Panel I: Surveillance and Detection

Moderator: Gao Fu, Institute of Microbiology, Chinese Academy of Sciences

As you know, I think we are very pleased to have the two clean-up speakers this morning and who summarized the strategies in China for the control of the effects of infectious diseases, and also some thoughts about disease control from the American side. So, I believe as you know, as the first moderator, I think I have five minutes to speak. So, what do I see? Because this is a dialogue, and also we are talking about emerging science and technology, the abbreviation is EST. As you know, what EST means – it means we are going to find some functional domain or module or whatever. I hope this is the target we are here to try to find something. We can work together between the Chinese science officials and the American scientists and American officials, to try to tackle this . . . – the EID -- emerging infectious diseases.

So, as you know, obviously we need the collaboration because the globalization – from my recall, I remember when I attended one of the global discussion meetings, the former President of the U.S., Bill Clinton, was there. He used the word not globalization, but he used the word "interdependency". So, we are living in a world so we are dependent on each other. I also want to remind you when we had SARS in 2003, one of the expressions I hope you still remember – once . . . . would make the . . . . be around . . . ambulance. So, I hope everybody remember that. Less globalization and interdependence . . . . So, I hope this is our dialogue today.

Again, I won't have . . . for everybody, so we want this not a symposium. We want this as an interactive dialogue between the scientists to promote as the ultimate goal, to promote the science, try to identify the common interests between the American scientists and the Chinese scientists to try to tackle the global infectious disease. So, we need to work together. So, I hope this is our target for the whole meeting. So, remember, globalization – interdependency, wants us to work together.

Now, I think we can start with panel 1. It will be the surveillance and detection. I'm very pleased to introduce the first speaker, Dr. Maria Elena Peñaranda, from Sustainable Sciences Institute of the USA. Her title for the talk is Practical Tools to Combat Infectious Diseases in the Developing World: A Model of Technology Transfer That Works.

Maria Elena Peñaranda,
Sustainable Sciences Institute

Practical Tools to Combat Infectious Diseases in the Developing World: A Model of Technology Transfer That works

I am very honored to be here and very happy to be part of this wonderful dialogue that we're going to have these days.

I'm going to talk about transfer of technology – not any new technology necessarily that you all are going to be talking about soon, but a model that we have and how to communicate the new technologies that are happening to the developing world. And, why is this important?

There is a disparity between what is in the developing world and the developing countries as potential years of lives lost in developing countries – 93% versus 7% in the industrialized world. This translates as research expenditure by purpose. So, for these the research funds to combat this is 90% of the research funds go to study diseases that effect 7% of the industrialized world, and only 10% of these research funds go to study problems that effect 90% of the countries and it is usually due to infectious diseases. So, there is a gap. The global forum for . . . strengthening research capacity in developing countries is one of the most effective and sustainable ways of advancing health and development in these countries, and of helping correct the 10/90 gap. This 10/90 gap, maybe the numbers have very little . . . 2000 and 2001 . . ., we are working to bridge the 10/90 gap.

How do we do it? In our institute, we are trying to bridge the gap by providing training, funding, networking and consulting and material aid. This is the Golden Gate Bridge in San Francisco where our institute is based. We aim to bring technical expertise in the developed world to where it is needed, access to the resources where there is limited resources, decide to apply this technology to where there is scientific isolation, research orientation when there is lack of research . . . . Our objectives specifically are to build scientific capacity in developing countries,

to make our medical techniques available to local scientists, and to support investigation of locally relevant public health problems.

How do we do this? We adapt the techniques to existing local conditions and local is going to be a word you are going to see often. We train scientists in the theory, practice and laboratory techniques, epidemiological methods, and proposal development to assist the local scientists to investigate relevant infectious disease issues of their own.

We have several processes to do this, and I'm mainly going to be talking today about our training workshops. We have on-site training and grants. Advanced workshops are usually done in countries where we are first going to bring the technology. So, we bring a big team of 6-7 researchers which transfer the laboratory techniques in . . ., and then we also train epidemiological methods and grant-writing skills. This is so that once they have the technology, they have to think about how they are going to apply this, what is the problem they want to solve and how they are going to solve it.

The groups that we visit are already have their work going, but they need an update, we send peers – people that are already trained, they have the ability to troubleshoot – this is what is best, this is what is fastest, and usually we focus in . . . to transfer and I will talk about it later. We provide small grants of seed money that hopefully they have learned how to put their ideas in a fundable grant, and we give smaller grants, $10,000 to get them started in the work. Then we have a new most popular one, . . . writing workshop. They have to publish the results. You know how important that is, and how difficult it is sometimes. Then they have to develop centers of excellence. Once we started going to a country and we find a nucleus of researchers that are motivated and are carrying on with the local problems, we keep giving them advice, we keep them in the support. So, this is done through networking and consulting which is what brings all this together. You cannot bring technology and then abandon these people to their own. So, we reduce scientific isolation, build partnerships, national, regionally and internationally.

Then we have programs called material aid, thanks to a grant from the Keller Foundation, that allows us to take equipment, especially in the area where we are . . all these biotechnologies that are used, but in good condition, but the companies want newer, faster equipment, if it is in working condition, we can then . . . to bring this equipment to the countries that need it.

So, this is an example of how we break down the technology and the . . . . on-site. PCR – you can do it . . . by hand, in extreme conditions where you don't have . . . and it works really well. You need someone with patience to sit and do the cycling. When you don't have an . . . centrifuge, you have the blendifuge – it is a blender that is adapted to spin your samples. An alternative, you can also collect samples, dry the blood in the field and bring it to the lab to be processed later. Usually, the DNA material is stable in these conditions.

So, here was have the laboratory training, the epidemiology. We usually try to teach epi-info which is a free epidemiological and statistical software provided free by the CDC, and then the funding for a small research grant.

Our work is mainly in Latin America. It started in Nicaragua and it has expanded through the rest. That is where we started calling Centers of Excellence. Nicaragua, and now Equador, Paraguay, Panama. Egypt is an exception and we are just starting to work in Peru.

Then when we were talking about . . . transfer, our people are experts now that are in Nicaragua are the ones that come and train people in Bolivia, Equador, Peru, because usually the problems are similar and they can understand better in language and conditions and it just promotes this divide is close.

