

# TRANSCRIPT OF PROCEEDINGS

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DEPARTMENT OF HEALTH AND HUMAN SERVICES

FOOD AND DRUG ADMINISTRATION

CENTER FOR DEVICES AND RADIOLOGIC HEALTH

MICROBIOLOGY DEVICES PANEL MEETING

OPEN SESSION - VOLUME II

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Pages 1 through 233

Gaithersburg, Md.  
July 28, 2000

MILLER REPORTING COMPANY, INC.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES  
FOOD AND DRUG ADMINISTRATION  
CENTER FOR DEVICE EVALUATION AND RESEARCH

MICROBIOLOGY DEVICES PANEL MEETING

OPEN SESSION - VOLUME II

Friday, July 28, 2000

9:00 a.m.

Holiday Inn Gaithersburg  
Gaithersburg, Maryland

MILLER REPORTING COMPANY, INC.  
735 8<sup>th</sup> Street, S.E.  
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Freddie M. Poole, Executive Secretary

MEMBERS

Margaret R. Hammerschlag, M.D.  
Carmelita U. Tuazon, M.D.  
Melvin Weinstein, M.D.

INDUSTRY REPRESENTATIVE

David T. Durack, M.D., Ph.D.

CONSUMER REPRESENTATIVE

Stanley M. Reynolds

CONSULTANTS

Ellen Jo Baron, Ph.D.  
Blaine F. Hollinger, M.D.  
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Steven C. Specter, Ph.D.  
John A. Stewart, M.D.

FDA

Steven Gutman, M.D.

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**Opening Remarks**

1  
2  
3 DR. WILSON: I would like to call the meeting to  
4 order. I would like turn the meeting over to Ms. Freddie  
5 Poole, who is the Executive Secretary, for the opening  
6 remarks.

7 MS. POOLE: Good morning. I would like to welcome  
8 everyone to our Microbiology Devices Advisory Committee  
9 meeting.

10 We have a few general announcements to make. At  
11 the restaurant for break, the back room has been reserved  
12 for the panel members. If you have a cell phone, please  
13 turn it off now or put it on vibrate. If you have a pager,  
14 please turn it off or put on vibrate.

15 On the agenda, we stated that Dr. Michael Wilson  
16 was the Acting Panel Chair. Dr. Wilson's appointment was  
17 approved and Dr. Wilson is now the Chair for the  
18 Microbiology Devices Advisory Committee. So, I would like  
19 to turn the meeting now back to Dr. Wilson.

20 DR. WILSON: I would like to begin with  
21 introduction of the panel members. We will start with Mr.  
22 Reynolds.

23 MR. REYNOLDS: Stanley Reynolds, Pennsylvania  
24 Department of Health, Consumer Representative.

25 DR. DURACK: David Durack, Industry

1 Representative, Becton Dickinson.

2 DR. SMITH: Margot Smith, Washington Hospital  
3 Center in Washington, D.C.

4 DR. WEINSTEIN: Mel Weinstein, Robert Wood Johnson  
5 Medical School, New Brunswick, New Jersey.

6 DR. WILSON: Dr. Mike Wilson from Denver Health  
7 and the University of Colorado.

8 DR. HAMMERSCHLAG: Margaret Hammerschlag from the  
9 State University of New York Health Science Center at  
10 Brooklyn.

11 DR. HOLLINGER: Blaine Hollinger, College of  
12 Medicine in Houston.

13 DR. TUAZON: Carmelita Tuazon, George Washington  
14 University Medical Center.

15 DR. STEWART: John Stewart, Centers for Disease  
16 Control, Division of Viral Diseases.

17 DR. BARON: Ellen Jo Baron, Stanford University  
18 Medical School.

19 DR. SPECTER: Steven Specter, University of South  
20 Florida, College of Medicine.

21 DR. GUTMAN: Steven Gutman, Director of the  
22 Division of Clinical Laboratory Devices, FDA.

23 DR. WILSON: Thank you. I would like Ms. Poole  
24 now to read the Conflict of Interest Statements.

25 **Conflict of Interest Statement**

1 MS. POOLE: The following announcement addresses  
2 conflict of interest issues associated with this meeting and  
3 is made part of the record to preclude even the appearance  
4 of an impropriety.

5 To determine if any conflict existed, the agency  
6 reviewed the submitted agenda and all financial interests  
7 reported by the committee participants.

8 The conflict of interest statutes prohibit special  
9 government employees from participating in matters that  
10 could affect their or their employers' financial interests,  
11 however, the agency has determined that participation of  
12 certain members and consultants, the need for whose services  
13 outweighs the potential conflict of interest involved, is in  
14 the best interest of the government.

15 A limited waiver has been granted for Dr. Ellen  
16 Baron that allows her to participate in the discussion, but  
17 not vote on the PMA before the panel today. A copy of this  
18 waiver may be obtained from the agency's Freedom of  
19 Information Office, Room 12A-15 of the Parklawn Building.

20 The agency took into consideration certain matters  
21 regarding Drs. Margaret Hammerschlag and F. Blaine  
22 Hollinger. These individuals reported financial interests  
23 in firms at issue, but not in matters related to what is  
24 being discussed today. Therefore, the agency has determined  
25 therefore that they may participate fully in today's

1 deliberations.

2 In the event that the discussions involve any  
3 other products or firms not already on the agenda for which  
4 an FDA participant has a financial interest, the participant  
5 should excuse him or herself from such involvement and the  
6 exclusion will be noted for the record.

7 With respect to all other participants, we ask, in  
8 the interest of fairness, that all persons making statements  
9 or presentations disclose any current or previous financial  
10 involvement with any firm whose products they may wish to  
11 comment upon.

12 DR. WILSON: Thank you.

13 There is an item of New Business this morning, a  
14 presentation from the FDA for the general public on  
15 information on two issues. The first is the issue of IRB  
16 approvals for clinical trials which will be presented by Dr.  
17 Jean Toth-Allen.

18 **New Business**

19 **FDA Presentation**

20 **IVD Device Studies**

21 **Jean Toth-Allen, Ph.D.**

22 DR. ALLEN: Good morning.

23 [Slide.]

24 As the bottom of the slide says, I am with the  
25 Division of Bioresearch Monitoring. Specifically, I am with

1 the branch that deals with IVDs. Also, I am Co-chair of  
2 BiMo re-engineering team, and one of the stakeholder  
3 meetings, in fact, several of them that we held early on in  
4 the process revealed that industry was indeed interested in  
5 seeing some definitive guidelines from the FDA with respect  
6 to these very issues.

7 Right now I am chairing a subteam that is in the  
8 process of producing a comprehensive guidance as to the  
9 studies of IVDs in general, and that obviously will be a  
10 part of it.

11 In starting to put this guidance together, we  
12 realized that a lot of the problem had to do with  
13 nomenclature and with the difference in the types of studies  
14 that are done when you are developing an IVD device versus  
15 the other types of devices that we look at. While all of  
16 this is familiar to you, I just wanted to make sure that we  
17 were all talking from the same sheet, so just a couple of  
18 quick outline slides.

19 [Slide.]

20 This was just my way of presenting and thinking  
21 about the differences in how we go through the development  
22 of an IVD product, that we have components that we are  
23 looking at rather than a true prototype at the very start,  
24 that we then have a feasibility study at about the same  
25 point in development. Where other devices might have a non-

1 clinical type of study and then the performance testing of  
2 the IVD devices is more akin to the clinical studies that we  
3 have, and, of course, all this leading to a submission.

4 [Slide.]

5 The point of what I am going to be talking about  
6 has to do with this type of layout with respect to that, and  
7 that is the fact that, for the most part, when you move from  
8 the idea stage to the initial component testing, we are  
9 talking about using products, such as analytes, the type of  
10 matrix that you might want to put into the device, and  
11 antibodies, most of which might not even be of human origin,  
12 probably are not, but once we go from the component to the  
13 feasibility stage, we now start using human tissue samples  
14 in the studies, and this is the part that is essential to  
15 what I am going to be talking about, and then, obviously,  
16 once we look at performance testing, we are definitely using  
17 human tissue samples.

18 [Slide.]

19 With respect to institutional review boards, the  
20 regulation that governs studies that are regulated by FDA is  
21 21 CFR Part 56. Now, that is important because the common  
22 rule that has to do with protection of human subjects that  
23 includes institutional review board issues is 45 CFR 46, and  
24 our regulation is a little bit more tight in a few areas,  
25 and I think this is where some of the confusion comes from.

1 For any study that is under FDA supervision in the  
2 sense that it is going to be a study that is going to be  
3 submitted to us in support of something, you have to have  
4 IRB approval. If, as I said, that submission is going to be  
5 sent in, in support of a product, and if you are using a  
6 human subject and/or a human specimen, there is nothing in  
7 the regulation that allows for an IRB to waive its review  
8 and approval.

9 They can do an expedited review if there is  
10 minimal risk, but they cannot waive it. The only person  
11 that can waive IRB review and approval of a study, even of  
12 an IVD study, is FDA itself.

13 So, this is I think the heart of the problem that  
14 we see, is that 45 CFR 46 does allow for waiver of IRB  
15 review and approval if there is minimal risk; 56 does not.  
16 The only one that can waive IRB review is FDA, and we are  
17 trying to get this word out to the IRBs, as well, because we  
18 are very well aware that they too confuse the issue and  
19 obviously don't help when a sponsor therefore is relying on  
20 them to make a decision.

