sure that they actually hybridize to the genome is that they will

at

label the genomic DNA and hybridize that to the array. Everything should light up when you hybridize the genome.

[Slide.]

What they found was that, in some strains, there are genetic deletions and they can be detected by hybridizing the genomes, for example, of the Oshkosh strain of M. tuberculosis, a very virulent strain, I understand, to the lab strain here. In comparing the two, you can see that this region right here is present. We would interpret that as being present. There is a row of perfect-matched probes and a row of mismatched below it. Perfect-match probes lighting up indicate that that gene is being detected.

Over there, you can see that there is an absence of hybridization in that area indicating that there is actually a gene deletion. Remember, this is the genome, not the expressed material from that. So this part of the genome is actually not present in this very virulent strain.

This is actually data from isolates from a San Francisco study. It turns out that each of these different isolates has a different deletion footprint that can be detected on the array in this fashion. So not only is this an expression analysis array but it is a gene-mapping array, or a deletion-mapping array as well.

at

It turns out that if you look at the number of probes that we are using to do the expression monitoring here, it comprises, it covers, about a quarter of the entire genome so that it really is quite a high coverage of the genome to do this kind of analysis.

[Slide.]

Another thing that the arrays are great is again looking at single-base changes in specific genes. The RPOB gene is frequently mutated. That is implicated in antimicrobial resistance. Very similar to the approach that we took with the HIV array, we can create an array that assesses the sequence variability of that RPOB gene. Here we are detecting a specific mutation that confers rifampicin resistance.

You can see in the data here that here we have an A-sequence in the mutant. Here we have a high peak in the mutant and we are not seeing that over here. So we can detect this quite easily with the array. In fact, we get information at all points of that gene.

[Slide.]

So, in summary, the gene chip arrays are great platforms to do this type of highly complex analysis. We can look at patterns of sequence to look for base changes. The conclusion here; we can see a mutation. That mutation,

at

such as in the case of HIV, may be associated with drug resistance. That is important information.

We can look at the gene expression patterns and we can do comparison analysis and tell whether that is a pattern that for each gene is the same or whether it is altered, et cetera. That is a very complex implication and sometimes the results are not that easy to come to a firm conclusion on without a great deal of research.

However, using that same array, we can do this full genome fingerprinting and sometimes we can see specifically, in the Mycobacterium tuberculosis experiments, the specific deletions that are associated with this increased infectivity. The basic need for this, though, is an index of these deletion mutants and their association with virulence.

That is my last slide. With that, I will just open it up, take any questions that you may have.

DR. KAPLAN: Thank you very much for your talk.

DR. ALLAIN: What do you think is the cutoff point if you want to look at mutations versus using the traditional methods like specific probes or RFLP and all that going to the expense of setting up a microarray? What kind of numbers you would say is the cutoff point going to that or traditional methods?

at

DR. HURT: Good question. So where does it become feasible or desirable to use this massively parallel format versus more traditional straightforward, uniplex or small multiplex experiments? That is a good question. The power of the GeneChip Array is a massively parallel analysis so our expression arrays are a great example, the human genome, yeast genome, rat and mouse, very complex genomes. We are using that massively parallel array of oligos to give us really nice data on that complexity, really deconvolve those genomes into individual datapoints that we can actually use and analyze and have the ability to do multiple determinations on each gene and do statistical analysis on those results.

If you are going to do an analysis of a single gene, for example, the protease and RT region in HIV. It is 1500 bases. We can do that with about 50,000 oligo probes. That is a fair number of probes, but there may be other ways to get that kind of complexity on other formats.

One of the advantages of the GeneChip is the ease of use. A single operator with some basic instruction can start cranking out this data really in a couple of days. There is no scale-up. You are not in the business of creating arrays. You are in the business of gathering data. It is Affymetrix business to create the arrays and make sure they work for you.

at

It is a good question. I am not sure there is any single answer. But, thanks.

DR. CHUMAKOV: Do you see any time in the future the situation when the end user will be able to create their custom chips with your technology?

DR. HURT: Oh; excellent question. You can do that today. We will work with you to create custom arrays. If you have proprietary genomes--for example, somebody wants to do a zebrafish experiment. A lot of people do zebrafish research. A zebrafish sequence database, genome database, we will work with you. We will take that information and create an array, a custom array, for that customer alone will be solely available there.

There is an investment, so there is a little bit of up-front. We have another program that allows researchers to either harvest probe sets from our commercial arrays--these are expression arrays that we are talking about here--so existing probe sets. We have already designed them. They are available in our commercial arrays in whole genome or very ambitious formats.

You may only be interested in 500 to 1000 genes. So we have a program to allow you to array up to 1000 genes either culled from our commercial array sets or proprietary

at

designs from your own proprietary sequence. So that is something that we are doing for our customers today.

DR. DUNCAN: I would just like to push you to think ahead on where the technology might go. The kind of question that an audience like this would be asking is when could this technology be used in a screening, in a clinical laboratory. So when I think about an answer to that question, I think about things like, how could the process of hybridization scanning and then moving on to the next sample be automated.

Is your company or anybody you know thinking about changes in the way the platform is put together so that a single chip, for example, could be reused sequentially one sample after another?

DR. HURT: Excellent question. We are definitely thinking along those lines. We are currently in the process of building partnerships with companies that are in the clinical markets today to help us along that path.

As far as the reusable array idea, we think of it this way. We want the best data that you can possibly have. If you reuse that array, degradation is going to occur. It is inevitable. Nucleic acid is not that sturdy. So the answer is make it affordable to use additional assays. Have a manufacturing process that is salable that you can generate as many arrays at an economical price as

at

are necessary so that you are not afraid to throw that array out, get out a fresh one.

That is something that our manufacturing technology is fully capable of. About two to three years ago, there was a great deal of bottleneck in the array-delivery process. People were waiting literally ten, twelve, weeks to get a delivery of a GeneChip array.

Of course, that was not satisfactory to us. Today, we are quoting a two-week turnaround with the caveat, order as many assays as you want. We can deliver them. So the capacity is there to deliver these arrays at an economical price. The price for academics, for example, has come down almost tenfold over that time period. So we believe that the format doesn't warrant reusing the arrays with the danger that that is going to taint your results, that you can use a fresh array every time. The manufacturing economies are there to allow that to be a very affordable process.

Thinking about the clinical market, we see--right now, gene expression is our main activity. As a field-application specialist, I spend probably 90 percent or more of my time supporting our expression customers. A lot of people are using our expression arrays.

Going forward, we are thinking about the clinical market by doing some basic research. We have spun out a

at

company called Perlegen. Perlegen's mission is to use this array technology, not small arrays like we provide commercially, but whole wafers containing 60 million unique probes to scan the entire genome looking for genetic diversity.

Their goal in the first year is to scan the entire genome of 50 individuals and bank that information, analyze it. Try to determine, for example, patterns or haplotypes of genetic variation that might be useful as, for example, in anticipating disease susceptibility or drug resistance or toxicity, those sorts of things.

But, really, that is an endless pipeline. Once we have that information, the capability to do that, then do you provide the full genome screening? What do you do with the information and how do you provide that? We are really in the vision process. Perlegen is about halfway through that first year.