The writing manuscripts – this is a new workshop that was developed in response to partners' needs. It is important because it boosts visibility and enhances chances of competing successfully for grants, helps gain ownership of the research, and contributes to scientific knowledge that influences local and national health policy. Usually there is a lot of data that is being generated by wonderful, and very good investigators, and it is not being published. So, our format is we provide the workshop, the participants . . . topics for manuscript. You have to have

some data that you want to write about. Usually sometimes it is even their thesis where they have a lot of data that you could extract a few articles from there. We select instructors with matching expertise. This is important and it is one of the hardest part – because if people are writing about tuberculosis or writing about . . ., we bring people that have experience in writing in the team, but also to help them analyze the data so that it is consistent and makes sense.

So, we have lectures in the morning and then we have one-on-one tutoring in writing for the rest of the day. That is when, for examples, the tutors will help people express best the result – what is worth publishing, and what is not; what is the best way to present these in figures or tables, and what should not be on a paper.

By the end of the week, we hope to have a very rough draft which is very ambitious and difficult, but they do. They work really hard and they make big progress.

We have done Panama, Paraguay, Guatemala, and Nicaragua, and now the Pan-American Health Organization sponsored us to go to Paraguay, but now we are going to Bolivia this year and the Panamanians, which is a nice development, are then teaching other teams of their country how to write scientific papers. They asked for assistance, just one or two instructors, to help out. But, it is their initiative. That is the kind of thing we want.

So, an example further of how this works, in the laboratory training we'll use . . . which we work in our lab at . . . There is 50 million cases of Denghi fever annually and a complication is that Denghi fever or Denghi shock syndrome, 250,000 cases annually. This is due to varying infections of different . . . types of Denghi. There is four. It is transmitted by . . . which is daytime biting mosquitoes, so the bed nets that are used for malaria don't work here. Typical . . . control and it produces urban epidemics. It is spreading rapidly.

So, what we did is in conjunction with Nicaraguan scientists, we design . . . for all Denghi types, and then for each . . . type that renders different . . . for each . . . so, in one tube, you can have what is the Denghi . . . type that is being circulating in the area. It is a quick diagnosis which we always stress the importance of conventional techniques as well. This is a quick way of finding

out what . . . type is circulating, but it is very important to confirm by . . . culture . . . and other types of techniques.

So, for example in Paraguay, there was a large epidemic of Denghi in 1999 and 2000, after a lapse of ten years. The mosquito had been almost irradicated in the Americas in the 60's and it reduced transmission of yellow fever and Denghi. But use in DTT, which is very bad for environment. So, the mosquito has reemerged and it is coming because the mosquitoes are spreading in the American nation and all over the world. So, the implementation of molecular and biologic techniques for identification of Denghi . . . so, they were allowed to identify the arrival of Denghi to . . . in May of 2000, leading to a rapid . . result in containing the outbreak. The virus comes from Brazil and there are a few cases that started coming to us in . . . and a similar scenario happened in 2002. So, now the team of researchers at the university work together with the Ministry of Health to stop the spread of these outbreaks and it is pretty much under control almost at all times.

Another case that happened in Nicaragua is . . . viral illness that was assumed to be Denghi, but we had a technique working and it was negative. There was no . . .. depressant. So, by international teams and also mainly the University of Berkeley, determined that it was not Denghi, but it was . . . which was treated by antibiotics. So, these kinds of intervention are important for these kinds of things as well.

So, an example of a Center of Excellence, we call, so we have trained these people in Denghi fever diagnosis. They timely detected collect and collaborated with the Ministry of Health to stop outbreaks of . . . . viruses from Brazil. Then we did technology transfer to other diseases that they request to us. We respond to local initiative and then they continue. There is a very active group which they have written grants, obtained presentations in other countries, collaborate among themselves with Panama and others, and then we helped create a molecular biology unit which is really nice. It is just a small room that is very clean that has thermocyclers and pipe . . and just simple equipment. Then we have a manuscript writing workshop, the . . . helps us with, and now our instructors are consultants for the Pan-American Health Organization as well. Now they are receiving grants as well.

So, we started from PCR to . . . the sky is the limit. In Latin America, we adapted a simple method in Nicaragua. It was used for molecular sub-typing and detect where . . epidemic is coming from, lab-based surveillance systems with full participation in the laboratory and clinical sectors. The clinics in the country are now joining with this program. Then the multiplier effect – the Nicaragua trainees are as instructors for other . . workshops in Latin America – not only in Central America. Then this experience is being repeated in Paraguay, . . . and Peru. We were in Peru in March last year, right before . . . Denghi came to Lima and they were fully prepared to answer the diagnosis in 24 hours. So, that was very satisfying.

Risk factors are now being studied in Nicaragua and what happened is now the communities participate in the mosquito control because it has become known to everybody, children in the school and mothers, and nowadays there is big participation that ended up with an international vaccine study in which now they are studying the response of children to different infections of different . . types of Denghi to prepare in case vaccine is ready because of the risks of hemorrhagic fever that could be caused even by a vaccine – this is a very touchy subject and it is being studied in Nicaragua.

Another Center of Excellence we have is in Egypt. We started . . . by a donation of scientists that are very interested in studying the Hepatitis C. That is a big problem in Egypt. As I mentioned before, the population of Egypt is about 30. . . . a vaccine to . . . . . The needles were tainted and a high percentage of the population was inoculated with Hepatitis C before it was known. Thirty years later, it is a big problem in Egypt. It produces many problems that end in liver cancer and cirrhosis as well.

So, we have been working with them. There was a conference and a consensus statement how to approach the subject. A technology transfer workshop . . laboratory went, and a small funding for different grants they have been receiving. Grant-writing workshops that were needed again for them to enter a second funding cycle, followed by a biostatistician, as I mentioned earlier. Biostatistics is very important to present to the science studies so they are valid later on when they are published.

Then we brought experts to bring them up-to-date on the epidemiological methods of a diagnosis, and we just finished a writing workshop in which they were publishing their results of this effort.

This is the one of the grantees of 2004 – the Association of Hepatitis C and Childhood Leukemia and Lymphoma – because this virus is highly associated with cancer.