21 [Slide.]

22 Obviously, joined to that are the issues of  
23 informed consent, and there is a separate regulation that we  
24 have, 21 CFR 50, that has to do with human protection.  
25 There are two areas with respect to IVDs that kind of

1 coexist here. One is repository collections and research  
2 samples that are used, and the whole idea of whether or not  
3 subjects can be identified.

4 [Slide.]

5 The National Bioethics Advisory Commission, called  
6 NBAC, has looked at this whole issue fairly recently, and to  
7 try to at least work from the same page, we are trying to  
8 use the same terminology as they are.

9 [Slide.]

10 There are a few problems, though. First of all,  
11 their report really is based on 45 CFR 46, and I have just  
12 said that we go by 21 CFR 56, so we do need to be careful  
13 how we use the entirety of it, but as both regulations do,  
14 the NBAC report is concerned with subject welfare, and it  
15 defines terms very nicely that now we can at least use  
16 commonly.

17 [Slide.]

18 They divide the terms into two big categories.  
19 One is with respect to repository collections where they use  
20 the terms "unidentified" and "identified," and the  
21 information is there, and I think it is fairly obvious that  
22 they mean just what the words say.

23 [Slide.]

24 Then, they use slightly different terms when it  
25 comes to research samples themselves. The main ones that

1 you keep hearing, I am sure, are "linked" and "unlinked,"  
2 and there is a slight difference between linked or coded and  
3 identified.

4           The unlinked are those that are basically  
5 unidentified, the linkages have been removed or there never  
6 was any, they are surplus samples that were done for general  
7 blood testing or whatever tissue testing it was, and never  
8 linked to a subject to begin with.

9           The linked or coded ones are somewhat different  
10 from identified in the sense that the investigators using  
11 the samples do not necessarily have right where they have  
12 all the information, the identification is what I am trying  
13 to get to. They have a code that was supplied to them by  
14 whomever supplied the sample, and they usually don't want to  
15 bother to even break the code, but it can obviously be  
16 traced back to a human being and the individual identified.

17           For the identified samples, it goes one step more.  
18 It is clearly known by everyone who the samples are from.

19           [Slide.]

20           Now, how does all of this involve informed  
21 consent? Well, as far as FDA studies, or at least studies  
22 that are going to eventually be submitted to us, if the  
23 study is prospective, obviously, that means that the  
24 subjects are right there, they are aware of the fact that  
25 they are in a study. We, of course, expect to see informed

1 consent and that there be an IRB-approved consent form that  
2 is used as part of the study.

3           When you are putting together some type of a  
4 repository--and this means everything from simple collection  
5 of samples by an individual who may be interested in  
6 research to the big repositories that exist out there  
7 commercially, it is advised by NBAC, and we too firmly  
8 believe, that at the present time there is little to no  
9 reason that informed consent should not be used.

10           Now, obviously, this is where a lot of the issues  
11 have arisen and we did have a guidance document that  
12 appeared last December. It is not new, it was just setting  
13 down, so that all of us within FDA do not confuse the issues  
14 here, and to our investigators in the field, as well, what  
15 we expect with respect to 56 and 50, and when it comes to  
16 these collections, this is where a lot of people had  
17 concern.

18           There isn't any problem in the mind of us who go  
19 out and look at studies if there is a general consent form,  
20 in other words, the sample was collected quite some time  
21 ago, but the individual knew that eventually their sample  
22 was going to be used in research of some sort.

23           How legal a general consent form is for the  
24 collection of samples is really something that the legal  
25 advisers have to do, but they are appearing, they are

1 becoming more and more common in some of the research  
2 institutions that have hospitals with them. You will see  
3 that these types of generalized forms are being used, and we  
4 would expect them from now on to be so.

5           There, of course, exists out there a large body of  
6 samples that have been collected under all types of means  
7 and that are very valuable because many of them can no  
8 longer be collected under the same conditions, and  
9 obviously, the first thing we would say to you is document  
10 or get as much documentation from such a sample that you  
11 would be using.

12           [Slide.]

13           Now, obviously, the bottom line as far as FDA is  
14 concerned, is what is available if you are going to send  
15 this information to us in support of either a 510(k) or a  
16 PMA PDP type submission.

17           Well, if it is a very simple 510(k), that is just  
18 taking the predicate and taking your device and using  
19 samples, comparing them side by side, if you should use a  
20 completely unlinked, unknown set of tissue samples, you may  
21 be okay, and I say "may" because you never know what issues  
22 might arise and you might need more information, that is  
23 really the bottom line - needing more information.

24           If you are trying to show a particular indication  
25 and the reviewers are not satisfied with the information

1 provided, and you have no way to get any further  
2 information, you are kind of sunk, and so the advice  
3 basically here is if you are going to be use samples that  
4 you have limited information about, have limited means of  
5 getting any further information or maybe no means, the best  
6 thing to do before you even start the study is to come in  
7 and to talk to the particular branch in the division that  
8 you are going to be working with and get an answer.

9           But the problems don't end with FDA. There are  
10 privacy laws that are sprouting up all over the place, and  
11 therefore, legal conditions, the considerations and  
12 conditions that go with that. HIPAA, the Health Insurance  
13 Portability and Accountability Act even has some issues that  
14 can concern you, and that is a federal one that is about to  
15 become finalized, and to make things even more complex,  
16 there are state and local differences from the federal laws,  
17 as well, and so while FDA might be willing to accept some  
18 samples in support, you may have issues that are legal  
19 issues with respect to informed consent here, too.

20           [Slide.]

21           Finally, just to give you some information as to  
22 where you can find some more of this, the IVD guidance  
23 document that started concern about informed consent,  
24 especially with repository samples and all, is found at this  
25 web site.

1 [Slide.]

2 This is the general web site for our division, and  
3 on it there are numerous links, which include the FDA  
4 information sheets and the comparison between 45 CFR 46 and  
5 21 CFR 56 is included within that, and there are also many  
6 other documents that are related to these issues.

7 [Slide.]

8 Last, but not least, there is an FDA web site on  
9 human subject protection, and this includes such information  
10 as the NBAC reports.

11 Hopefully, this is the type of information you  
12 were looking for, and I would be happy to answer any  
13 questions.

14 DR. WILSON: Do any of the panel members have any  
15 questions? Dr. Weinstein.

16 DR. WEINSTEIN: Are you saying that for a clinical  
17 investigator who is evaluating for a company a new device,  
18 and is collecting microorganisms from clinical specimens,  
19 that regardless of what that individual's own IRB says about  
20 informed consent, you are saying that there has to be  
21 informed consent for those specimens?

22 DR. ALLEN: We right now believe that if you are  
23 prospectively collecting samples, and you know you are going  
24 to use them for research, you should have the individual  
25 sign an informed consent, yes.

1 DR. WEINSTEIN: I think you are going to get a lot  
2 of clinical research if you do that, if you interpret things  
3 this way.

4 DR. ALLEN: We know we have a lot of education to  
5 do out there because we know that IRBs have somewhat  
6 traditionally waived their need to review them because of  
7 the multitude of reviews that they need to do and the fact  
8 that they have felt that IVD type studies are minimal risk,  
9 but the problem is going to be even worse in the future, and  
10 I think IRBs are beginning to become more and more savvy on  
11 this, because very shortly, if not already, it is almost  
12 impossible to truly have an unlinked sample, so therefore,  
13 the subject is not completely protected by the fact that you  
14 have removed them.

15 DNA analysis is common. DNA profiles are being  
16 amassed by all types of groups, and they are going to  
17 continue to be so. So, even though we may be willing to use  
18 some of them in very limited places--and again that is up to  
19 the Division to decide--the protection of the individual's  
20 rights and welfare has become a prominent issue here, and  
21 even if we were to waive something because it already  
22 existed and we felt there were protections, that doesn't  
23 mean there won't be legal issues.

24 So, things are being looked at on a case-by-case  
25 basis as things evolve, but it is going to be very difficult

1 to justify having collected samples from individuals,  
2 knowing you intended to use their samples and not have asked  
3 their permission.

4 DR. WILSON: Dr. Durack.

5 DR. DURACK: Just to continue the question raised  
6 by Dr. Weinstein, it occurs to me that the issue is  
7 particularly difficult for microbiology specimens because in  
8 that case, the way the specimens come in, there may be no  
9 prior indication that it contains an organism that might be  
10 very important or useful in research at the time the  
11 specimen is collected, maybe more so than with blood  
12 samples, for example. So, I would really ask that the  
13 microbiology issue be carefully considered.

14 DR. ALLEN: Again, I think the legal issues are  
15 going to drive this even more so than our considerations.  
16 The point is, is that there are many institutions for just  
17 that reason are going to generalize types of informed  
18 consent if the individuals or the institution systematically  
19 does or, on a regular basis, collect interesting  
20 information, then, they know that that possibility exists  
21 whenever they take a sample of an individual, and therefore,  
22 there isn't any reason that they couldn't have their  
23 institution put together a general informed consent with  
24 that type of information in mind, and that's the types of  
25 things that NBAC was talking about and what the privacy laws

1 are indicating. So, I mean that's what we are talking  
2 about. We are not talking about, you know, something  
3 extremely specific necessarily.