But we are looking to loop that. That is an independent company. We supply them with these arrays and they supply us and share the data with us and work to gain synergy from that research and development. This is never going to be a commercially available product.

This is a very industrial-strength type of gene chip. But my understanding is that one technician can run three of these experiments in an afternoon and that that is

at

an equivalent data output to 196 well sequencers running 24 hours. So it is really a mind-boggling amount of data.

Where is that going to lead us? We really don't know yet because we have to look at the data. But that is the kind of basic research that we are using to gauge our forward-looking vision.

DR. TABOR: Ed Tabor, FDA. Saying that it is the equivalent of sequencing so many million hours or sequencing on amino-acid--I mean a sequencer, an automated sequencer, is meaningless for what we are talking about in here and saying that the price has come down tenfold from three years ago is meaningless in here.

If you are going to use this technology to screen blood for viruses, for instance having all of your NAT screening on one chip, maybe having several different gene sequences for each virus on one chip, first of all, it is probably going to have to be a lot faster than the rate that it is being done now.

You are going to have to get the price way down. I don't know what tenfold lower than three years ago means. If you are going to have chips that have to be thrown away after each blood sample, are you going to be able to get the price down so that a company that makes NAT tests can provide these to blood banks around the country and for

at

12 million units of blood to be screened, 12 million of source plasma to be screened every year?

DR. HURT: I think the answer is yes, we can. The current list price for those HIV arrays that we talked about is about $100. It takes about 45 minutes to run that test on the system once you have labeled nucleic acid. The labeling takes about three or four hours prior to that.

DR. TABOR: So, for that hundred bucks, today, which is only materials, not technician time, how many samples can you screen?

DR. HURT: That is an array cost. Then there is a cost for labeling. So probably $150, $200 for the--

DR. TABOR: For how many--if you were using--

DR. HURT: For a single sample.

DR. TABOR: You are talking about a very expensive screening test, I realize. I am trying to get an idea of where we are in terms of--you are describing something that it still in the very early stages; right?

DR. HURT: Yes; absolutely. As I have said, we are very much in the vision phase of our clinical corporate development. We have actually partnered with some clinical diagnostics companies to help us along that road.

DR. TABOR: For that to be feasible for blood screening in the blood bank--

at

DR. NAKHASI: But I think the question, and what you are trying to ask, is that, in that situation, you can--and if it is a $100 chip, if there are ten or twenty or thirty pathogens at one shot, you can do that. So, therefore, the cost is really--

DR. TABOR: Is reduced; that's true.

DR. HURT: That is one element. The other element is--the HIV product that I just quoted you a price on is really a proof-of-concept product. It has been out for about four years now. Frankly, it is not going like wildfire so there is not a lot of volume there. That doesn't allow us to gain synergy from that manufacturing strategy.

All of the investment to make these assays is up-front. When we design the arrays and make those photolithographic masks, that is the investment. The more assays we sell, the more we have ties to that up-front investment out. These are literally going to continue to go down for the foreseeable future.

DR. TABOR: You are still talking about something that has to be thrown out after each unit that is screened.

DR. HURT: Absolutely. We firmly believe that the quality, just as it is if you strip a Southern Blot, you probe it again, you get a result. But it is a little bit dirtier than it was the first time. Sometimes, a lot

at

dirtier. You are not sure if it was completely stripped or not. All those sorts of things.

Our goal is to make this economical enough. We feel your pain about the price. We want it to be in every lab as well. We are working toward that goal and we think we have come a long way and we are going to continue to make those efforts to bring those prices down and to arrange, and we continue to work to make the assay, the preparatory side, as economical in time as we can.

DR. KAPLAN: We have one last short question from Dr. Asher.

DR. ASHER: I just wanted to make sure I understood. The labeling is PCR labeling--

DR. HURT: For the HIV assay. We have many assays and the labeling is unique for each one. As you can imagine, there are multiple ways to get a label onto a PCR product, et cetera.

DR. ASHER: But each viral gene would require a separate label?

DR. HURT: Yes; that is probably true. We don't have that multiple virus array. Building the array is not, frankly, the challenge right now. It is building the array, as was pointed out.

DR. KAPLAN: Thank you very much.

at

We are closing this session. We invite you to the panel discussion starting a few minutes from now.

[Break.]

IX. PANEL DISCUSSION

Panelists: J. Allain, C. Bianco, M. Busch, I. Hewlett, S. Kleinman, H. Nakhasi, M. Nubling, J. Saldanha, S. Stramer, E. Tabor

DR. TABOR: Good afternoon. I would like to welcome you to the panel discussion. I think we will begin by going around with some introductions. Even though there are name plates here, I think, perhaps, they can't be seen from the back of the hall.

I am Edward Tabor from CBER at the Food and Drug Administration.

DR. STRAMER: I am Susan Stramer from the American Red Cross.

DR. KLEINMAN: Steve Kleinman from Victoria.

DR. HEWLETT: Indira Hewlett from CBER, FDA.

DR. NAKHASI: Hira Nakhasi from CBER, FDA.

DR. NUBLING: Micha Nubling from the Paul Ehrlich Institute in Germany.

DR. BIANCO: I am Celso Bianco from America's Blood Centers.

DR. BUSCH: Mike Busch, Blood Systems, San Francisco.

at

DR. SALDANHA: John Saldanha from the National Institutes for Biological Standards and Control in the U.K.

DR. ALLAIN: J.P. Allain from the University of Cambridge in England.

DR. TABOR: I would like to invite the audience to participate in this. I am going to try really hard to be a little controversial. I am going to start out by bringing up NAT testing for parvo B19 and HAV. I thought the presentations in the session on parvo and HAV were excellent. There was a lot of data presented about how much parvo there is and how much HAV there either is or isn't, but where I thought we were left a little high and dry was nobody really said what they are planning to do.

What is going to happen in the blood community? What is going to happen in the source-plasma and fractionation communities? Maybe we can start out with the panel members and, since someone from the Red Cross is sitting next to me, we can start out with her. But then I would like to ask the audience if anybody is willing to talk about what they see coming down the pike in terms of testing because what a lot of people do will force everybody else to do likewise, probably.

DR. STRAMER: Thanks, Ed. Or I am not sure I should thank you. Clearly, the source-plasma industry has adopted the route that they do in-process testing only.

at

Most of the industry, I think all of the industry, resolves to individual donation. They manage the positive product. I don't believe they do any product retrievals unless they are within the six-month time line of the PPTA standards and they don't do donor notification.

Perhaps the simplest route for the whole blood industry would be just to follow that same pathway. The complication is that we have red blood cells and platelets that are components of the blood collection. That is why, in at least my presentation, I presented the two-phase approach to meet our recovered plasma needs. Our plasma operations folks are adamant that they want to be at parity with the rest of the industry and have recovered plasma screened for parvo and HAV.

That is why we are going to have to start by outsourcing testing. By doing outsourced testing, the time line involved in doing that will cover the period where our red cells and platelets will expire naturally. So it won't be something that we are methodically doing to plan to have those whole-blood recipients get the parvo-positive products and not have plasma components be parvo-reactive.