So, this it the model we have. It works because it is hands-on, its local relevance and initiative and ownership. The groups are responsible for a long-term approach to continuing to study the problems, and the proposal development and grant-writing skills is very important. Regional experience, interaction, training of local technical experts.

Beyond the workshops, there is a long-term technical, scientific and material support which we continue. Access to electronic communication and resources – we try to supply. The small grant funding for the work to continue. Partnerships with more developed countries for them to expand. Beyond the projects of public health practice, we form links between our . . . clinic, epidemiologic, and health policy sectors through active projects and published results. When the data comes out, it interests the authorities and there is a collaboration that is sometimes difficult to obtain between universities, centers of research and the Ministry of Public Health. It has a direct effect on health by supporting local application of the results to solve the problems.

. . . as I stated genomics and world health in October 2002 . . . has a model that works. The work of . . . has demonstrated how the techniques of modern molecular biology may be readily transferred and adapted to local conditions in developing countries and harnessed to address local priorities for improving health research.

. . . messages – human capacity. Focus on the human component. Technology alone is not a magic bullet. You have to create a nucleus of researchers.

On-site – to address the local priorities that the research is considered important in the countries. True partnerships, either . . . to. . transfer, collaborations, local collaborations between the Ministry of Health and universities, which is difficult, and knowledge into action dissemination to other country researchers which is happening very much in Peru. After they were trained to quickly detect the presence of Denghi, they are going to other communities in the jungle to train them to do this as quickly as well. And, small is beautiful – avoid the developing country divide. It is important to keep it simple.

Now, I'm going to switch gears just for three more slides and I'm going to talk about a bit of new technology that is being developed at U.C. Berkeley in conjunction with us and the engineering department. So, they came to us because they wanted to do analysis on a chip. They couldn't make it work, so they . . . to do it. An analyzer, you know you have the capture of an antigen, an anti-human antibody, linked to . . . . and you get . and you get results and you know . . . Well, the thing can be done on a computer chip on the . . . surface. It binds agents as well and instead of having color developed, you have an electromagnetic bead and this bead is excited by a magnetic field and you can read the results on a computer, and ideally on a PDA device that you can bring in the field. So, you could do this in the field and it is being worked at U.C. Berkeley at the moment.

We started with a prototype, went to a flip chip which is a smaller one, and now we are working in . . chips that are really small and the idea is to have different agents and different chips that you can read in the field. So, this is being adapted to use with nucleic acids as well. So, this will be the gold chip. It is activated by . . . . .. XNO which will then bind a probe to improve the kinetic mixes longer, that has attached a sequence, and another sequence that is linked to the target probe. So, these are comparable to these homologous and they hybridize and they provide . .. probe, the specific probe that is going to notify the target . . . . or recently has been developed to work with the electromagnetic bits also. So, it is called a universal biosensor in which the . . . of the chip can have an . . . . and . . can read the signal, or it could have DNA or RNA binding to detect DNA presence of an agent.

This is in development at the University of California at Berkeley and they hold the patent. So, through SSI, we negotiated a compromise or a development agreement. The idea is to promote global . .. ability of technologies invented at universities through royalty-free agreements and revenue sharing to contribute to the public good rather than to maximum revenue. So, we negotiated with a university, held patents with companies in the for-profit world, while allowing the therapies and diagnostics to be made available at-cost in developing countries. In other words, with the technology that has been developed at Berkeley is used in the developed countries and the United States by companies that what to profit from it, they are free to do so. But, if this is being used in developing countries like Nicaragua to bring in the field, they will give it at-cost, and we are hoping to be able to do this by $1.50 per assay when ready.

This is our collaborators. . . . Harris, our president of the Board, and . . . which is here in the audience, is also part of the board of directors and he will be talking about his work. Then our collaborators in different countries and University of Berkeley, engineering department. And, our supporters – and the way to contact us. I brought a brochure and newsletter for those that are interested in learning more about us.

Moderator – Thank you, Dr. Peñaranda for your wonderful talk. Because we have a very limited schedule, I think as a moderator I'm supposed to introduce all the speakers because we are far behind the schedule. So, everybody want to see the biography, you have the book. So, our next speaker, can I ask all the speakers to try to limit your time within 20 minutes because we want to dialogue.

The next speaker is Dr. Yang Ruifu from Bejing Institute for Microbiology and Epidemiology, and his talk is Microarray-based methods for detecting and tracing bacterial pathogens.

Yang Ruifu, Beijing Institute for Microbiology and Epidemiology

Microarray-based methods for detecting and tracing bacterial pathogens

Thank you, Chairman. Good morning everybody and also I would like to thank the meeting organizer to invite me here to give this talk about microarray-based method for detecting and tracing bacterial pathogens.

We . . . increase . . not only from the emerging or reemerging infectious diseases, we have talked a lot about SARS this morning. Also, after SARS follows the avian flu and stratocaucous . . . infection and also the concern about terrorism and biological warfare. Terrorism has become a real situation after 911's attack in the U.S., and the anthrax spores were sent by conventional mail system and also we are concerned about the advances of biotechnology because if we put the . . . of human genome as a hallmark of the genomic era, before that the only concern about agent itself . . concerned about . . . . or the genetically modified and conventional agents. But, after the genomic era start, we also concern about another kind of biological agents because . . . biological agents is not agents-based, but is material based. We can use our advanced technology to modify the organisms itself to destroy our computer system or communications systems, not targeted to our human . . .

In our situation of birthright, the early detection and the . . . of the pathogen is quite important for control of epidemics conventionally we use EIA or PCR to detect a pathogen or other antibodies. Other measures . . .

In the last ten years, we have witnesses the rapid progress of microarray technology. Now, we can develop . . . protein microarray for detection of multiple pathogens in one assay and also we can use analytical chemistry-based matter such as mass spectrometry, TC mass spectrometry or infrared mass spectrometry to identify bacteria. And, with the advances of the genomic research, they can develop genetic or bacteria mass spectro or finger printing of fatty acids databases for tracing the bacteria, tracing the source of the bacterial.

In this talk, I would like to talk two parts. The first is a microarray developed in our laboratory for detection of the bacteria of pathogens, particularly for the important . . bacterias, and also I will show you how we combine genetic polymorphism database and the microarray database to develop another kind of microarray for the source tracing of the . . . .