4 DR. DURACK: I certainly see the point, but it  
5 could become extremely burdensome if you needed a general  
6 consent form for every microbiology specimen.

7 DR. WILSON: Dr. Hammerschlag.

8 DR. HAMMERSCHLAG: What I am concerned about is  
9 let's say you have specimens that have been collected for  
10 culture for what organism X is part of a study, and that  
11 have been now archived and banked, and then a new test comes  
12 along for evaluating organism X and you want to go back to  
13 these specimens and see whether this new non-culture assay  
14 will detect it, and maybe they have been in your freezer for  
15 two to three years.

16 Does that mean now we can't do it or we actually  
17 have to go back and get informed consent from these  
18 individuals who were seen several years prior?

19 DR. ALLEN: That goes along with that comment that  
20 I made about repositories that were already out there. Even  
21 NBAC took a look at that and realized the onus that that  
22 would take, and, in fact, there is legal concerns about  
23 privacy laws being invaded if you were to try to go back  
24 now, after you have somebody's sample and they didn't know  
25 it and ask permission.

1           Again, what I would say is what you need to do is  
2 go to the Division or have the sponsor go to the Division  
3 that is going to receive the submission, and discuss the  
4 issue with them, why they want to use the samples, how they  
5 were contained, and all that, and solve the issue.

6           We are obviously in a place in time where things  
7 are changing, and there is a lot of information out there  
8 that was not collected in a way that the law and  
9 regulations, and even just privacy issues would say we  
10 should do today, but that doesn't mean we should discard  
11 them, because we just can't, we can't afford to lose that  
12 kind of information.

13           DR. WILSON: Dr. Baron.

14           DR. BARON: To clarify for me, many laboratories  
15 do comparisons or validation studies when they are bringing  
16 a new technology into the lab. This is required by CLIA.  
17 We normally don't ask permission of patients when we are  
18 going to be splitting their samples and testing them in two  
19 systems. Sometimes those are for publication, which you  
20 could call research. Mostly they are not for FDA  
21 submission.

22           Are you suggesting now that we just have a general  
23 consent form for every specimen that is collected for  
24 microbiology, for every patient?

25           DR. ALLEN: That basically is what NBAC was

1 suggesting, and it is because of the increase in the concern  
2 for privacy and subject rights, that anytime you take a  
3 sample that could be used in any way other than for that  
4 individual's own diagnosis, that you should have their  
5 permission to do so.

6 No one said, you know, that there wasn't going to  
7 have to be some major changes, but, yes, that was the  
8 initial idea, if there was at all that potential, that they  
9 should have the right to agree or not to it.

10 DR. WILSON: Mr. Reynolds.

11 MR. REYNOLDS: In a hospital setting where a  
12 patient may be in the hospital for a period of time and have  
13 multiple specimens or cultures collected, would one general  
14 waiver upon admission be acceptable, or would you have to  
15 have a waiver for each specimen collected?

16 DR. ALLEN: Common sense would say probably the  
17 first under the circumstances, but that is something that  
18 the lawyers need to work out. I am not a lawyer and I  
19 couldn't tell you the legal things, but in the discussions  
20 that were made on this, that I have heard at several  
21 symposiums with lawyers, they felt that that was a very  
22 definite, easily-solved problem.

23 The bigger problem comes in, for example, if you  
24 have collected, say, samples of someone who had diabetes,  
25 and so you are going to possibly store them and save them

1 for developments that come down the line for testing  
2 subjects that have diabetes, but then you find something  
3 else unique about someone who had diabetes and you want to  
4 go off on a tangent and do some other tests, would this  
5 general consent form cover that.

6           That was what their bigger concern was, and some  
7 lawyers said, yes, they thought it was if the individual did  
8 it or not. So, you know, there is ramifications in there  
9 that a lawyer needs to work out as far as the pieces, but  
10 they seem to say at many of the conferences that development  
11 of some fairly simple, all-inclusive type ones, when you are  
12 taking samples, and, of course, that would mean if you are  
13 going to have multiple samples at a stay, that you would  
14 have a form that says, you know, any sample collected during  
15 my stay as part of my care might potentially be used  
16 eventually, and, you know, I give my permission for it type  
17 of thing.

18           They are already appearing. I had had several  
19 people tell me that they have signed it, and I myself have  
20 signed one that gives permission for my sample to be used.

21           DR. WILSON: Dr. Gutman.

22           DR. GUTMAN: Folks in the Division appreciate  
23 quite keenly the tension that is being generated here and  
24 the importance of having access to this material. The  
25 spirit of this rule is to protect human subject privacy, and

1 we certainly aren't going to support anything that would  
2 break the law, so what Jean is right, that lawyers are  
3 probably more important to talk about the edges of this  
4 policy than scientists, although perhaps scientists should  
5 them honest and make sure that research isn't impeded.

6           From my perspective, the spirit of the law here is  
7 to protect the identity of the patients, so that whether it  
8 is repository or whether it is a new sample, as long as  
9 there is no possible link, you are going to destroy the  
10 link, I am not at all bothered by the use of samples that  
11 have no link.

12           That still is problematic because sometimes you  
13 are going to want to go back and have access--that is  
14 problematic at least for us in submissions, because if you  
15 have destroyed the link and suddenly you need to know what  
16 the glucose level was or whether there was a cross-reactive  
17 disease process, that can be problematic, so even protecting  
18 the notion that you have a delinked sample and allowing it  
19 to be used doesn't necessarily make this an easy process.  
20 It is probably more complicated now than it was before the  
21 NBAC report, but the NBAC report is only marking a  
22 heightened consumer consciousness about what is being done  
23 with people's sample.

24           So, if people here have great ideas on how we  
25 ought to interpret it, the reengineering process that Jean

1 is heading is ongoing, and you are certainly welcome to  
2 become involved in that dialogue.

3 DR. WILSON: We need to move to the next  
4 presentation, which will be CLIA waivers and be presented by  
5 Ms. Clara Sliva.

6 **CLIA Categorization at the FDA**

7 **Clara Sliva**

8 MS. SLIVA: Hi. I am Clara Sliva. I am the  
9 Acting CLIA Coordinator. I hope all of you got this handout  
10 that I prepared. I want to just you know to publicize, we  
11 have got a CLIA web site, all the information that is in  
12 your packet is going to be--and the slides actually--will be  
13 on the CLIA web site.

14 [Slide.]

15 I wanted to acknowledge some of our CLIA team  
16 members who are actually in Microbiology - Kathy Wright and  
17 Marian Heyliger, Liz Rogers. They are all part of our CLIA  
18 team, and also obviously Dr. Gutman and Don St. Pierre, who  
19 is our deputy. Then, the real stabilizing influence has  
20 been Dr. Hackett, and I guess he is back holding the fort  
21 down, so he is not able to be here, but he is the Associate  
22 Director for CLIA.

23 [Slide.]

24 I am going to talk a little bit about the history  
25 of it. I don't know if a lot of you know that we did take

1 the responsibility for CLIA categorization from CDC in  
2 January of this year, and we are responsible just for  
3 categorizing commercially marketed test systems.

4 These are test systems that come into the FDA for  
5 clearance or approval including products from the Center for  
6 Biologics, and CDC still remains doing laboratory procedures  
7 like Gram's stain.

8 Again, I am going to give you the current status  
9 of the DCL CLIA team, talk about our partners in CLIA, HCFA,  
10 and CDC, give you a little refresher on what CLIA '88 is. I  
11 will talk about the progress of it, the waived tests, the  
12 new technology.

13 [Slide.]

14 The history. It was transferred from CDC on  
15 January 31, 2000. Again, FDA is only responsible for the  
16 commercially marketed product. It was at the behest of  
17 industry and Congress that this change was actually made,  
18 and it really does make more sense. It really is - we call  
19 it one-stop shopping. You get your clearance and your  
20 categorization at the same time.

21 Again, CDC is responsible for the categorization  
22 of laboratory procedures, Gram stains, provider-performed  
23 microscopies. HCFA pays the bill for us, so they pay us to  
24 do the CLIA categorizations, and we do have an interagency  
25 agreement with them.

1 [Slide.]

2 Again, the CLIA team, Miin-Rong Tsai, Arleen  
3 Pinkos, Carol Benson, Dr. John Hyde, who is a statistician  
4 and a medical officer, Jim Callahan, who is our computer  
5 person.

6 Hematology/Pathology, we have Louise Magruder,  
7 Deborah Moore, Josie Bautista, Maria Chan, Michelle Clark-  
8 Stuart.

9 [Slide.]

10 Marian Heyliger, Liz Rogers, and Kathy, who are  
11 here at the meeting today. Then, obviously, the real brains  
12 behind this whole thing is Dr. Gutman. I am the Acting CLIA  
13 Coordinator. Marina Kondratovich is a statistician. Joe  
14 Hackett is our Associate, Don is our Deputy Director, and  
15 also Candy Chun, who is our systems analyst. She is also  
16 part of our CLIA team.

17 So, it is big and it is getting bigger because  
18 business is booming.

19 [Slide.]