Whether that is the correct paradigm, Kevin Brown is out here and he certainly can comment more on the clinical features of parvovirus and at-risk recipients.

at

But I think many of the presentations did cover that there would be some patient benefits.

We can't quantify that. I don't think there have been really good prospective studies done with parvo to know really what the clinical burden is on the transfusion community. So I think, for now, to answer your question, Ed, what the Red Cross will do is, as we talked about a Phase I approach, will be to outsource testing but eventually we would like to, as we did with HIV-1 and HCV, have control of our own testing, be able to do testing in real time and control all products based on positive results.

DR. TABOR: I know you sort of addressed this, but could you just comment on what your future policy would be in terms of donor notification?

DR. STRAMER: In the phase 1 approach, we wouldn't resolve to individual donation. So we would have no donors to notify. It would almost be identical to what happens with SD plasma at Vitex where resolution only goes down to a pool of 20 and nothing happens beyond that.

DR. TABOR: But, in phase 2?

DR. STRAMER: That, I think, still has to be negotiated with FDA. A memo was written at the request of Celso which he will obviously comment on as well, Celso Bianco, and Dr. Nakhasi had basically addressed some of the

at

concerns and said we would consider the way we outline testing that it would be considered donor screening by the FDA and that would include donor notification, actions on previous collections, some period of donor deferral.

DR. BIANCO: So it is my turn? I think that we have to ask, as we start that, what are the issues that we are trying to address. There are two different issues here. One is the issue for the plasma industry. The other one is the issue of patients that would be at risk of receiving a unit with a high titer B19.

For the plasma industry, it is very clear. There are limitations to current inactivation procedures. The pools are large and the chances of having a pool contaminated with B19 are very high. So there a simple approach that can be implemented fast and is being done by several of the companies, as we heard here, and saw a lot of very nice data, can reduce the burden tremendously and be of immediate benefit to patients receiving those products.

From the point of view of the whole-blood donor, and Sue raised very well the issues, the impact is much smaller. The patient population that really is at risk is small, well-defined or at least mostly defined. It will take a while for us to get there.

at

So, while the Red Cross is thinking about the phase 1 approach, we are thinking about the one-phase approach; that is, the in-process testing without resolution of pools and without donor notification. As we learn more about the assays and process and can come to the level of individual donor screening, what I foresee is different. It is not a general--but that is an opinion and an opinion of a few of us--is screening for specific use, the same way that we do screening currently, for instance, for CMV virus, the same way that there have been discussions that we should do screening for Babeosis, for Babesium microti or other Babesia agents.

When we are going to transfuse units to patients that could be very susceptible, an AIDS patient or a pregnant woman, that we would then select units that are negative or have a lot titer of B19. Actually, at that point, I don't think that we can even talk about a 10^4 cutoff. What I would like to transfuse into a pregnant woman is a unit that is negative for B19.

So I hope that we can--and also I said that yesterday I am very happy that Mei-ying presented to us actually a table of what would go into an IND and how we would approach that from our point of view.

at

DR. TABOR: Thank you. Is there anyone from the fractionation industry who could comment on B19 screening or HAV screening?

DR. ZERLAUTH: With respect to parvo B19, I think the way is fairly clear. Not only Baxter, but the whole industry, PPTA, has decided to add parvovirus screening to their voluntary standards that are be introduced beginning next year.

So, for the industry, I think this route is clear. Some sort of algorithm, one or two we have seen, will be enough to then we try to reduce the intake or the rate of virus in pools.

DR. TABOR: To what extent is parvo and HAV screening been done today? What percentage of blood and what percentage of plasma?

DR. ZERLAUTH: Baxter does every drop. Aventis, the same. To my knowledge, Alpha is--you can--

DR. PEDDADA: Lorraine Peddada from Alpha Therapeutics Corporation. Maybe you missed my presentation, but we initiated screening December, 2000. So all plasma is currently being screened.

DR. HURT: We have accounted for Baxter, Bayer, Aventis and Alpha. I think that covers the U.S. source plasma. I think there is zero blood being screened. I think the parvo discussion is really very complex and very

at

difficult. Some of the issues that I thought of that Celso discussed is the challenge becomes if we have a test available and we have the ability to test in-house, that is where the conundrum sets in. Is it ethical to wait 42 days to test and release components without any screening when we clearly know there would be benefit?

We have patient groups identified, just like we do for CMV, who would be at risk but we really don't know how far that extends. I really think, and that was an outcome of the NIH workshop that we had in December of whatever year that was, 2000 or 1999, that more studies need to be done in the transfused community. We really don't have the answers to those questions.

But, if we do have a test in-house, certainly we do know a unit that has 10^9 or 10^{13} virions per ml that is antibody-negative is going to transmit infections of any recipient and recipients are not typically healthy individuals. That is why they are receiving blood.

So I would say that we would be doing a disservice to our recipients with that type of model. The other question is will we ever be able to have a parvovirus screen that goes down to zero copies per ml. After someone is infected, there is a long period of time where virus tails or trails off for maybe up to six months, maybe even longer. In that state, it is heavily complexed with

at

antibody. We have no idea if those units would be infectious. Data from Japan suggest that, in the presence of IgG, such material wouldn't be infectious and maybe only the IgM-positive units would transmit.

So I just think there are a lot of issues that need to be discussed and I don't know what the correct pathway, meeting with the manufacturers, the regulators and the whole-blood industry to determine what type of pathway there is. So I don't know if we can all agree on what pathway we would choose to use, but, obviously, that would be a desired outcome.

DR. TABOR: I see two hands in the audience. Dr. Kleinman, you wanted to comment?

DR. KLEINMAN: I was just going to comment on the selective approach and say something similar to Sue. I don't think that is a good model. I think it is a model that we had for CMV because the only way we could screen for CMV is to do antibody in half the units. We couldn't supply CMV-negative units to everybody so we had to make choices.

But I don't find that preferable at all to--we can't really define immunosuppressed groups as well as we might like. It is logistically complicated for hospitals to actually execute that reliably. In fact, that is one of the arguments that proponents of universal leukoreduction

at

have made, give it to everybody because you can't assure that you do it correctly.

So I think it is a model that worked, but I don't think we should move that way. So either we should provide parvo-negative blood or we shouldn't. That would be my view. So it still doesn't answer the question as to which approach we should take, but I don't think that middle-of-the-road one is a really viable option in my opinion.

DR. BIANCO: Just to complete that. When I make these considerations, Steve, it is not just as--if the assays were here and easy and we could just implement it, that's fine. We are going to go through a process and the process of implementing parvo in whole blood will take a year, a year and a half, for us to get there. The process of getting into the clinical trial and real development of a BLA and going for a licensed product for blood screening is three or four years ahead.

So I wanted us to do it in a timely manner and probably, in between, as we get the information, an intermediate step would be the specific population.

DR. TABOR: There were two hands raised in the audience. Yes; in the middle?

DR. WENNER: Albert Wenner of Aventis Behring. I just would like to emphasize what Gerold Zerlauth said about PPTA. There is the voluntary industry standard for

at

PPTA to reduce the amount of parvovirus B19 entering the manufacturing pool by up to the end of this year that each donation pool, sample pool, will be tested and the middle of next year that each vaccination pool will be released when the titer will be less than 5--or up to 5 log International Units. Then the fractionation pool will be released.