First, I'd like to introduce you to microarray developed in our laboratory. This microarray can detect 80 bacterias in one . . . We designed . . . targeting for . . . . RNA genes to detect the bacteria. We . . . 4-5 . . . programs for each pathogen and we can use a legal profile to identify the bacteria. By using this technology, we can distinguish the very closely related bacteria such as bacillera anthraxes and . . . Also, we can correctly identify the bacteria in a mixture of two bacteria like this picture shows, and also we can detect one-eleventh of the target bacteria in a mixture of two bacteria or three bacteria. We can identify them.

Because the micro-technology is familiar to everybody, it is not so complicated. So, I don't want to talk to much about microarray technology itself.

Next, I'd like to move my talk to another microarray developed in our laboratory for the detection, identification, . . . analysis and the source tracing of . . . In this chip, we use different reagents or DFRs . . . . mutations and . . . genes in this shape for the detection and identification . . . analysis of the . . . .

First, I'd like to introduce you to how we identify the DFRs and the . . . sequences by using the whole genome macroarray based hybridization and also the SS . . . . . hybridization.

Before we go into detail for technical, I'd like to introduce you to some background about . . . That is why we are interested in this deadly bacteria. We all know . . . is a . . . literally circulating around rodents and the fleas. Sometimes flea can bite humans and cause bubonic plague. If the human develops the pneumonic plague, the . . . can transmit from human to human and also this type of bacteria can be used for bioterrorism or biological warfare purposes.

The plague is now distributed in Asia, Africa and also America on these three continents. After 1980, the number of cases reported to . . . are increasing, so . . . disease as a reemerging infectious disease and this figure shows you the . . . in China. . . . . . and this region is the plague for . . . and now it is disappeared due to the change of the environment or the . . . disappearing of the host. The other regions are not as active . . . in China and for . . . . is divided into three . . . The . . is from . . . . Also, another . . . is the . . . . This pink circle shows another . . . . newly defined by our laboratory. Other areas . . . . belongs to . . . . .

We used the strains from different regions of China to analyze the genomic content by the whole genomic DNA microarray. This microarray contains . . . . five genes and also we use different serotypes of . . . closest ancestor of the . . . . to hybridize this . . . to identify the . . . segments in the different regions of China. Also, we use SSI to identify the DFR or the . . . sequence.

This figure shows you the 40-64 strains of . . . from different regions of China and this shows the . . . five genes. The red one denotes the loss of the gene, and the blue one is the presence of the gene. The green one is data missing. So, we use the . . . to verify the gene laws or gene . . . or the data missing.

Finally, we find 22 DFRs and if we use the PCR to study the distribution of these DFRs in different regions and different . . from different regions in China. Totally we investigate about 264 strains. We divided all these strains into 14 . . . .

Another technology we use is SSX because . . . have been . . . by different groups. This one is . . . . by our group. So, at the time we did SSX. There is no sequence information for the . . . strains, so we used . . . strain from the . . . area because we believed the strains from this area . . . in China . . . . So, we used this strain as a tester and another known segment . . strain . . . SSX and we find a new DFR is DFR 23 and we use the . . . to start the distribution of DFR in China. We find it is only distributed in the . . . area, only in the . . . strain in this area.

Then we used the . . . to start a distribution of this 23 DFRs in different regions of China. We finally found the . . . evolution of the . . . in China follows the way of the laws of DFRs – it is in very good condense with . . . .

We also use this . . . microarray to hybridize ways of DNAs from different groups, different . . . groups of . . . . for tuberculosis. We found significant . . . 13 genes only present in the . . . not present in different serotypes of the . . . tuberculosis. . . . . . belongs to the DFR1 and the DFR3 which we have identified before.

Another technique used for the genomic polymorphism analysis of . . . in China is the . . . mutation profiles. When we compare these three seconds to genome, we find a lot of rearrangements in the genome . . . and there are four kinds of . . . sequences in . . . About 4% of the . . . sequence present in the genome of . . . We designed primers to detect all the possible rearrangement in events for the . . . . and then we found a very good correlation between the rearrangement profile and origin of the strains.

This table shows the detailed data. I don't want to waste our time and explain this in detail.

This figure shows you that we find a very good correlation between the different . . . . and also the region of the . . . . in different regions of China.

Until now, we find 18 DFRs from the 23 we have identified can be used for genotyping . . . . in China and also we select four . . . sequence . . mutations in this . . . . can be used for genotyping of . . . . in China. So, we combined all these markers to do the genotype analysis of 902 strings of . . . isolated in different regions of China with the . . . 59 genome . . .

I'm sorry this slide is in Chinese, but I will give some English here. Here is tuberculosis' ancestor or . . . and this . . . . When they put our DFR and the . . . sequence . . into this map, we find a very good correlation between the . . . . and the origin of the . . . The different color denotes different . . . in China for . . . . .

So, finally, we choose the DFRs . . . for each DFRs and also four . . . sequence genes and mutation. We designed 12 . . . and . . . . . and also . . . virulence genes together and also put some control . . . . in this microarray about 270 genes in this microarray and each gene has three duplicates in this microarray. Then we used this microarray to identify the different strains of . . . . from different areas of China. It can correctly identify the origin of the . . . in China.

So, thanks.

Moderator – Thank you, Dr. Ruifu for your adequate control of the time. So, our next speaker, we have to move on. Our next speaker is Dr. Charles Chiu from the University of California at San Francisco. The title of his talk is Using a Microarray to Detect Viral Pathogens Associated with Human Disease.

Charles Chiu,
University of California at San Francisco

Using a Microarray to Detect Viral Pathogens Associated with Human Disease

First I want to thank the organizers of this event and to say that I'm very privileged to speak to you today.

Today, I'm going to talk about microarrays for viral pathogen detection and discovery. I work in the Duresee Laboratory at the University of California at San Francisco.

Briefly, a summary of the presentation I'm going to start out describing what is the viro chip. Then, I'm going to discuss E-Predict which is a computational tool that we have developed in our laboratory for virus prediction. The last thing that I will discuss is a recent case report where we actually applied the viro chip in a clinical setting.