20 Again, our partners are Health Care Financing.  
21 What we do is when we get a waiver notification in the  
22 instance of Microbiology, we have waived Strep A and H.  
23 pylori since FDA has been in charge of CLIA. What we do is  
24 we notify HCFA, and then we provide them with a package  
25 insert, so then their inspectors will know exactly which

1 package insert people are supposed to be using. That is one  
2 advantage, I think, that we have over CDC, because a  
3 document number, an FDA document, but not document number,  
4 will be aligned with that.

5           Again, we have constant contact with CDC. We are  
6 still following their policy for the criteria for waiver and  
7 we consult with them on waiver applications. For the  
8 moderate and high, we really don't have that much. I think  
9 we are doing pretty good on those, too.

10           Also, we keep them apprised of new technology.

11           [Slide.]

12           We are going to have a CLIA Waiver Workshop, which  
13 is going to be August 14-15, and in this we will discuss the  
14 criteria for waiver that were published by CDC in 1995.  
15 There is a lot of interested parties in this - consumers.  
16 When CDC first published their waiver criteria in 1995, the  
17 Public Citizen had a question because they thought that  
18 these should be based on risk.

19           Obviously, the medical community is interested  
20 because physicians really base a lot of their tests that  
21 they offer in their laboratories, physicians' office  
22 laboratories, by what is waived.

23           Laboratorians are certainly interested because  
24 this somehow may affect some of the business that they are  
25 doing, too. I mean people may be going to the smaller

1 unitized devices.

2 Obviously, the manufacturing industry is very  
3 concerned about it because they have told us that they want  
4 more tests to be waived. The interesting part is what we  
5 are seeing is a lot more microbiology tests that they are  
6 asking to be waived.

7 [Slide.]

8 Again, why are we having this CLIA Waiver  
9 Workshop? It had been out five years by the time we have  
10 the meeting, and FDA really needs to implement its  
11 responsibility for CLIA waiver, and it is going to be  
12 beneficial to all groups.

13 It is going to be beneficial to FDA because we  
14 will know exactly what the rule will be and manufacturers  
15 will know exactly what they need to do to get something  
16 waived, it really won't be something that a notice  
17 published.

18 The interest in waived tests is very high right  
19 now, and we get calls every day from someone that wants to  
20 have another waived test. In fact, a lot of physicians only  
21 offer waived tests, and they will call and say, you know,  
22 which tests are waived now, because I am going to put that  
23 on, you know, if I can, I am going to put that in my  
24 physician's office. They have no intention of having that  
25 extra layer of regulation by running moderate or high

1 complexity tests.

2 [Slide.]

3 Again, let's talk about the Clinical Laboratory  
4 Improvement Amendments of 1988. This specified that  
5 laboratory requirements be based on the level of complexity,  
6 and on February 28, 1992, the regulations were published to  
7 implement CLIA.

8 There actually were three categories of test -  
9 moderate complexity, high complexity, and waived. The most  
10 interesting ones are the waived tests. Originally, in 1992,  
11 the regulation said that if it was cleared by FDA for home  
12 use, it was waived, and if it was simple and accurate to  
13 render the likelihood of erroneous results negligible, it  
14 was waived.

15 [Slide.]

16 Then, on September 13, 1995, there was a lot of  
17 interest from people because what happened is CDC actually  
18 had a moratorium and they did not allow any tests to be  
19 waived, even those that were home use. After that, they  
20 went ahead and published the criteria, and the criteria is  
21 actually in your packet.

22 Then, in November of 1997, the CLIA waiver  
23 provision was actually revised by Congress. This was put  
24 under the Food and Drug Modernization Act, and it did  
25 clarify that all tests approved by FDA for home use are

1 automatically waived. It did say that the tests will need  
2 to be simple and accurate, but they needed to render the  
3 chance of erroneous results by the user negligible, which  
4 basically means if a laboratorian can run the test, if the  
5 untrained user can reproduce those results, then, the tests  
6 should be waived. I mean there is a lot of controversy  
7 about that.

8           Then, also, it says no reasonable risk of harm if  
9 performed incorrectly.

10           [Slide.]

11           We have been very, very busy. We have categorized  
12 over 800 products since we started, which is pretty amazing.  
13 We have waived 90, and the majority of them have really been  
14 over-the-counter or prescription home use.

15           They have actually taken very little time. The  
16 categorizations usually take about the same amount of time  
17 as getting a 510(k) out, and the waived products may be a  
18 little bit longer, but still I know that it is probably 50  
19 percent faster than it was at CDC at this time because we  
20 are just administratively set up to do it. So, we have  
21 streamlined the process somewhat.

22           [Slide.]

23           Again, there is some source of controversy because  
24 waiver allows labs to perform the test without having to  
25 meet any personnel, quality assurance, or proficiency

1 testing standard. It is really quite interesting because  
2 when manufacturers come in and they want to test their test  
3 with a non-laboratorian, sometimes they actually go to  
4 seventh grade classes and have them run Strep A test or H.  
5 pylori, so we have had them done in car dealerships, we have  
6 had them done in--so the way that you evaluate a test for  
7 waiver is completely different than the way that you are  
8 evaluating this PMA, for instance, right now.

9 It is quite different and we do separate those two  
10 processes because we have got FDA laws that regulate  
11 clearance and approval, and then we have got the Public  
12 Health Service Act, which regulates CLIA waiver criteria,  
13 and that is one of the reasons that we are going to be  
14 having this workshop where we are going to be getting  
15 additional comments on these criteria.

16 But like I said, we may be meeting before the  
17 Micro panel again because there is lots of interest in micro  
18 devices.

19 [Slide.]

20 Again FDA, the majority of tests that we have  
21 waived have been over-the-counter or prescription home use,  
22 and when CDC originally put out their waiver criteria, they  
23 received about 44 comments, and practically everybody said  
24 that they had no problem with waiving tests that were home  
25 use, and I don't know if these people that were responding

1 really understood what FDA did.

2 All we do is we consider that they are simple and  
3 they are reliable, and that they can be performed by the  
4 patients themselves or untrained family members. But we  
5 also consider the health benefits to be gained, you know,  
6 that they do outweigh the risks, so it is a completely  
7 different review than a waiver review.

8 DR. WILSON: We are going to have to wrap up here  
9 to keep on schedule.

10 MS. SLIVA: Okay, great.

11 [Slide.]

12 Like I said, a growing number of lab tests have  
13 been cleared over-the-counter, and these are the example,  
14 and the list is going to expand substantially in the next  
15 few years.

16 [Slide.]

17 Again, waiver tests may be simple to perform, but  
18 they may be driven by complex technology, and industry has  
19 proven the technology is reliable.

20 [Slide.]

21 In the future directions, do we want to expand the  
22 list? Do we want to limit the test list? Or do we want to  
23 say, as Dr. Gutman says, do we want a damn near perfect test  
24 for waiver?

25 Thank you.

1 DR. WILSON: I think we have time perhaps for one  
2 question from the panel.

3 DR. SMITH: I have one question.

4 DR. WILSON: Dr. Smith.

5 DR. SMITH: I was just briefly looking at the list  
6 of tests that are already waived. I do a lot of medical  
7 education, and one of my concerns over time, over the last  
8 decade or so, things have changed. We are not able to do a  
9 lot of microbiology, looking at Gram stains on the floors  
10 with residents, it all has to go through laboratories.

11 One of our recent contentions is doing hemocult,  
12 but it seems that that is a waived test, so there would be  
13 no legal reason that the medical residents, for example,  
14 couldn't do a hemocult test on the floor, is that correct?

15 MS. SLIVA: Exactly

16 DR. WILSON: Thank you.

17 The issue for today are two premarket approval  
18 applications by Roche Molecular Systems, one for the  
19 AMPLICOR HCV test. It's a nucleic acid amplification test  
20 for the in vitro diagnostic qualitative device to detect  
21 hepatitis C virus RNA and an automated version, the COBAS  
22 AMPLICOR HCV test also to detect HCV RNA. Neither test is  
23 intended for blood donor screening.

24 I would like to ask that the panel hold questions  
25 until after all four presentations have been completed. I

1 would also like to remind the audience that only panel  
2 members can ask questions of the speakers.

3 I would like to introduce David Thomas, who is the  
4 Vice President for Clinical and Regulatory Affairs for Roche  
5 Molecular Systems, who will introduce the manufacturer's  
6 presentation.

7 **Manufacturer's Presentation**

8 **Introductions**

9 **David B. Thomas**

10 MR. THOMAS: Dr. Gutman, members of the  
11 Microbiology Devices Panel, FDA colleagues: It is my  
12 pleasure to present the sponsor's summary of the two PMA's  
13 in consideration for the Amplicor and Cobas Amplicor HCV  
14 Version 2.0 HCV RNA tests.

15 [Slide.]

16 These two second generation assays have been  
17 available as research products since 1998 in the United  
18 States and internationally, have been approved by all of the  
19 major developed countries that have such approval processes.

20 [Slide.]

21 In our agenda today, Dr. Karen Gutekunst, who is  
22 the Director of Product Development, will review the  
23 technology of the assays, as well as the non-clinical data.  
24 Dr. Michael Fried, who is the Associate Professor of  
25 Medicine at the University of North Carolina, and one of the

1 principal investigators in the clinical trial, will discuss  
2 with you the use of the assay in clinical practice, as well  
3 as something about the procedures used in the trial and the  
4 patient population.