DR. HEATON: Andrew Heaton, Chiron Corporation. For us, as a manufacturer, there really is a time and a cost issue here in that we are ready and prepared to manufacturer an ASR-specific reagent next year which would be manufactured to GMP standards and which would allow a user to test in real time for parvo B19.

For the blood centers, they are selling their plasma onto fractionators who make products that complete with a product sold by Baxter and Aventis and others. Indeed, Aventis has recently been approved to make a product claim that its product has been screened and tested for parvo B19.

So, for the Red Cross and Blood Systems and others, if they cannot test for parvo, they will be forced to sell their plasma at a discounted price. So, if the FDA applies one standard for the plasma industry and a separate standard for the whole-blood industry, you are going to transfer a cost to the whole-blood industry which will be

at

disproportionately greater than the cost that will be borne by the source-plasma industry.

So, from our perspective as a manufacturer, we are seeing FDA guidance as to whether this is really an in-process test to improve the quality of plasma or whether this is a donor-screening test to improve the safety of blood for blood recipients.

At present, the agency appears to have taken the position that this is an in-process test to enhance the safety of plasma. If that is the case, we can provide a test next year which would be very good value and very specific and very easy to implement.

But, if you take the position that this is a donor-screening test, then you are talking about a two- to three-year BLA process and/or the associated expense. So, as manufacturers, we need your guidance on that issue.

DR. TABOR: Dr. Dodd?

DR. DODD: I agree with what you are saying, Andy, but I think we have also heard from Dr. Epstein and other people in the agency that if you do some screening for plasma purposes, and you know that you have a pool of samples or a pool of donations that include one with a high titer of B19, it has been made quite clear to us that, if the products are available, we have an obligation to get them and not transfuse them.

at

This also complicates the issue because it means that you are either purely in process or you are doing something artificial and frankly logistically very difficult which is waiting 42 days before you do your testing.

Or you are back in the situation where you do need to think about individual donations. I don't think any of it is simple but we have got some guidance that also makes the choice of approach somewhat difficult.

DR. TABOR: A comment in the back?

DR. GALEL: Susan Galel from Stanford. I think what Sue Stramer said is the key and that is that we don't really know about the transmissibility of the agent and what titers will transmit disease. If all of the different viral concentrations will transmit disease in the whole-blood setting, then you will not get incremental safety just by screening for high titer units. It is silly just to only discard those units.

So then we should be screening all of the units. Then that is where you get into the need for selective testing because the prevalence of viremic donors is so high. So I think we really need to know much more about what dose of virus is infectious in the recipient population.

at

DR. ALLAIN: I wanted to come back to this issue of infectivity because we heard, on several occasions, this magic number of 10^4 as being something that corresponds to noninfectious. In fact, I think, rather than that, it reflects the amount of virus that can be effectively neutralized by the antibody in the pool. I don't think it has anything to do with infectivity.

It is the same if somebody has been infected and recovers and, from 10^{13} again to 10^4, it is no longer infectious because there is neutralizing antibody. So I think there is no magic in 10^4. The real question is what number of infectious particles are necessary to be transmitted.

It is like for HCV. In a chronically infected individual, only about 5 percent of the viral particles you detect by RT PCR are infectious because they are free particles. The other ones, being complex, are noninfectious. I believe it is the same for B19.

So I think we are talking about something that has very little to do with infectivity when we give a number in DNA copies. I don't think it means anything. If you wanted to really do infectivity studies, it should be by taking the sample from someone who is newly infected during the window period and make dilutions of that and use

at

it to infect. Then you would have something real about what the virus infectivity is.

But if you take something with antibody, you don't have any answer at all.

DR. BUSCH: I think the issue, to me, is would we consider screening for B19 or hep A of blood donors if there weren't this evolving pressure from the plasma side. I think we have been transfusing units for decades with a prevalence of these agents. They are very common. The disease penetrance is extraordinarily small. From a qualities-gain perspective, we are going to be talking about minuscule safety advantage to the general population.

I think we have obviously had to deal with the first HCV, HIV, pressure coming from the plasma industry and successfully responded to that. But I think developing the paradigm of delayed testing and resolving only down to a minipool context will help us in the future have an alternative strategy to deal with what are appropriate issues coming from the pooled derivative side.

I think putting into place the logistics, the ability to store the units and to do the delayed testing and deal with what other regulatory issues come out of that is a good thing because I think these agents that we are going to need to test for for derivatives, we need to be

at

able to separate what is justified for derivatives versus what is really justified in whole-blood screening.

We are not going to be able to do on-line whole-blood screening for these agents for less than $3.00 or $4.00 a donation. I have talked to the companies about that. We are going to be looking at the same kind of infrastructure costs as there are for these NAT assays. To me, it is just extraordinary that we would consider doing that.

DR. TABOR: I would like to change to a different subject now and jump ahead to GeneChip microarray technology. I feel that the last session ended without really focusing on what the microarray technology means for the blood and plasma industries in the coming three or four years.

I would like to point out that Dr. Peterson showed a slide with, I think, about six panels on it, or was it nine panels--six panels of six different things that can go wrong with the microarray test that will prevent, for the short term, its being used in blood banks. It included things like scratches on the cassette, dust, too little nucleic acid and so forth.

In addition, we heard Dr. Hurt, I think, saying that to do microarray testing on blood and plasma donations, you are going to have to throw away the chip

at

after each donation is screened. Although, as Dr. Nakhasi pointed out, you do have some cost savings by having all the tests for all of the analytes on the same run, you are talking about in excess of 24 million chips a year.

I also heard--in the break, somebody told me that, at one or more of these companies, the chips are still being made by hand. The manufacture of them has not even automated yet. So, 24 million is only if you never have to throw any out. You are talking about a very large production goal for something that is still being done by hand.

I was wondering if I could get some discussion from Dr. Peterson and maybe Dr. Chumakov, Dr. Hurt, about whether there is any likelihood that our NAT testing of blood and plasma could be done with this technology in the next few years or is this something that is going to be done ten years from now.

Dr. Peterson, could you comment?

DR. PETERSON: For right now, I would say it is still in the research phase. But I would anticipate within five years, we will see some prototypes, maybe not for testing for disease, per se, but I think they are working already on testing for patients who are suitable for certain kinds of drugs and what kinds of adverse reactions they may have. The technology will evolve, I believe,

at

within the next five to ten years but, at this stage, it is purely research.

DR. TABOR: You mentioned that there is a company that is focusing on blood testing.

DR. PETERSON: Yes; Motorola is developing a device called the E-Chip. It basically only has a couple hundred DNA probes on it but you can use it to detect electronically whether or not there has been a hybridization event. Their first markets will probably be for testing for suitability for drugs. Again, a lot of it is expression assays, expression profiles, that people are looking at.

DR. TABOR: Dr. Nakhasi?

DR. NAKHASI: Ed, I think, as you have pointed out, this is just basically what--it is at the infancy, I would, say in regards to clinical use. I think we need to keep our mind open about how we can, and think how we can go in the future, how we can utilize it.