So, Dr. Ahlquist has already talked about this in great detail, but I'm just going to reiterate that there are several reasons why we are interested in looking for new pathogens. One is that many common infectious diseases really have unrecognized viral causes. It is still thought that over 25% of respiratory infections are caused by some yet to be characterized virus. The second is that many especially chronic diseases that were once thought to be non-infectious are actually thought to have a viral origin – for example, cancer. As you can see here, Dr. Ahlquist has already talked about it, but many different viruses have been associated with cancers.

There are many limitations to current detection methodologies for viruses. One is simply that many viral viruses are simply not culturable. Hepatitis C, for instance, had not been cultured until last year.

The second is that amino acids really require that you know what you're looking for and require special reagents that have been tested in the laboratory.

RTPCR and PCR are tremendously useful, but again, you have to know what virus you're looking for.

Subtract hybridization techniques have been used. They tend to be pretty laborious and not really useful in a clinical setting, but very useful in a research setting.

So, our solution was to develop a comprehensive and a high through-put way to detect viruses directly from clinical samples. Our solution is called the viro chip. It is essentially a CDNA microarray that allows psy-mutaneous screening for all known viruses. The idea is that it provides a global and a non-biased approach at detecting viruses, and gives us a comprehensive picture of which viruses are present. The general idea is that you take basically Field's virology, which is the bible of virology – it was supposed to turn into a chip. I guess it didn't. That's okay.

This is what we describe as the viro chip – it is thought to be really an inter-disciplinary – it basically integrates many different disciplines in clinical investigation, genomics, virology, epidemiology, and bioinformatics.

You are real familiar with the viro chip, but this is actually a picture of part of the viro chip where essentially every spot is essentially CDNA. By the way, this is an atomic force micrograph of the actual chip. It was done in Texas.

This the microarray and my mentor, Dr. Joseph Deressi sitting here on the left, dressed in his laboratory wear, basically. This is the microarray.

So, how did we basically select viral sequences to include on the chip? What we did was we took the sequence of every known virus and did an alignment. We deliberately selected sequences that were the most conserved among the virus families. To give you an example, this is basically an alignment showing all of the members of the . . . virus family, essentially all of the entro-viruses. You can see here that the most conserved regions are really in the five prime, untranslated region of the five prime UTR, as well as some other areas further downstream. You see here, the red arrows are basically where we selected the oligonucleotides to include on our microarray.

Our oligonucleotides are essentially seven demurs. You can see here, this is the coverage provided of the polio virus genome in a way that we can detect polio virus. You can see that most, where each line represents a single spot on the microarray, you can see that most of the representation is really here in the five prime region.

So, here is how we actually process the samples. We actually take our sample, the samples shown here basically, and we isolate RNA from the sample, reverse transcribe it into . .. DNA. We then couple it and basically hybridization it to the microarray. The amplification is done by random PCR method. Basically, the red channel is used to indicate a hit on the microarray. The green channel, unlike in traditional genomic expression arrays, is simply used to indicate the location of the spot.

So, to give you an example, this is our current version of the microarray. You can see here you see a bunch of red spots. These are actually human DNA controls because usually with randomification, we also have amplification of human cells. Where every red spot here actually . . . .

(Tape 3)

. . . it calculates what the theoretical hybridization pattern would be on the microarray for any particular virus, generates a theoretical energy profile, then compares it to the actual hybridization pattern that we see from the sample. By doing so, we can actually rank the viruses as well as provide a probability estimate of how likely it is that this particular virus is in your sample.

So, here is an example from the clinic. This is basically a pediatric patient who came in with fevers and a rash and this is actually a nasal . . . aspirate from this patient. You can see here that the number one prediction by E-Predict is measles virus and the intensities are very high. They range from zero to 65,000, where 65,000 is the maximum intensity of the microarray and you can see that E-Predict detected measles virus successfully.

What is very interesting is also the second hit on E-Predict was canine distemper virus which is a related virus but is not the same as measles. So, the idea is that you can actually detect viruses that are related and potentially detect divergent strains of known viruses.

This is to give you another example and this is how we detected SARS in the virus. This is basically E-Predict as well, and you can see here that many of the oligos correspond to corona viruses and astro viruses. SARS is not an astro virus so you might ask why did it detect an astro virus? Well, as it turns out, there is a conserved three-prime motif among both corona viruses and astro viruses, and that was detected by the microarray.

So, we want to apply this microarray in the clinical setting. The important thing is to select what diseases should we target. What we define is really six key selection criteria. One is there has to be some kind of clinical entity as well as an epidemiological evidence for an infectious ediology. It would be very useful if we had some evidence of pathology, if there was some inflammation suggesting that it could be from an inspection, sample availability and finally clinical relevance.

I'm not going to discuss any of these, but these are current viro chip projects that are ongoing. We are doing a pilot study of pediatric respiratory infections. We are also very interested in cancer, especially looking at associations of novel and known viruses in both lymphoma and other types of cancer. In fact, in our lab we have recently published online and . . . as a potential retrovirus that may be associated with certain cases of prostate cancer.

I'm very interested personally in a study of aseptic meningitis and encephalitis because it is thought that over 50% of cases are caused by viruses that have yet to be characterized. And, we are also looking at basically cases of lymphoma as shown here in a patient who has an enlarged lymph node.

We are also doing a collaborative study with Dr. Eva Harris on looking in the setting of febrile illnesses in tropical regions -- other illnesses besides Denghi virus.

In addition, we are also looking at again respiratory infections. This is a view of an inflamed bronchial in the setting of asthma. We are looking at viral corlets or viral agents that may be implicated in asthma exacerbations.

Finally, and this is what I will talk about – we are interested in basically novel viral pathogens in intensive care setting. So, essentially this is what I describe as hospitalization from critical illnesses. I'll give you three examples here. This is the SARS corona virus, avian influenza H5N1, and honta virus.

Really, this study came about partly as a result of some previously published data already about the CDC Unex model. What the United States CDC is involved in is a population-based

surveillance program looking for critical illnesses that are thought to be from infectious causes in young, healthy individuals. What is very interesting is that they did a study of over 100 cases and they really found an infectious agent in only 28% of cases, suggesting that well over the majority of cases are still remain to be detected. Our idea is to use this viro chip as a comprehensive screening tool to better identify infectious agents in such cases.