5           Finally, Dr. Alison Murray, who is our Director of  
6 Clinical Affairs at Roche Molecular Systems, will discuss  
7 and summarize the clinical results.

8           [Slide.]

9           Several considerations that you are probably aware  
10 of, that we would like to point out. The first is this is  
11 the first product seeking approval as a direct test for HCV  
12 RNA. Therefore, we will be discussing with you both the  
13 specifics of the product and what will presumably be the  
14 class labeling for such products in the future, which is, of  
15 course, a matter of considerable interest and concern with  
16 industry.

17           The second issue is the limitations of available  
18 methodologies for characterizing HCV RNA, particularly  
19 quantification. The methodologies that are available, first  
20 of all, are not in all cases independent of PCR itself,  
21 which is the product under review, and, secondly, there is  
22 limitations in the precision, particularly for  
23 quantification of the virus that make the design and  
24 interpretation of experiments somewhat difficult.

25           We have had considerable discussion about these

1 matters with the FDA staff, appreciate their concern and  
2 counsel on this, and will look forward to any advice you may  
3 give us on these matters.

4           Finally, the lack of an independent gold standard  
5 for the assessing of clinical infection, again independent  
6 of PCR, which is something that we will discuss with you  
7 regarding the clinical outcomes.

8           [Slide.]

9           The proposed indication for use of the subject  
10 product, which we are pleased to say we are in agreement  
11 with FDA on this intended use, is as follows. The Amplicor  
12 Hepatitis C Virus Test, Version 2.0, is a qualitative  
13 diagnostic test for the detection of HCV RNA in human and/or  
14 plasma specimens.

15           The Amplicor HCV Test is indicated for patients  
16 who have liver disease and antibodies to HCV that were  
17 detected by enzyme immunoassay and by immunoblot assay, and  
18 who are suspected to have active infection.

19           Detection of HCV RNA is evidence of active HCV  
20 infection, but does not distinguish between acute and  
21 chronic states of infection.

22           Obviously, after this presentation, we will be  
23 happy to take your questions.

24           Dr. Gutekunst.

25           **Product Description/Non-Clinical Performance Data**

1                                   **Karen Gutekunst, Ph.D.**

2                   DR. GUTEKUNST: Thank you.

3                   [Slide.]

4                   We will be presenting two tests to you today, both  
5 the Amplikor and Cobas Amplikor HCV tests. These are both  
6 qualitative in vitro diagnostic tests for the detection of  
7 hepatitis C virus in clinical specimens.

8                   Both of these tests detect the viral nucleic acid.  
9 The technology that we use is reverse transcription -  
10 polymerase chain reaction amplification--sometimes you will  
11 hear this referred to as RT-PCR--and we have colorimetric  
12 detection of the amplification product.

13                   [Slide.]

14                   Just to get everybody onto the same page,  
15 hepatitis C virus is a member of the Flaviviridae family.  
16 It is a single-stranded RNA virus with a genome of  
17 approximately 10,000 nucleotides.

18                   [Slide.]

19                   The genome of the virus contains both structural  
20 and nonstructural genes, as well as untranslated regions at  
21 both the 5-prime and 3-prime end of the genome. We will be  
22 focusing on the 5-prime untranslated region for the  
23 amplification and you will see why in the next slide.

24                   [Slide.]

25                   I apologize for the colors here. The 5-prime

1 untranslated region is one of the most highly conserved  
2 region of the genome, and although there are three regions  
3 with some degree of heterogeneity, we have chosen our  
4 primers KY78 and 80 and the probe to be outside of those  
5 regions of heterogeneity, so they are very highly conserved  
6 over the known genotypes of HCV.

7 [Slide.]

8 Some of you may be aware of a previous version of  
9 test that Roche had produced, the Version 1 test, which had  
10 demonstrated some genotype bias. We have made some  
11 modifications to that test including a modification to the  
12 sample preparation method to improve the analytical  
13 sensitivity of the test.

14 We removed manganese from the specimen  
15 resuspension diluent in order to improve the reagent overall  
16 stability, and we added a co-solvent to the master mix,  
17 which we believe was what enabled us to improve the  
18 amplification of the different genotypes.

19 [Slide.]

20 Now, you will remember in the previous slide that  
21 I said that the primer and probe sequences were very highly  
22 conserved, and they are, but the 5-prime untranslated region  
23 also demonstrates a high degree of secondary structure, and  
24 our primers and probes are embedded in this secondary  
25 structure.

1           We believe that the addition of the co-solvent to  
2 our amplification reaction helps to destabilize this  
3 secondary structure and make these primer sequences more  
4 accessible for amplification.

5           [Slide.]

6           We will be talking about two kits today, but these  
7 kits are more similar than they are different, and that is  
8 by design. The two tests were designed to look like each  
9 other and to perform like each other. So, I will describe  
10 where the tests are different and we will focus primarily,  
11 when we present data to you today, on just the Cobas  
12 Amplicor unless there are differences between the two tests.

13           The kit is comprised of four sub-kits - a specimen  
14 preparation kit which is identical for both tests. It  
15 includes a lysis reagent, an internal control, and the  
16 resuspension buffer, which we call specimen diluent.

17           We include a controls kit that has both a positive  
18 and negative control, as well as negative plasma in order to  
19 process these controls in a background that is similar to a  
20 clinical specimen.

21           The amplification kit contains the master mix,  
22 which is the primary amplification reagent that contains the  
23 enzymes, primers, DNTPs, et cetera, and then manganese is a  
24 cofactor for the enzyme that we use, which is rTth DNA  
25 polymerase. This is an enzyme that has the unique ability

1 to perform both the reverse transcription and the PCR  
2 amplification under the appropriate buffering conditions.

3 Now, the two detection kits do have minor  
4 differences, and I don't know how well you can read this.  
5 They both contain a denaturation solution, which is a  
6 chemical reagent that will denature the double-stranded  
7 amplification product, a hybridization buffer, conjugate,  
8 and a substrate.

9 Where they differ is on the Amplicor test. We  
10 have a microwell plate that has the probes bound to the  
11 surface of the microwell plate, but on the Cobas Amplicor,  
12 we use magnetic microparticles that have the probe bound to  
13 these magnetic particles, so we have a solution  
14 hybridization that occurs on the Cobas Amplicor test.

15 Then, down here at the bottom you see--if you  
16 could read this--would say STOP solution. The reaction is  
17 stopped on the microwell plate, which gives a yellow color,  
18 and that color is read at 450 nanometers as opposed to the  
19 reaction on the Cobas Amplicor, which is not stopped. We  
20 therefore are reading a blue color, which is read at 660  
21 nanometers. So, in your packages you may see slightly  
22 different cutoffs for the two assays, and this is due to the  
23 fact that they are being read at different wavelengths.

24 [Slide.]

25 This shows a brief schematic of the test

1 procedure. As I said, the sample preparation for both tests  
2 is identical, so the prepared sample could be used to go  
3 either to the Amplicor test procedure or the Cobas Amplicor  
4 procedure.

5 For the Amplicor test, the amplification occurs on  
6 a Perkin-Elmer 9600 or 2400 thermal cycler. After  
7 amplification, the reaction is then manually denatured by  
8 the addition of denaturation and the user adds the solution  
9 to the microwell plate and completes the detection portion  
10 of the assay.

11 The Cobas Amplicor, on the other hand, once the  
12 prepared sample is added to the amplification, it is loaded  
13 onto the instrument and the amplification and detection  
14 occur automatically on the same instrument platform.

15 [Slide.]

16 This is a brief schematic of the sample  
17 preparation procedure just to show you that it is a fairly  
18 simplified sample preparation that occurs in a single tube.

19 We start with whole blood and separate into either  
20 serum or plasma. A small aliquot of this is then added to a  
21 lysis reagent to which the internal control has been added.  
22 So, from this point on, the internal control is now  
23 processed through every step of the reaction in each  
24 individual sample.

25 We then precipitate the RNA with isopropanol, wash

1 briefly with ethanol to remove residual salts, and then  
2 resuspend the RNA, so now we have both targeted internal  
3 control RNA resuspended in a specimen diluent which is now  
4 ready for amplification.

5 We add the master mix reagent and then go either  
6 to the Amplicor test format or the Cobas Amplicor.

7 [Slide.]

8 As I mentioned, we include an internal control in  
9 each specimen, and this diagram is intended to show you that  
10 the primer sequences of the internal control are identical  
11 to the primer sequences that we use for target  
12 amplification.

13 There is a unique probe region, however, which  
14 enables us to discriminate product resulting from  
15 amplification of the target cDNA as opposed to the internal  
16 control.

17 [Slide.]

18 Also included in the amplification--again, this is  
19 a little difficult to see, I think, so you will just have to  
20 trust me--we include an enzyme that we call AmpErase, and  
21 this enzyme is Uracil-N-glycosylase.

22 There has for a long time been concern that  
23 because PCR generates so many copies of amplified DNA that  
24 there is a high potential for carry-over contamination. In  
25 order to minimize this, we have included this enzyme in the

1 amplification reaction along with a substitution of dUTP in  
2 the place of TTP.