The interesting part, what I see in this whole issue, is making a multiple complex. You can increase the complexity in the thing. However, there is a caveat to that, also. How many can you make a multiplex and still maintain the specificity and the sensitivity. I think that is one other issue that needs to be understood.

at

I was having a side conversation with Dr. Hurt on the outside. They are already thinking in those directions. May Dr. Hurt can put a light on that conversation.

DR. HURT: One thing I would like to comment about. The manufacturability issue was raised. We, at Affymetrix, have spent many years focusing on that as a core competency. We believe that, today, the assays are deliverable in literally any volume that is desired.

We have a great deal of excess capacity built into our system, a great deal of scalability in our manufacturing plant planning. So, as far as being able to deliver a million arrays, or whatever, we have been shipping hundreds of thousands for arrays for a couple of years now. We are really up to speed.

So, in terms of volume and robustness of manufacturing, I think that that, as far as Affymetrix goes, is a very solid part of the puzzle that is already in place.

A side conversation. We have been thinking very carefully about how to make an entry into the clinical marketplace. Frankly, this GeneChip Array, the complexity and the highly dense nature of the oligos make it very uniquely suitable for certain things but it also means that it is too big of a gun for certain other things. It is

at

certainly not going to displace every other technology that is available in labs.

It most likely will not be economical for very low-complexity testing. 100 oligos? The GeneChip is probably not your platform. A million oligos? I don't know of another platform that I could think of. So I will just leave it open to the discussion.

DR. TABOR: Dr. Chumakov?

DR. CHUMAKOV: I think, at this point and, perhaps, for the foreseeable future, this gene-expression analysis is a discovery tool. It is very good for looking for patterns. As soon as you identify a pattern that is associated with a certain condition or presence of a certain virus, then you want to downsize the chip or maybe even more to an entirely different type of assay and just follow the marker that you discovered by using this technique.

It can be even not a nucleic-acid-based test. There are a number of other platforms, microchip platforms, that are reusable. In addition to Motorola, there is NanoGen, a chip that I think is about 10 by 10. It is like 100 elements but it can be reused many times. So there are a number of ways to address this, but I don't think that this generic 10,000 or 20,000 gene chip is a viable routine test. It is more for research, for discovery of markers.

at

DR. TABOR: Thank you. Dr. Dodd?

DR. DODD: There seem to be a number of ideas that have crept into our field. I am not sure where they have come from--that really need a lot more careful thought. I think the GeneChip technology for screening blood is one of these ideas. I am not sure how these things arise. Another one is that everything is going to be great when we have a test for vCJD and another one is that virus inactivation is the answer to all ills.

I think we need to be much more critical about thinking through these concepts. I think the message that I got was exactly the one that has just been presented to us by the people who discussed chip technology, that this is not the way to go at the moment, but maybe some directions and ideas can come from that. But we need very critical about "this will save us all," because I am not sure that a lot of these ideas really will.

DR. NUBLING: Maybe I can give a comment, a quick point. I think, for diagnostic purposes, the array technique works, it works very good. But, for screening, we are looking for pathogens for viruses which are not normally distributed. They are distributed after Poisson. There are few particles in one ml. I have a number of doubts about the sensitivity that we need for screening what you have to offer with the array technique.

at

DR. ALLAIN: My understanding is that one of the main uses of microarray is when you have substantial genetic polymorphism. I think, in the area of blood banking, testing for HLA genotyping could be the first area I would go for, if I was going for any--in our discipline.

DR. NUBLING: But the genomic background. In the genomic background, it is enough material. You have no difficulties, then, to discriminate between phenotypes and so on.

DR. NAKHASI: I think, if I may add to that, it is right. You have to be creative in thinking what kind of techniques or technologies you can exploit in the sense--like I was just talking to somebody--drug resistance is another issue. If you know where the drug markers are, you can utilize those types of things.

So one does not have to think of it in the way that this is the solution for all the ills but has to keep on thinking open about it, how we can utilize it. I was not involved when the NAT testing came into being and I am sure the same questions would have been raised at that time, is it possible, is it feasible.

But, as Dr. Duncan earlier pointed out, I think we have to keep thinking how we can use it, be critical, as Roger said, and also think what would be the best way you can utilize it.

at

DR. HEWLETT: Actually, I would just like to add to that. Back in 1994, when we had our NAT workshop, I think the general sense was that nucleic-acid testing is a good tool but we were not ready to implement NAT in any fashion at that point for donor screening.

But, thanks to the help of NIH, in a couple of years, was saw INDs and we have succeeded in putting nucleic-acid technology in our blood banks. Of course, that is done in a different format. That is basically in a ELISA type of format which has already been put in blood banks previously.

So the next and natural progression seems to me going in the direction of perhaps exploring other platforms. That is when chip technology becomes one of the candidates that would be worth exploring. Of course, it would become useful only if one could put on, as was pointed out by the experts here, if you could put on more than 20,000 or a million targets in there, it would become useful and it would become cost-effective.

So I think the issue is really whether we should explore that. I think what you are hearing is that, perhaps, we would start by looking at HLA and making some inroads into actually putting this platform into the blood-bank setting and then going from there as to whether it could be expanded to detection of pathogens.

at

DR. TABOR: Celso?

DR. BIANCO: I want just to give a little bit of a customer point of view. I think that what we are looking for is not necessarily the chip technology. I think that what was sought is a possibility to automate, simplify and increase the number of sequences that we can examine.

We are looking very specifically for high sensitivity. It is not genomic DNA. I think that that is a very good point. So I think that it is worthwhile looking not only at a chip but anything that can facilitate that process by which we can examine, with high sensitivity, a large number of sequences in an automated fashion.

DR. TABOR: Dr. Kaplan?

DR. KAPLAN: One point I would like to make is that there is a general consensus that it is a combination of techniques. Okay, you can amplify your sequence using PCR and just detect it with a chip using specific oligos. But there are many other ways of amplifying very low copy numbers that you can use a combination of hybridization techniques and PCR amplification.

So I don't think that the point that was made here of the sensitivity has any impact on the use of the chip, as Celso was doing, as a general hybridization

at

platform to look at a large number of pathogens at the same time.

The other thing, I couldn't ask a question before, but one of the problems that I see on the previous question, on the B19 and HAV, is that both viruses have been bundled. They are two completely different problems and they have two completely different answers. I don't think we talked about HAV at all. Basically, our conversation was on parvo B19. So I don't know if the panel would be interested in bringing it up again or not.

DR. TABOR: Dr. Busch.

DR. BUSCH: Something I have thought about in the past but Celso just triggered again. The concept of multiplexing is innovative and, obviously, appealing in concept. But, when that hits the regulatory reality, it is not very easy to bring forth. GenProbe Chiron have developed a multiplexed HIV, HCV. But when the issue came forward about adding HBV to that, they have taken that multiplexed dual assay through clinical toward licensure. In essence, they would have to go back to step 1 and create a triplex. It turns out the Roche users are in a much better position because they simply have a third assay to add to an extract.