So, I will basically end the talk by going over a case report and I would like the physicians in the audience to see how they would approach this and what they would do.

This is a very interesting case about someone who presented to our university hospital in the U.S. She was a 28-year-old, very healthy female. She had a ten-day history of fever, cough, night sweats and bloody sputum muscle pain. She came to the doctor and they thought, maybe she had an early pneumonia, so they gave her some oral antibiotics – Zithromyacin. Unfortunately, she came back three days later feeling much worse. She started getting extremely short of breath. In presentation, she had fevers to 39º, she had hypoxy and her oxygen saturation level was down to 80%. She also had a leukocytosis about 17,000. You can see here on her admission chest x-ray she has a very fine reticular nodule infiltrate. You can't really see it too clearly as well as . . . consolidations on her chest x-ray.

She had a CT scan also when she came in and basically this is one representative slice from the CT scan and what is really noticeable is you can see basically here evidence of bronchialitis or bronchial inflammation. These bits here are actually characteristic of what they call a "tree and bud distribution" -- that is characteristic of a diffused bronchialitis.

So, she was treated with antibiotics for community . . . pneumonia, but unfortunately by day three she got much worse and she developed acute respiratory failure. She was actually – this is a healthy 28-year-old woman and she had to be intebated. They changed her antibiotics to Moxyfloxicin for additional coverage and they also started her on Acyltomavieur in the case that this was some unusual influenza case. They also gave her some high dose steroids as well.

They did a bronchial viral lovage and a bronchoscopy in the hospital. Basically the results were negative.

Finally, because she was getting much worse and she started requiring pressers as well because she was getting clinically hypotensive, they finally did an open lung biopsy and again keep in mind this is a healthy 28-year-old woman up until this point She had an open lung biopsy which showed this and briefly, the pathologic reading was this is a bronchialitis. There was no evidence of any viral infection, no viral inclusions, no . . . cells and no vasculitis.

So, these are just some of the diagnostic tests that were sent. There were over 200 microbiological tests that were sent. Blood, urine, sputum, and viral fungal cultures, legional antigen, rheumatoid factor, . . . antigen, coxyhisto. . . antibody titers, HIV antibody. They did PCR and DFA for both . . . They looked for PCP or PJP by . . fluorescence. They looked for a bunch of unusual diseases including blastomycosis, . . . and . . . DFA tests were sent for all of the known common respiratory pathogens, RSV, adno virus, influenza A and B, Para influenza 1-3. They sent a test for CMV. They sent a PCR test for . . . virus which had been recently described. They also sent a PCR test for the SARS corona virus as well as an . . for honta virus.

All of the tests returned negative. So, I just want to ask, is there anything else that should have been sent? What would you do at this stage, given that you have a woman who is about to die and had previously been healthy and you really want to do something to save her. Anybody from the audience?

Well, what I wanted to get from the physicians in the audience was that one idea is that since this was thought to be possibly a virus infection, is there a possibility of giving an impuric antiviral treatment such as intravenous . . . vuren. That would have been an option.

So, what we did is we took a look at this viro chip. This is a diagnosis. Human Para influenza type 4. What was very interesting was that the signature was extremely weak, so I did mention that we had this took called E-Predict. We had other ways of analyzing the microarray, but unfortunately all of those tools were actually negative. We actually had to devise another tool

called a single oligonucleotide analysis which actually looks at statistically significant oligos of the microarray. If you actually look at the six most significant oligos, it turned out to be all from human Para influenza type 4.

You can see up here are basically the Z scores of the significance of these particular oligos and you can see those oligos essentially span the whole genome.   This result was confirmed by specific PCR of all of these regions across the HP. .. genome. There was additional confirmation that was done afterwards, and we confirmed it by IFA using her hospital patient . . .

So, one possible alternative had she been doing worse would have been to start intravenous rabuvirun. By the time we got the diagnosis, actually she had been doing better and I'm happy to say she was extabated and now she is completely healthy and well.

So, I'm not going to go over Para influenza type virus too much. I'm just going to say the type 4 has always been traditionally thought to be jus mild respiratory infections. In fact, to give you an idea of the standard DFA kit which is sent for a standard clinical diagnosis of respiratory infections, does not include Para influenza 4. It really has only Para influenza 1 to 3.

So, I would like to end by just acknowledging my mentors were Dr. Joseph Deressi and Dr. Gannum at UC San Francisco as well as our collaborators on this study looking at hospitalization for clinical illness. We actually plan to expand this study to look at a number of diseases, usually about 2-3 cases of this come every month and we are looking at the viro chip as a potential way to be part of this clinical algorithm for work-up.

Thank you.

<u>Moderator</u> – Thank you, Dr. Chiu, for your wonderful talk. We will move on to our last speaker for this morning's session, Dr. Kan Biao who is from the Institute of Infectious Disease Prevention and Control, Chinese CDC. The title of his talk is laboratory-based subtype surveillance of infectious diseases.

Biao Kan, Institute of Infectious Disease Prevention and Control,
Chinese CDC

Laboratory-based subtype surveillance of infectious diseases

Good morning. In this session, I would like to talk about the laboratory . . . I come from China CDC. . . . laboratory-based surveillance. So, now some of the talkers has talked about the spread of infectious disease. Infectious disease has spread more rapidly and is now a global problem. This is an example for food-borne diseases and food-borne pathogens which can cause dire diseases.

We have observed a shift from the type of . . . source that . . . . is a party and then the outbreak occurred in this church supper. But, now the outbreak may occur in different regions and we may see that the pathogens . . . rapidly.

Here is a simplified example in the pathogen evolution is an example that we may find different outbreaks in different regions and in different time and also we can find more patients. We can . . . the different isolates. Here, we may find, for example, there is three outbreaks occurred in different regions. Outbreak A, B and C. Maybe there is an . . . in its evolution – the new . . may occur in different regions or in different areas, and . . . because this outbreak . . . Maybe there is a connection of the relationships between these two outbreaks. This patient may cause a new outbreak in another region or another village such as, for example.