3           So, any amplified DNA from the Amplicor  
4 amplification reaction will contain dU in the place of T's.  
5 In the presence of AmpErase, these dU's will be cleaved and  
6 then during the subsequent PCR amplification cycles, when  
7 the reaction is heated in the alkaline pH of the  
8 amplification reaction, the DNA will fall apart and is no  
9 longer amplifiable, so it is no longer a template for  
10 amplification. This is the first step in the reaction.

11           We incubate with AmpErase and then we go on to  
12 perform the RT-PCR reaction.

13           [Slide.]

14           I will talk briefly now about the detection  
15 chemistry.

16           [Slide.]

17           We start with an amplification reaction. We have  
18 added our target and internal control that we have isolated  
19 from the specimen, and the master mix reagent now contains  
20 the enzyme, the primers, one of which is biotinylated, and  
21 the dNTP's. Amplification occurs, and now at the end of  
22 amplification we have double-stranded DNA which contains a  
23 biotin tag at one end due to that incorporation of biotin  
24 onto the primer.

25           This material is chemically denatured and then

1 added to a well on the microwell plate. The microwell plate  
2 is coded with a probe that is specific for HCB. We have a  
3 separate plate that has a probe that is specific for the  
4 internal control.

5 The amplified DNA will selectively hybridize to  
6 the probe. Then, after an hour incubation, this is washed  
7 with an automated plate washer to remove any unbound  
8 material, and then we add an Avidin-horseradish peroxidase  
9 conjugate.

10 By virtue of the high affinity that Avidin has for  
11 biotin, this conjugate will bind to the target, the  
12 amplified DNA that is bound to the probe on the microwell  
13 plate.

14 Again, after an incubation, we wash to remove  
15 unbound conjugate and then this conjugate will catalyze the  
16 oxidation of tetramethylbenzidine to form a blue color.

17 We add on the microwell plate a STOP solution,  
18 which is a weak acid. This blue color turns to yellow, and  
19 then that color is read on an automated microwell plate  
20 reader.

21 [Slide.]

22 Now, the Cobas Amplicor Analyzer is an instrument  
23 that was 510(k)-cleared in 1997, and this instrument  
24 combines five separate functions onto a single platform  
25 including the thermal cyclers, automated pipetting for the

1 addition of all of the detection reagents, an incubator to  
2 perform those incubations, as well as a wash station, and an  
3 onboard photometer to automatically read the reaction at the  
4 end.

5 [Slide.]

6 This is a top-down view showing the thermal  
7 cyclers, reagent bottles. These are detection reaction  
8 tubes, the pipetting unit. This is the wash station, and a  
9 photometer is back here in the back.

10 [Slide.]

11 I know you can't see this slide. The point that I  
12 wanted to make on this slide is that the chemistry that is  
13 performed as part of the detection on the Cobas Amplicor is  
14 identical to the chemistry on the microwell plate assay with  
15 the exception that the probe is bound to magnetic  
16 microparticles. It is not fixed onto the surface of the  
17 microwell plate.

18 [Slide.]

19 So, now I would like to briefly review some of the  
20 non-clinical studies that we have performed. The reason  
21 that I have these highlighted in different colors, those  
22 that are highlighted in yellow, I will go into a little more  
23 detail on, and those that are in white, I am not going to  
24 spend much time just in the interest of time, and if there  
25 are questions at the end, of course, we have the data with

1 us and would be happy to share that with you.

2 [Slide.]

3 The first study was to determine the preliminary  
4 cutoff for the assay. We did this by analysis of 530 anti-  
5 HCV negative specimens, and we had 236 anti-HCV positive  
6 specimens, 154 of which were confirmed as being antibody  
7 positive by RIBA.

8 These specimens were analyzed on both test  
9 formats, and then we looked at the specificity and  
10 sensitivity versus serology using these 530 and 154 EIA  
11 positive specimens. This is shown on the next graph--

12 [Slide.]

13 --which is a cumulative distribution analysis showing  
14 specificity and sensitivity versus cutoff, and you can see  
15 that for a wide variety of cutoffs, the sensitivity and  
16 specificity virtually do not change.

17 So, for the non-clinical studies that we  
18 performed, we chose a preliminary cutoff of 0.15 absorbance  
19 units on the Cobas Amplicor to conduct our non-clinical  
20 studies. This other line here represents an upper limit of  
21 an equivocal zone that was added at the time of our clinical  
22 study, and Dr. Alison Murray will talk a little bit more  
23 about the equivocal zone, but for the purposes of this  
24 study, and the studies I will describe, we used this cutoff  
25 of 0.15.

1 [Slide.]

2 I just want to briefly mention, as Dr. Thomas  
3 mentioned, the need for standardization. There is currently  
4 no gold standard for HCV RNA assays. This is because you  
5 can't visualize the virus by electron microscopy, and you  
6 can't grow the virus in culture.

7 So, in 1997, a collaborative study was undertaken  
8 by the WHO International Working Group on the  
9 Standardization of Genomic Amplification Techniques for the  
10 Virologic Safety and Testing of Blood and Blood Products,  
11 and this group is called SoGAT.

12 They performed a collaborative study where they  
13 sent three candidate materials to a number of laboratories,  
14 22 of which actually sent data back, so the data that they  
15 analyzed was from 22 laboratories.

16 Each of these laboratories performed endpoint  
17 dilutions on each of the three candidate materials, and upon  
18 analysis of all of the information, a single material was  
19 chosen. It was assigned a value of 100,000 International  
20 Units per mL. This was accepted by the WHO and has become  
21 the first International Standard for HCV RNA nucleic acid  
22 test assays. It is No. 96/790.

23 [Slide.]

24 This material is described in a publication by  
25 Saldanha and his colleagues, and their recommendation is

1 that International Standards are used to provide a common  
2 standard unit of measurement, the International Unit of a  
3 material, and that all other standards and working reagents  
4 are assigned a value using this same biologic unit of  
5 measurement.

6 So, we have tried to incorporate this into some of  
7 our analytical studies.

8 [Slide.]

9 The first study that we have performed was a limit  
10 of detection study using the actual WHO International  
11 Standard. I should point out that this material is a  
12 genotype 1 specimen that was diluted into HCV-negative  
13 cryosupernatant.

14 We diluted down to low copy numbers, and you can  
15 see that at concentrations of 50 International Units or  
16 higher, we had 100 percent detection rate with both the  
17 AmpliCor and Cobas AmpliCor test.

18 [Slide.]

19 We also did a similar analysis on another reagent  
20 that is available from the National Institute of Biological  
21 Standards and Controls in the U.K. This is called the NIBSC  
22 Working Reagent. This material is a genotype 3 specimen.

23 It was originally characterized using the branch  
24 DNA technology, so the concentrations are reported as genome  
25 equivalents per mL. It has later been recharacterized in

1 reference to the WHO standard, and those International Units  
2 per mL are shown in the parentheses below the genome  
3 equivalents.

4           You can see that at a concentration of  
5 approximately 100 genome equivalents per mL, or less than 50  
6 International Units per mL, we had approximately 95 percent  
7 detection rate on both assays.

8           [Slide.]

9           We also tried to look at the sensitivity of the  
10 test in reference to antibody formation by analyzing a  
11 series of seroconversion panels. We analyzed nine  
12 seroconversion panels that were comprised of different  
13 numbers of specimens.

14           This column shows the day of the first HCV RNA  
15 results. So, for example, this sample was positive on all  
16 days. It was from day zero on it was positive on the assay.

17           This column shows the day that we had the first  
18 positive HCV EIA, whether or not that was confirmed by RIBA,  
19 and then the difference between the first RNA result and the  
20 EIA result. You can see that in all cases, the RNA was  
21 detectable on average 20 to 40 days before the formation of  
22 antibodies.

23           [Slide.]

24           We looked at the specificity of the test by  
25 analyzing 29 unrelated viral isolates and 4 bacterial

1 isolates. We saw no cross-reactivity with either test in  
2 any of these isolates. We looked at 10 clinical specimens  
3 from individuals infected with hepatitis A virus and 10  
4 individuals infected with hepatitis B virus. Again, we saw  
5 no cross-reactivity to either test.

6 We evaluated potential interference of the assay  
7 by looking at clinical specimens from individuals with  
8 elevated levels of albumin, hemoglobin, triglycerides,  
9 immunoglobulin, and bilirubin. We saw no interference with  
10 the ability to detect HCV RNA in these specimens.

11 We evaluated the potential interference by  
12 therapeutic drugs including those drugs used to treat  
13 patients infected with hepatitis C, HIV, hepatitis B virus,  
14 and CMV. We tested each of these drugs at three  
15 concentrations and saw no interference with the ability to  
16 detect HCV RNA in the presence of these drugs.

17 Finally, we looked at co-infection of HCV with  
18 hepatitis B virus, HIV, both HIV and HBV, and HBV and HAV,  
19 and again saw no interference with any of these co-  
20 infections.

21 [Slide.]

22 A reproducibility study was conducted at four  
23 sites. We included four HCV RNA positive specimens ranging  
24 in concentration from approximately 200 to 50,000 copies per  
25 mL, and a single negative specimen.

1 Each of these panels was analyzed twice a day at  
2 each site for a minimum of 4 days, and we are presenting the  
3 results as the number of correct positive and/or negative  
4 results.

5 [Slide.]