So, as you get into the regulatory and licensing side of this, you lock yourself into a system that is

at

multiplexed in one configuration and isn't flexible toward subsequent modification and licensure.

DR. NAKHASI: I think you are right. I think we need, as regulators, also, to keep changing our thinking also because I think that is an important issue. I think we have to keep up with technology. We have to keep up with what is out there and what kind of impact it will have and not go by the "classical" thinking that we cannot do that thing. I think that is very important point.

DR. TABOR: Dr. Zerlauth?

DR. ZERLAUTH: I would like to add a more general view on this technology, the microarray or MicroChip technology. I think it is an attractive technology and we certainly can overcome--or not we, but those gentlemen can overcome the problems still involved. But, basically, we are targeting very few targets in millions of samples. This chip technology offers us a million targets in one sample.

I think this is the crucial point and I would follow Chase's wording in saying we should rather pursue the simplification and the automation of what we have because now we are going for five viruses, we could easily add five more, because once you have installed that system, it is actually a matter of--it is no big deal to add further tests.

at

I think if we automate that and if we make it really stable and sturdy, we are better off for the moment. I don't mean we should not pursue these microarray techniques, but, for the moment, I would buy an automated technique rather than microarray systems.

DR. HURT: I just want to sort of amplify on Mike's point on the point about bringing the right gun for the game. The GeneChip platform, the array, is simply a detector. It depends on what you use it for, what are you applying to that detector. Making the detector is no longer the game. How do you prepare a biological sample and make use of the wonderful multiplicity of that platform. That is what we need to think about and integrate into our strategies.

So, if we want to look at a million viruses, maybe a year from now, we will be making that million-virus array. No problem. But how do you effectively label and prepare a million different virus targets for that platform. So we need to really focus on the front end, not really the back end. It is neat. It's great. It's new. But we need to use it appropriately and we need to use it towards its strengths and not try to apply it towards something it might not be so suited for.

DR. TABOR: I would like to ask some of the test manufacturers if they could comment on this type of

at

technology. I don't see the people from NGI anymore, although they were here quite late today. But I see someone from Chiron. Is it possible to get you to just comment on where you see the future of this type of automation going?

DR. PHELPS: Bruce Phelps from Chiron. I think the chip technologies will definitely have a place in the future. I would agree, I think, at this point that they are useful for research purposes. I think if you can pick the right sequences and you pick a few sequences that will determine with certainty that you pick up all genotypes, for example, for HIV, HCV, HBV, that you could do it with a limited array and that might be what we would want to implement first.

I am not sure that the manufacturing capability is there to make the large numbers yet. I think we would have to evaluate that. But I think that, in the future, that that potential would be there.

I think Indira's point about 1994, when we were all thinking the same thing about NAT technology is a very good one. If we apply the right focus and the right support for these types of projects in industry, we can come up with the tests that are needed. I think that the multiplexing of the test is something that we have all been

at

thinking about. We would all like to see the right way to implement it.

The implications, I think, have been raised already with respect to the ability to expand the technologies to include new viruses and new entities. But we also have to think about the regulatory approval cycles that we go through and how we can expand those and change those over time.

So I think that the doorway is just now being opened. I think there are some good lights at the end of the tunnel that we can come up with products. Whether it is five years from now, whether it is ten years from now, I don't think we can tell.

DR. KLEINMAN: I am just thinking about the clinical trial that one would have to do to validate a technology that tested for every virus that we are screening for and what kind of submission would go to the FDA reviewers and how they would review something.

So I think we would need some--if testing for multiple agents in a given run, and I mean beyond two or three, ever becomes a reality, the FDA would have to think about its approval mechanisms because I just can't see a manufacturer wanting to go down that path of regulatory approval.

at

DR. TOBLER: Listening to you guys talk, it almost sounds like some people are advocating changing the way we do blood banking to fit this new technology and somehow that doesn't seem right to me. We do blood banking quite well.

DR. HEWLETT: I think that is definitely true. I think we do blood banking very well. We are doing very efficient infectious-disease testing. I think the question is we are always faced with this question of what do we test for next. Do we go in for bacterial NAT, for Babesia? Do we go in for malaria? It is the multiplexing capability, as had been pointed out by a number of people here, that makes this technology interesting and something worth looking at. It is something that we should at least investigate to see whether there is any value in putting something like this in place five or ten years down the road.

We are not talking about next year.

DR. TOBLER: But we already have a shrinking number of donors. The more and more infectious agents we test for, the less and less donors we are going to find that will qualify to be repeat donors.

DR. NAKHASI: I think, in response to that, it will be interesting--also, you heard this morning that we are deferring a lot of donors on the basis of false-

at

positive results. Now, if you have technologies out there which you can get them back into the pool, you will have those pools. Plus the fact is more and more pathogens will be tested, whether it is not now, people, down the road, demand because people are aware--vCJD is a classical example of that. We do not know yet whether it is transmitted, but we have deferred it.

So I think more and more things will be coming. So we have to be prepared for the future.

DR. TABOR: Dr. Busch.

DR. BUSCH: This is a good transition to a topic I hope could be brought forward and more focused on the realities of NAT screening, the issue of reinstating these very large numbers of historically deferred donors for HIV and HCV. In terms of changing the FDA paradigm, what we heard yesterday was a kind of classic, any donor who is deferred prospectively and presumably has been deferred in the past will need to come back and go through a separate sample-test reentry algorithm.

I think we have completely changed the paradigm of screening with the addition of NAT testing, more sensitive serologic testing. I would hope maybe you could address whether you have any real concerns about an alternative strategy which would allow us, with the licensure of NAT and the incredible array of technologies

at

we have and all the enhanced even donor-history questioning, can't we just send letters to all of historically deferred donors, the confirmed negatives and indeterminates, the neutralization donors and say that, "We are now in a new era. All of you are eligible again."

The cost of trying to reenter all these people, the complexity, basically we are not going to get any significant number back unless it is done this new way.

DR. TABOR: Let's talk about that. But I would like to talk about it not in the context of ask the FDA but more in the context of what can be done and what the different alternatives might be related to that suggestion. Is there anyone who would like to comment on that?

DR. HEWLETT: I think it is certainly doable. I don't think it is something we have discussed at length within the agency in terms of regulatory current thinking type of thing. But certainly, if one has a large amount of data, a lot of test results and a history of the donor and so on, and repeat testing, I think it is feasible.

I would not want to say at this point, without knowing the specifics of the different categories of donors we are looking at or we are talking about that it can be done right away.

DR. BIANCO: Philosophically, I agree with Mike in the sense that our testing systems are so good that we

at

are going to pick these donors up and we are going to resolve it no matter what the past history was.

However, I think that we--and that burden falls on us, not on FDA--I think that we have to convince you that our systems are good enough that we are not going to mix up tubes, we are not going to mix up donors and you are going to be comfortable about the fact that you may have a potentially risky unit in the system that could be mistransfused.

I think that we are close to that in the blood-center environment. We are not close to that in the hospitals and in the systems and the errors and fatalities that are reported to you. So I think that that has been the focus, to review these measures and things so that you feel more comfortable that secondary errors are not going to put a recipient at risk.