So, what we should understand is that if there are some relationships or associations among these outbreaks, what we can answer this question, then based on the . . . investigations and also another proof should come from the laboratory evidence. That means we should do the pathogen surveillance, then we may find relationships between these two outbreaks. Because there is a

difference, these two pathogens are different, so these outbreaks is different with outbreak A and B.

So, in the infectious disease control and . . system, what the laboratory tasks – laboratory plays a critical role in public health based on . . . specific epidemiology for different syndromes. For this laboratories, we mean clinical laboratories which are located in . . . locations. Another is a . . . in different regions, provinces, and the center of the whole country.

So, there are tasks in the disease control and prevention includes simple collections. . . . investigation of the outbreaks. Also, in these laboratories, the tasks that should be done in different methods, but because we in the disease control system, we may have other peripheral level of labs and intermediate levels and a central level. In the . . . level, the central laboratory is . . . province of CDC . . in the whole country. Such as in China, we have our own central laboratories. All these laboratories have an organized network . . . do the tests and the reports and also . . . transport of the messages and the pathogens or materials collected from the patients.

What is the surveillance of an infectious disease? The . . . collect the information from the patients from the population. But for infectious diseases, the disease is caused by the pathogens and the pathogens will be maintained in the environment and in their host, such as the animal . .. virus, and also these pathogens are transmitted to humans . . population and then cause the outbreak of epidemics of some diseases.

So, in this circle, we should understand which steps we . . which information we will collect from the surveillance. Now, most of the information comes from the population, but I think we should understand that because of the pathogen evolution, it is maintained in the environment of the host, we should collect information of the pathogens transmitted to humans.

So, laboratory surveillance network in the infectious disease surveillance network, we are . .. epidemiological information and . . laboratory information to do the surveillance.

I would like to talk about the molecular subtyping. Molecular subtyping methods in the laboratory surveillance network – for subtyping, there are phenotype methods and genotype methods. For phenotype methods, sometimes it is unstable. So, in this meeting I think most of the people are talking about the molecular genotyping. So, here is the least of the genotype methods, some . . methods such as RFLP . . . and . . . . . and other PCR-based methods in . . . typing. . . . . EFRP and advanced DNA sequencing . . .

For different pathogens, they have for one pathogen with a different . . they have the backbone, but it is mostly genome. This is an example that is published by one of our laboratories. Here is an example . . . different isolates. Here is an example. The results we obtained with the CGH of . . . isolates.

(not transcribed)

Question and Answer Panel Discussion

Moderator – Thank you, Dr. Kan, for your wonderful talk. I think this session is open for comment, discussion and questions. Any questions, comments or discussion? Anything . . .

Question - . . . from the Genomics Institute. Have you ever compared . . . results with the sequencing of the whole genome . . . ?

Kan – No, because we cannot get much moral sequences of different strains. With the PFG, we need to . . . strains, but we cannot obtain sufficient amount of strains which the whole genome . . was obtained. But, I think the PFG can only find some size, some variance in the whole genome so . . . messages. So, I think for the whole genome . . . is the best way, but the cost is too high for public health control in this work.

Question – Dr. Mary McBride from Lawrence Livermore National Lab. I have a question for Dr. Peñaranda. I'm curious to know how you initially recruited scientists that were willing to go and train the trainers, and also how you were initially funded to do your work?

Peñaranda – We initially were funded because of the work of Eva Harris in Nicaragua. She got a McArthur award or Genus Award in 1997 and she started a non-profit organization ever since. We get funds from Rockefeller Foundation, Kellogg Foundation, and many other sources that we constantly apply for grants. In order to recruit people, it is a slow process of training the trainers. We started in Nicaragua because that is historically where we were. People by context of knowing us, come and write because they want some sort of health intervention. We try to match, as best as possible. So, my job would be to find if there is a problem in tuberculosis, there are people I know in the United States that are really good at the epidemiology or whatever is the . . I try to match them. There is no problem finding people that want to go to developing countries to help. That is the great part. They usually all know English or Spanish or Arabic and I failed to mention before we are expanding to Africa. We hope to expand to China as well. Thank you.

Question – Eric Eisenstadt from the Institute for Genomic Research. I have another question for Dr. Peñaranda. How do you – I don't recall you addressing the issue of reagent stability in the field. I wonder if you could comment on how much of an issue that is for what you're trying to do and what you are doing to address it, in the context of using molecular – doing molecular-based diagnosis.

Peñaranda – Reagent stability -- we usually work with central labs where the samples are brought to. The reagents are carried by hand, usually by myself or the instructors, properly cold. It is a problem and we are now trying to establish links with local companies that will make and sell these reagents. It is a big problem because the companies that represent Latin America charge three times more what the reagents would cost to buy in the United States because of, I imagine, representatives that need to be paid down the line in developing countries. And, it is a big ordeal that I will open to discussion or if anybody has any solutions to how we can provide

reagents that are available and at lost cost. At the moment, we make them and we carry them ourselves.

Question – I have a question for Charles Chiu. First, I'd like to congratulate you and your boss for some groundbreaking work. My name is Steve O'Brien and I'm from the National Institutes of Health in the USA. First, I would like to congratulate your group for raising some ideas and thoughts that I think all of us are thinking about and ways of trying to identify these pathogens. But, I do have some questions about sensitivity with respect to the success that you've had.

With respect to both SARS recognition and also the most recent xenotropic mouse virus in prostate, are you able to detect sequences related to either of these in serum or in blood at all?

Chiu – That is a very good question and the sensitivity has always been an issue with us because we do not use specific primers. It is clear that the microarray is less sensitive than specific PCR for a virus. So, to answer your question is no, we have not detected sequences from either SARS or this recently characterized xenotropic leukemia virus in serum. It does not appear that the microarray is sensitive enough to do so, which is why we have been very careful to select primary material that is probably potentially the highest yield, that has a high titer of virus.

O'Brien – Do you have any hope for – we know that there is circulating RNA, for example, from HIV in infected patients and in many other infectious diseases. If there is circulating PCR detectable nucleic acids in serum of some of these patients, how would you suggest that these discoveries that you're making be turned into diagnostics or screening by being able to detect that. What is the best way? Just a PCR test?