6 You can see that with one exception, we had one  
7 false negative here, the results were completely concordant  
8 with those that we would have predicted.

9 [Slide.]

10 We performed two studies to evaluate how well the  
11 assay performs on serum versus plasma. In the first study,  
12 we had matched specimens from 34 anti-HCV positive  
13 individuals and 10 anti-HCV negative individuals.

14 So, from each of these individuals we had a  
15 matched serum, ACD plasma, and EDTA plasma tube drawn. In  
16 all cases, all of the anti-HCV positive specimens were  
17 positive and all of the anti-HCV negative specimens were RNA  
18 negative regardless of whether that material was collected  
19 as serum, EDTA plasma, or ACD plasma.

20 [Slide.]

21 We followed that up with a second study where we  
22 took 10 sets of matched specimens and we diluted those. We  
23 initially analyzed those specimens using a quantitative HCV  
24 RNA PCR test to estimate the starting concentration of  
25 material in those specimens.

1                   We then diluted those specimens to near the limit  
2 of detection of the assay as determined with the WHO  
3 standard, and then looked for the number of positive  
4 results. Again, we did not see differences between the  
5 different matrices.

6                   [Slide.]

7                   Finally, I would like to talk a little bit about  
8 HCV genotype detection. Again, I want to remind you that we  
9 have some challenges here because there is currently no gold  
10 standard for non-1 genotypes of HCV. So, we have a  
11 difficult time estimating what the true concentration of RNA  
12 in specimens is in order to ask the question are we  
13 detecting the same number of molecules in each specimen.

14                   But in spite of that, we tried to conduct some  
15 studies to address this issue, and I will describe four  
16 studies.

17                   [Slide.]

18                   In the first study, we have a series of HCV RNA  
19 transcripts that were generated from seven different  
20 subtypes of HCV. This RNA was purified and then analyzed  
21 spectrophotometrically. So, in this study, we have a  
22 somewhat independent method of quantitation although these  
23 transcripts do not represent the entire genome of HCV. It  
24 only represents a subgenomic region of the virus.

25                   These transcripts were diluted to very low copy

1 levels representing 200, 100, and 40 copies per mL of HCV  
2 genome. We performed a series of replicate analyses on each  
3 of these dilutions and then did a probit analysis of the  
4 dataset to determine the concentration at which there was a  
5 95 percent probability of getting a positive result.

6           These concentrations ranged from 5 to 11 copies  
7 per PCR for each genome on the Cobas Amplicor test, all with  
8 overlapping confidence intervals, and this data is shown on  
9 the next slide.

10           [Slide.]

11           You can see that these are the different  
12 genotypes, the predicted concentration at which we would  
13 have a 95 percent detection rate, and the confidence  
14 intervals are overlapping for all genotypes.

15           [Slide.]

16           We then tried to confirm this result by using  
17 clinical specimens. We had eight clinical specimens  
18 representing eight subtypes of HCV. Again, we performed a  
19 quantitative PCR test on these specimens to try to estimate  
20 the starting concentration of material that was in the  
21 clinical sample, and then based on that quantitative RNA  
22 result, we diluted these specimens to near the limit of  
23 detection of the assay.

24           All of these specimens demonstrated 100 percent  
25 detection rate at what we believe was close to 100 IU's per

1 mL. These data are shown on the next slide.

2 [Slide.]

3 We performed three dilution series, one targeting  
4 100 International Units per/mL, one 75 International Units  
5 per mL, and 50 International Units per mL. I have put  
6 ranges associated with these because the inherent  
7 variability of quantitative RNA PCR tests, I believe doesn't  
8 allow us to precisely say that each of these samples had  
9 exactly 100 International Units. We believe it is  
10 approximately 50 to 200, and approximately 36 to 150,  
11 approximately 25 to 100.

12 So, you can see that at the higher concentrations  
13 that we tested, which are very close to the limit of  
14 detection, we see no differences. We may start to see some  
15 differences at this very lowest copy level, but we can't  
16 really discriminate whether these differences are due to the  
17 ability to detect the RNA or whether these are, in fact,  
18 differences in the true concentration of RNA that is in each  
19 of those specimens.

20 [Slide.]

21 We next performed a study with 11 clinical  
22 specimens, again representing a variety of HCV genotypes.  
23 We analyzed the amplification product from each of these  
24 reactions on an agarose gel to see whether they produced an  
25 amplification product of the expected size.

1           You will see on the next slide that, in fact, all  
2 of these specimens produced the same size amplification  
3 product. This gel is frowning a little bit, it's curving  
4 down, but these are all of the predicted size. The controls  
5 were also frowning.

6           [Slide.]

7           Finally, in the last study, we looked at 87  
8 clinical specimens from a U.S.-based anti-HCV therapy trial.  
9 These represent 15 subtypes of HCV, again, a wide variety of  
10 subtypes tested, each tested with both assays, and we had  
11 100 percent detection of each of these specimens.

12          [Slide.]

13          In conclusion, we are presenting two tests to you  
14 which have a limit of detection of approximately 50  
15 International Units per mL in reference to the WHO  
16 International Standard for HCV-RNA.

17          We did not observe any cross-reactivity to other  
18 unrelated microorganisms or viruses. We did not observe any  
19 interference by either endogenous substances, exogenous  
20 substances, co-infections or non-HCV viral hepatitis  
21 specimens.

22          We believe we have demonstrated comparable  
23 detection HCV in serum, ACD plasma and EDTA plasma, and  
24 comparable detection of the known genotypes of HCV.

25          Now, I would like to turn it over to Dr. Fried,

1 who will talk about the applications of these tests in  
2 clinical practice.

3 **Device Use in Clinical Practice**

4 **Michael W. Fried, M.D.**

5 DR. FRIED: Thank you very much and I greatly  
6 appreciate the opportunity to be here today.

7 [Slide.]

8 I have been asked by Roche Molecular Systems to  
9 discuss the standard evaluation of patients with hepatitis C  
10 and to place into context the clinical utility of PCR assays  
11 for the detection of HCV viremia.

12 Because PCR assays have really become an integral  
13 part of our management and diagnosis of patients with  
14 chronic hepatitis C, I think much of what I say will be  
15 second nature to many of you on the panel and in this room,  
16 as well. In fact, barely a single day goes by when I do not  
17 order an HCV RNA assay in order to diagnose and manage my  
18 patient with hepatitis C in my clinical practice. So, the  
19 clinical utility of hepatitis C RNA really cannot be  
20 underestimated.

21 I have had the good fortune really to work in  
22 hepatology really since the earliest days, shortly after the  
23 discovery of the hepatitis C virus, to the present time when  
24 our therapeutic interventions are really quite effective  
25 now, and I grew up in the era of the "home brew" PCR assay.

1 I have personally performed those assays in the  
2 laboratory and I have also lived through the anxieties of  
3 trying to determine whether a PCR test was a true positive  
4 result or the result of carry-over and whether a true  
5 negative result was truly negative or whether someone forgot  
6 to add the TAQ polymerase or other critical reagents.

7 So, I bring to the panel a real understanding of  
8 the importance of a standardized reproducible assay that we  
9 can use in clinical practice for the diagnosis of hepatitis  
10 C infection.

11 [Slide.]

12 Now, when we first meet a patient with suspected  
13 hepatitis C, there are a number of questions that we must  
14 answer which are shown on this slide. The first question,  
15 is it really hepatitis C, how severe is the patient's liver  
16 disease, should we treat it with interferon, is it  
17 responding to treatment, and ultimately, is it cured?

18 I will spend most of my time discussing the first  
19 question, is it really hepatitis C or how do we confirm the  
20 diagnosis, and corollary to this is what are the potential  
21 pitfalls to the diagnosis of hepatitis C and what are the  
22 limitations of the various assays that we currently have  
23 available, how do all of these fit into our diagnostic  
24 algorithm.

25 [Slide.]

1           This slide shows the basic principles of patient  
2 evaluation that include history, physical examination,  
3 laboratory testing, and ultimately, liver biopsy. I would  
4 like to stress that clinicians do not rely on a single test  
5 to diagnose hepatitis C infection or the presence of liver  
6 disease. A full evaluation encompasses multiple modalities.

7           The history is very important. We try to elicit  
8 from a patient a history of exposure risk, which I will  
9 discuss in detail later, and believe it or not, we do see  
10 other diseases besides hepatitis C, so that the  
11 symptomatology, the family history, social history, such as  
12 alcohol use, concurrent medications, et cetera, are also  
13 very important questions.

14           The symptoms are not helpful at all in the  
15 diagnosis of hepatitis C either in the chronic state or the  
16 acute state since, in fact, most patients are asymptomatic,  
17 and if they do have symptoms, they are usually very  
18 nonspecific, such as just fatigue.

19           On exam, we look for signs of chronic liver  
20 disease, such as palmar erythema, spider angioma,  
21 hepatosplenomegaly, et cetera. The laboratory studies that  
22 are helpful in establishing the presence of liver disease  
23 include ALT and AST, which are markers of hepatocellular  
24 injury, and we also use serum albumin and the prothrombin  
25 time as a measure of liver function.

1                   Finally, the only way to stage liver disease and  
2 confirm the presence of chronic hepatitis is by liver  
3 biopsy, which remains the gold standard by which we judge  
4 the presence and severity of liver disease.