DR. TABOR: Comment from the back?

DR. STRONG: Mike Strong, Seattle. I would just like to echo the plea for simplicity. We all have these complex reentry algorithms for our donors. But none of us really use them. We don't want to have to deal with the irate donor that we have deferred now after they have donated 200 gallons of blood and now they have their second core-positive false-positive test.

at

So we really need a mechanism that is simpler to allow us to go back to these donors that we have lost who are our most dedicated donors and try to bring them back into the system and eliminate these false positives.

DR. TABOR: Dr. Stramer.

DR. STRAMER: Regarding a follow-up sample, I think traditionally the way the FDA has thought is that it starts the sample neutral on a clean bill of health and basically starts the donor again once the follow-up sample has tested negative.

It is probably well known that the American Red Cross does not do reentry for anything except for p24 antigen. That is a process that we have monitored very closely because it is the only test that we do reinstatement for and we want to make sure we get it right.

So, looking through the data, as I do, for p24 antigen, we have just switched test manufacturers from Coulter to Abbott. I just want to address the feasibility of having a follow-up sample for our dedicated donors. We have just entered, I counted, over 200 donors in the last month who we have just reinstated by just changing p24 antigen kits and letting them follow up on the new antigen test as opposed to the old antigen test.

So, even having a follow-up sample and starting the donor at a neutral ground was feasible. It worked. I

at

think it was very effective. So I am not opposed to changing the paradigm but I think the paradigm does work. So I just wanted to add that.

DR. PHELPS: Bruce Phelps, again, from Chiron. One more comment. I think with the potential for initiating parvo B19 testing, I think we may be embarking on another tack here where now, all of a sudden, our donor population may be notified that they have been infected with a virus but they are not sick and it is okay, "but you are only deferred for a short time and now you can donate again."

I think what you are doing is now highlighting a fact that there is a potential infection with an agent which you have detected and you are finding it necessary to notify the donor, but yet it is not a life-threatening situation. There is no treatment for it. They don't need to go to a doctor. They most likely have resolved by the time they are being notified.

Now, what are you doing to that donor? Is that donor really going to go back again and risk being tested for potentially another viral agent that is also relatively innocuous as far as he is concerned?

I think there are a lot of issues that are coming out when we are looking at expanding the numbers of agents that we are looking for and the implications that it has on

at

the donor. The recipients are a different story. But the donors, I think, are now having to be considered getting a positive test and not being prepared for it at all.

DR. TABOR: Dr. Dodd?

DR. DODD: Maybe a somewhat more modest proposal in response to Mike's comment and something that we, perhaps, could do going forward and might help a lot is to reevaluate the concept of the indeterminate result. The indeterminate result is not doing anybody any good. We saw two huge data sets for HIV and HCV. It is very clear with the way that supplementary tests are now read that almost none of the indeterminates have the slightest chance of actually being infected.

We have to acknowledge that there were some indeterminates for which NAT was positive, but I think it is high time to rethink the concept of the indeterminate result, move it or most of it over towards the nonconfirmed side of the equation. I think that is something that could be done a little bit more readily right now.

DR. ALLAIN: Being totally unfamiliar with the internal American politics in blood banking, I just wanted follow up on Roger's comment. I wonder whether it wouldn't be also time for the manufacturers of serological tests to relook at some of their assays which are currently used because we have seen, for instance, for HBsAg a fantastic

at

difference in sensitivity and with very clear clinical consequences.

What Sue presented was quite clear. I also heard that one particular confirmatory assay was less sensitive than the screening assay. Also, it seems to me that the antigens, for instance, that are used on RIBA 3.0 at the moment, some of them are poorly reactive and should be eventually reconsidered and try to improve the performance. I wonder whether that couldn't impact considerably on the issue of indeterminates that Roger was mentioning.

DR. STRAMER: Having been working for a diagnostic manufacturer in a previous life, as you have, and now sitting where I am sitting, we are going to use the test. The problem is we are going to use the test whether the specificity improves or not. We have no choice.

We have been using anticore, one manufacturer in particular in anticore assay, that, until recently had very, very poor specificity. But there are only limited choice. The manufacturers know they are going to use the test anyway. So, for like the Western Blot or for RIBA, I am not sure what financial motivation the manufacturers have, especially when they have to do million-dollar clinical trials, wade through the regulatory hurdles to give improvements through the system.

at

So it is very frustrating but I completely agree with Jean-Pierre. And another point I wanted to make when we are talking about reentry and catchment, the area that we have lost the most donors, or where we would get the greatest bang for the buck, is anticore. So what we should do, and we have been working on it with AABB TTD in a small working group is looking at reentry algorithms for anticore reactive donors and being able to get those masses of donors back into the system.

I don't know if the number I am going to quote is correct, but I think we have 1.3 million donors in the Red Cross's DDR. I would venture a guess that 60 percent of them are in there alone for anticore. So if we focused at least on that one test for which we don't have a supplemental test, for which we have indeterminates and all the noise associated with that, and be able to work with the agency and get an anticore reentry algorithm, I think that probably would do us a lot of good.

DR. BIANCO: As a follow up to that, I want to touch on two issues. One, while we, as customers, do not have the power to change those companies because we don't have the choices, the agency has some power to set standards, a minimum standard for HbSAg assay, for instance, that would be more compatible with where we are today instead of ten years ago.

at

The other thing on what Sue just touched, the anticore, where the nonspecificity of one of the assays has been critical, we have great opportunities as new assays are developed, as we change assays. That is the greatest opportunity to reenter donors. That is when nonspecificity is changed and we have a new--and I think that is where, again, the agency could give some thinking to that concept that comes from the early '80's, '84, '85, and the licensure of the first HIV test, of blaming somebody, or even the hepatitis test, blaming somebody for life with a positive screening test and then creating a complex reentry algorithm.

A simple retest. It doesn't have to be a sample, but a new donation will give the opportunity today for a much more complete reevaluation of the donor. Again, the issue that we have to resolve for you is to make you confident that we are not going to screw up.

DR. TABOR: Let me just say that when the data from the study that we conducted in collaboration with Dr. Busch and some of the manufacturers that Dr. Stramer was talking about this morning was presented at BPAC by Dr. Biswas. Part of the purpose was to inform people about the data but part of the purpose was to give a heads-up to the test manufacturers that the standards for HbSAg test will

at

be ratcheted up and that the CBER lot-release panel will be made more stringent.

It obviously takes time to put together a panel but that is in process and that is something that will occur.

With regard to anticore and reentry, this discussion is very informative. I think it is something that all of us ought to discuss further. I think, if you don't hear from us because of all of our distractions with bioterrorism and so forth, you should come back to CBER again and again to make sure that the reentry issue does get discussed further because there are a lot of facets that, perhaps, we are not fully aware of.

DR. BIANCO: Thank you for the offer. This is another aspect of terrorism. We terrorize the donors.

DR. TABOR: Dr. Busch.