Chiu – We've just actually started working with serum. I can tell you that we have looked at control samples. We are able to successful detect, say, Hepatitis B and Hepatitis C in serum. But, these are patients who have high viral loads. So, it is probably a matter of the amount of virenia that is seen in these patients. The answer to the question probably – this would be probably the best way to be able to simply get a serum assay and be able to find viruses, but I think in practice it is more likely that there is really more fruit to be found in looking at primary

materials such as tissue specimens. One way would be, and this is what we propose as actually finding new viruses by looking at primary material and then being able to design, say, a serologic assay for that particular virus that is very sensitive. We are currently working, for example, on an XNOV seriologic assay to be able to screen a large population for this virus.

Question – Arnold Monte, University of Michigan. Just to put in a further word of caution, as an epidemiologist, I think you need to make a distinction between the clinical application of a technique such as this to help in making decisions about treatment, and the claim that you have an ediologic relationship, because as somebody who has worked many years ago with Para influenza 4, I firmly believe that Para influenza 4 does not cause this kind of a syndrome with a 17,000 leukocyte count and that it might simply be a passenger or an agent which is infecting that individual. I know of no data to suggest that ribovirun is particularly good in Para influenza infections. So, I think clinicians need to go with what they've got, and this will be something new and something very important for them in making a diagnosis, but I think we need to stop there.

Chiu – Your point is very well made and that has always been the issue – if you find a virus that does not, by any means, establish a link to the disease, so there are many ways of indirectly looking at that. In fact, I think the serologic evidence is actually quite convincing. We show some evidence . . .

Monto – . . . Which just means this was an infection, but it doesn't mean it is ediologic.

Chiu – That is true. Especially you can't make conclusions from, say, a single case report such as this. But, the next step which is what we are doing is we are screening a large population of patients to see if there is a relationship to severity and human Para influenza 4 infections.

Monto – Para influenza 4 could well be one of these agents which is present, and never detected, because even in the days we were doing virus in isolation in monkey kidney culture, it didn't even . .. that well. So, it could well be missed. You may be right.

Chiu – I think that further epidemiological investigation needs to be done. This single case report does not establish a definitive link.

Comment – . . .. the initial infections were implicated to be responsive, caused by meta-pneumo virus which was isolated from those patients. So, it is important to be able to differentiate between an isolate and disease caused by that isolate.

Question – This is . . . from Chauman University. I do concur with the previous speaker very much so. I think it brings to us one question. Where does direct detection of viruses – what sort of role do we really have in the clinical setting? I'm not asking any specific speakers, but I think it is a question that has been playing up in my mind for a very long time. I would like to throw this question open for discussion.

Comment – This is George Atkinson. To follow-up the last comment, I would like to ask all four speakers, maybe, just before we adjourn, to give us some sense of what you think your current capabilities in surveillance and detection are, relative to what you perceive the current need is in terms of infectious diseases. So, maybe a simplistic model on a scale of 1 to 10, where 10 would be somewhat comforting, idealistic, where are we with the current capabilities that each of you spoke about, both the technological capability of the viro chip and the human capability of dealing with the sociological – the delivery of these to the various societies. Could you each comment briefly?

Peñaranda – I think we are very behind. On a scale of 1 to 10 – 4 or 5 I hope, optimistically. There is a lot of work to be done to deliver the technology and to detect emerging diseases.

Ruifu – As microarrays is a powerful technology for us for screening pathogens and also for tracing the source of the pathogens, but now it is still in development. We need a lot of work to improve these technologies. I would say a 5 - -in the middle.

Chiu – I agree. I think it is about a 5, simply because the technology is there, but currently there are few methods that allow us to do this in a high throughput manner. Second, we still have issues, as brought up, sensitivity as well as establishing link to disease.

The one other point I wanted to make is that we must also distinguish between the search for the novel viruses and searching for existing viruses or known viruses in clinical specimens. The issue is with novel viruses, it becomes ironically actually easier because it is more likely that especially in diseases for which nothing has been characterized, that this could potentially be an ediologic agent. But, again, I think we are not quite there for a clinical setting.

Kan – **(not transcribed)**

Question – Bruce Taillon . . . Life Sciences – I was wondering if any or all of the speakers could comment on essentially genetic changes in emerging pathogens or known pathogens and what role they are going to play and what the needs are in the detection surveillance technologies to identify those?

Chiu – One thing I can say is that we have done studies of rhinovirus using the viro chip and we are able to distinguish rhino viruses on the species level. There are some that have essentially only a few nucleotide changes and based on intensities as seen on the microarray, we can actually distinguish those particular viruses. So, it is hopeful in that setting to be able to detect a single nucleotide or other small changes.

Moderator – I think Dr. Gary Anderson will have a talk about another kind of microarray that can detect . . . . by his technologies.

Question – Just one quick question for Charles. Have you actually detected any with your array experience over the last several years in your lab, any virus relatives which are outside of the diversity space of known viruses – that is, have you detected anything new?

Chiu – The answer to that is no, and I think that really the problem is the technology. I don't think a microarray-based technology is based on the conserved sequences or homology is able to detect new viruses in principle. Probably what is currently being done is essentially shotgun sequencing and random PCR techniques which will probably be more useful in terms of leading to the discovery of truly novel viruses.

Comment – But I was very encouraged by your detecting of canine distemper with the . . . virus probe because that is a pretty good distance away, and yet it is in the same family. So, if there were first and second cousins of known viruses out there, I would have hoped you would have seen a few, but you're saying you haven't?

Chiu – Absolutely for things that are just somewhat divergent or at least moderately divergent, it is clear that the virus chip is able to detect that. The truly novel virus would probably be difficult.

Moderator – We have to adjourn this session. As you know, you always have endless speeches from the politicians. You always have endless arguments as discussing from the scientists. So, because we have a very short lunch break, we have to stop here. Can I ask all of you to join me to thank all the speakers in the morning session and all the audience? Thank you.

Comment – May I take this opportunity to make an announcement. The lunch is going to be served in the café on the first floor, outside this hall. During the lunch, please take your precious belongings with you and for your information packages, you are more than welcome to leave them here. We are going to lock the hall during the lunchtime. The panel discussion for this afternoon, the second panel, will start at 1:20. See you this afternoon.