DR. BUSCH: Again, this is a transition on the other topic that I had that I would like to have discussed. We are talking about supplemental testing and donor reentry. We have complained, me and many others, about the fact that the manufacturers of supplement assays don't have the motivation, have not brought forward improved tests to match the sensitivity of the screen. We don't even have a licensed test for HTLV.

at

We have complained and FDA, to some extent, heard us that, as they license new screening tests, they should require that the manufacturers develop appropriate supplementals. That is the one time that there is some leverage. Unfortunately, you seem to be doing that with NAT and it may be a problem in that the companies have tried to build some sort of home-brew supplemental capacity to support their clinical trials. But I think you will hear from all the manufacturers that those assays are not GMPed, are not sustainable, are not comparably sensitive.

Many of have discussed, and I would like to hear FDA's sort of considerations about instead of trying to develop some independent supplemental, this is the perfect arena where we have the optimal supplemental assay and the alternative screening assays. What process--because the companies are not going to do INDs to get a claim for supplemental for their screening assays. Even though they are not going to have to change the reagents, it is just the trials to demonstrate that.

So, hopefully, there will be a receptive ear to a simple way that the industry can validate a cross supplemental claim.

DR. TABOR: I would like to propose that we discuss this and that it be our final topic for the panel. However, I would like to limit the discussion to

at

supplementals for NAT testing so that we get away, a little bit, from a regulatory question-and-answer session.

Would anyone like to comment on NAT supplemental testing?

DR. STRAMER: I agree with Mike and I would just like to put out for consideration--I don't mean this to be "ask the FDA." This is just something to consider. For HTLV, because we don't have a licensed supplemental test, what we have been doing as an industry is using, without an IND and without a clinical trial, the FDA-licensed screening second HTLV test.

We have two tests that are both nonspecific. So the only advantage of that is that the nonspecifics of one assay are not reactive on the second assay. As I just said, when we brought all of our Coulter repeat-reactive antigen samples in, they were nonreactive and reentered successfully on the Abbott test.

The July, 1996 memorandum from FDA on product retrieval talks about use of a second licensed EIA to clear products for anticore and HTLV. So even though this is a discussion we have had, I would propose that we don't need to do anything. We have a licensed screening test that has proven sensitivity, reproducibility, manufacturing, all the requirements that go into an FDA license for screening which are more stringent than the supplement test.

at

So I would propose, for example, we have a licensed screening test for NGI, that that could be used as a legitimate supplemental test for NAT or when the other assays finish their review cycle and get FDA licensed, that we automatically have those built in.

DR. HEWLETT: Yes. I would agree with that. I think there has been precedent for using two EIAs, licensed EIAs, as supplementals for each other, especially if there are no specific supplemental tests that have been developed or are in the process of being validated as supplemental tests.

Clearly, in the case of NAT, you have multiple platforms. You have got NGI. You have got Roche. You have got all these kits that are under review. The data is being collected nationwide. So I think there is going to be a lot of data that would be supportive for that type of a situation.

The issue is they would essentially have to be licensed. At this point, there is only one licensed test.

DR. BIANCO: I want to support the points of view here. There is one little piece that is missing here and maybe Mike can help us is that the clinical trials have been going on different datasets and different specimens. In order to feel a little bit more comfortable about these types of decisions, I think that we will have to have at

at least one good experiment in which the same samples are subjected to the test from different manufacturers and see how they behave.

DR. TABOR: Dr. Rios.

DR. RIOS: How could we use NGI as a supplemental test if we know that NGI PCR for HIV does not detect most of the different strains as shown here. It mainly doesn't have any O or any M, and et cetera. So how could one use a much more limited specificity PCR for confirmation of a TMA, for instance?

DR. HEWLETT: At this point, with NAT testing, we have taken the view that it is really Clade-B sensitivity that we are looking for. We want manufacturers to test as many isolates of different subtypes that they can get their hands on. We realize that there are very few of these subtype isolates that are out there.

That is one of the reasons we are building a panel. We have referred manufacturers to foreign study collaborators to acquire these subtypes so that they can test them with their test. That is as much testing as has been done, certainly, with the licensed test and we would assume, and we would hope, that the other manufacturers would come in with similar datasets.

But you are right. These tests are not completely or fully validated for, for example, with HIV,

at

the non-B subtypes and with HCV, all of the other genotypes. It is the predominant genotype in the U.S. that is being tested. But we do have data from manufacturers that indicate that they can pick up five out of the five isolates of genotype 6a, for example.

So it is really a labeling--you will notice that, in the datasets, what we presented are mostly data on Clade B but we do say that they have tested various subtypes although the test is not fully validated for detection of the subtypes.

That is partly because it is very difficult to get these isolates. So you sort of have to work with what you have.

DR. BUSCH: Just to answer Celso's point, we do have a study that it actually took a year to get all the companies to agree, but NHLBI is funding, through the NAT study group, a head-to-head comparison of the assay platforms, neat versus minipool, multiple reps, multiple seroconversion panels. I think that is the critical data to show the essentially identical sensitivity of all these platforms.

The specificity side, in terms of crossover, the problem is there is not enough volume left after the supplemental testing that is being done now to be able to have reserve volume to be able to test on the other

at

platforms now that the companies are willing to allow this to happen, with the exception of some Red Cross samples where they have managed to carve away the plasmas.

DR. STRAMER: We retrieve, as part of our requirements, the plasm unit from each NAT-reactive index donation.

DR. HEWLETT: I was just going to say that I think we are aware of the studies and we have talked about this. I think these are the types of studies we are going to see where there is a nice common set of samples that are tested, as Celso was saying, and those types of data would be very useful in validating the test for this purpose.

DR. TABOR: Dr. Kleinman.

DR. KLEINMAN: I am going to say the same thing, but just to reiterate that the more readily available samples are the ones from seroconversion panels and so those could easily be tested under code, head-to-head, and one would have the results and show, hopefully, concordance. If you have to test NAT-positive window-period cases that you got from whole-blood donor screening, you are going to have far fewer samples. So I think, hopefully, that won't be a requirement. One could it with the seroconversion panels and then one could get a much larger dataset to evaluate.

at

DR. NAKHASI: I think it reminds me of the same HBV study with I think Ed was mentioning, too. I think that a similar type of thing can be done so that you can compare the sensitivity of the test.

DR. HEWLETT: Going back to the seroconversion panels, sure. Those panels exist so they can be used in this context. We realize that it is not easy to get a lot of samples in your prospective studies. So that data is, then, further supported by data that you get from seroconversion panels. So, definitely, that is very useful.

DR. TABOR: I would like to try to end the session now. Is there anyone on the panel who has anything they would like to say?

DR. BIANCO: I want to make a speech. This, I think, was--I want to thank all the organizers. This has been a very useful meeting. We could air a lot. I think that Indira reminded is of '94, September '94, September 24, '94 and we, six years later, are at a complete different stage. We are extremely comfortable with both assays that are being used in the donor screening, the whole-blood donor screening.

We heard a lot of the plasma industry with a comfort with the NAT. And I learned a lot of stuff, including microarrays. So think you.

at

DR. TABOR: I would like to thank everyone for remaining throughout the afternoon. I want to thank the members of the audience for participating in the last discussion. It was a great addition to the discussion.

With that, I will close the session. Thank you.

[Whereupon, at 5:00 p.m., the meeting was adjourned.]

- - -