5                   [Slide.]

6                   I mentioned that history is important in  
7 establishing a diagnosis of hepatitis C, and this slide  
8 shows the generally accepted risk factors for acquisition of  
9 hepatitis C infection.

10                   In the United States, past use of intravenous  
11 drugs is the most common exposure risk, and other risk  
12 factors include transfusions, occupational risk, sexual  
13 transmission in about 10 percent of patients, and about 10  
14 percent of patients don't have any identifiable risk factors  
15 based upon intense epidemiologic studies performed by  
16 numerous investigators in the CDC.

17                   [Slide.]

18                   The CDC has established guidelines concerning  
19 which patients are considered to be at high risk for  
20 hepatitis C infection and have made recommendations for  
21 appropriate screening, so that any patient that we see who  
22 has an abnormal ALT, regardless of how mildly abnormal that  
23 is, or any patients who has ever used intravenous drugs,  
24 even on one occasion, should be screened for hepatitis C.

25                   In addition, patients who have had blood

1 transfusions prior to 1992, hemophilic patient, hemodialysis  
2 patients, and the others shown on this slide all should be  
3 considered for screening for hepatitis C infection.

4 So, a physician caring for any patient with  
5 evidence of liver disease and any of these risk factors  
6 should have a strong clinical suspicion that the patient has  
7 chronic hepatitis C, and it is this clinical suspicion that  
8 really drives our next step, which is screening for  
9 hepatitis C.

10 [Slide.]

11 Well, we are all well aware that the best  
12 screening test for hepatitis C remains anti-HCV antibody.  
13 The limitation with this assay is that it does not  
14 distinguish between the acute, chronic, or resolved  
15 infections, and false positives may occur particularly when  
16 we are dealing with low risk populations, such as volunteer  
17 blood donors.

18 The CDC recommends supplemental testing for  
19 patients who do have a positive anti-HCV antibody, and RIBA  
20 is one such available test. RIBA is a very important test  
21 to demonstrate true exposure in the low risk patient. For  
22 example, a volunteer blood donor who otherwise has  
23 absolutely no risk factors for having acquired hepatitis C  
24 and comes up positive on an anti-HCV screening.

25 Once again, this test alone cannot discriminate

1 between acute, chronic, or resolved infections, and  
2 additional testing for HCV-RNA is necessary, and in reality,  
3 HCV-RNA testing is ordered much more commonly for our  
4 patients in whom we are establishing a diagnosis of chronic  
5 hepatitis C.

6 [Slide.]

7 This slide shows the CDC recommended algorithm for  
8 the diagnosis of hepatitis C with the screening for anti-HCV  
9 antibody, and I agree completely with this algorithm except  
10 there is one practical point that I would like to point out.

11 This algorithm gives the impression that RIBA and  
12 PCR testing are both equally used as supplemental assays for  
13 patients who test positive for anti-HCV antibody, and as I  
14 mentioned before, in clinical practice, RIBA testing should  
15 be reserved for the low risk population whose anti-HCV has a  
16 probability of being a false positive test.

17 If we did RIBA preferentially, regardless of risk  
18 stratification, then, we would still be in the position of  
19 needing another test to determine viremia in order to answer  
20 the question whether the patient is still infected.

21 So, essentially, all patients who we see who are  
22 anti-HCV positive get follow-up testing to determine viremia  
23 instead of RIBA except in that low risk population that I  
24 mentioned, and as a real world example, at the University of  
25 North Carolina liver program, we probably order about the

1 ratio of PCR test to RIBA test that we order is probably  
2 about 6 to 1. Again, we are only using those for the  
3 selected low risk patient.

4 [Slide.]

5 So, then, how do we diagnose hepatitis C infection  
6 and liver disease in the patients that we are seeing in our  
7 clinics? Well, we have a history of exposure in most of the  
8 patients, as I mentioned before. We screen for anti-HCV in  
9 all of those, and in the selected few, we use RIBA testing.

10 We have evidence of hepatocellular injury in most  
11 of the patients who have an increased serum ALT, for  
12 example, and we perform liver biopsies to stage the degree  
13 of chronic hepatitis, determine the grade of necro  
14 inflammatory activity, and also the stage of fibrosis.

15 Of course, we use HCV RNA to determine if a  
16 patient is viremic. Again, my patients don't care if they  
17 had hepatitis C in the past. What they really want to know  
18 is whether they have hepatitis C today, and the only way we  
19 can tell that for sure is by doing tests for HCV RNA.

20 So, all together, this makes for very compelling  
21 evidence for the diagnosis of chronic hepatitis C.

22 [Slide.]

23 Now, there are other clinical scenarios where HCV  
24 RNA testing remains important for the complete diagnosis and  
25 characterization of the patient's disease. Approximately 20

1 percent of the patients we see may have normal ALT despite  
2 being positive for anti-HCV and RIBA.

3 So, the question is does this patient have  
4 resolved hepatitis C infection or is he chronically infected  
5 with hepatitis C and remain at risk for progressive liver  
6 disease.

7 HCV RNA testing is the best way to distinguish  
8 these scenarios, and I would like to add here that a single  
9 negative HCV RNA determination does not guarantee that the  
10 patient has permanently cleared their viremia, so that we  
11 would recommend follow-up testing in the future.

12 Another scenario is the patient who is anti-HCV  
13 positive and RIBA indeterminate. Again, the question is, is  
14 this a false positive anti-HCV or does the patient have  
15 chronic hepatitis C infection.

16 Although rarely this may happen in the acute  
17 setting during seroconversion, we also see it commonly in  
18 the asymptomatic patient who is routinely screened for  
19 hepatitis C, maybe on the order of about 10 percent.

20 Indeterminate RIBA may be more common in  
21 immunosuppressed states, such as after transplantation or  
22 following chemotherapy. Here again HCV RNA is really the  
23 only way to distinguish these two clinical scenarios.

24 [Slide.]

25 Well, several investigators have looked at the

1 issue of interpretation of indeterminate RIBA, and this is  
2 an example of interpretation of various RIBA tests. You can  
3 see here on the left side a 4 antigen-positive RIBA, which  
4 is considered a true exposure to hepatitis C.

5 If only one band is positive, as shown in the to  
6 right-sided panels, RIBA is considered indeterminate and  
7 additional history and testing for viremia is required. In  
8 a low risk patient, indeterminate RIBA is likely to be  
9 indicative of a false positive anti-HCV.

10 In contrast, among high risk patients, an  
11 indeterminate RIBA may be associated with the presence of  
12 viremia by PCR in anywhere from 20 to 50 percent of  
13 patients, and this is particularly true when antigens from  
14 the structural region are positive in that indeterminate  
15 RIBA.

16 [Slide.]

17 Well, the next area that I would just like to  
18 touch upon deals with hepatitis C genotypes. Now, based  
19 upon sequencing various regions of the hepatitis C genome,  
20 there are six major genotypes and multiple subtypes that  
21 have been identified.

22 The recognition of hepatitis C genotypes has  
23 really provided a fertile area for research into their role  
24 in disease outcomes. These genotypes have definite  
25 geographic distribution with genotype 1a and 1b accounting

1 for roughly 70 percent of the U.S. population who are  
2 infected with hepatitis C, whereas, in Japan and Europe, 1b  
3 is the predominant genotype.

4           The most important clinical implication of  
5 genotypes is the recognition that certain genotypes are  
6 definitely less responsive to therapy. Patients with  
7 genotype 1 are much less likely to have a sustained response  
8 to any of the available therapies that have been looked at  
9 compared to genotypes 2 or 3.

10           So, for example, for the combination therapy, for  
11 patients with genotype 1, only about 25 percent of those  
12 responded with a sustained response compared to about 60  
13 percent of patients who had a genotype 2 or 3.

14           Now, numerous studies have also looked at the  
15 association between genotype and disease severity, but there  
16 is really no convincing evidence that genotype plays a major  
17 role in the outcome of hepatitis C infection, and if you  
18 look at genotype distributions at transplant centers, they  
19 are very similar. Where you see the most endstage liver  
20 disease, they are very similar to what we would expect in  
21 the general population.

22           [Slide.]

23           The next question then is whether genotypic  
24 differences will alter our ability to diagnose hepatitis C  
25 infection, and it appears that they would not. There was

1 some concern raised early on during the early days of  
2 quantitative testing where there were definite instances  
3 where genotypes 2 and 3 were underestimated by anywhere from  
4 0.3 to half a log, and sometimes to as much as a logfold  
5 difference.

6           However, later versions of various assays have  
7 demonstrated that quantitation appears to be consistent  
8 regardless of the genotype.

9           [Slide.]

10           This is a recent study from France that compared  
11 HCV quantitation using three assays that had been used in  
12 clinical practice - namely, the superquant, which is the NGI  
13 assay, the bDNA assay from Bayer, and the Roche Amplicor  
14 Version 2 monitor assay.

15           As you can see, there is very good agreement  
16 across the genotypes using the newer versions of these  
17 assays, suggesting that the genotypic differences are no  
18 longer of significance.

19           [Slide.]

20           The next slide of evidence suggesting that  
21 genotypes will not affect our ability to diagnose hepatitis  
22 C viremia comes from a review of baseline HCV RNA levels  
23 before therapy from several large multi-center treatment  
24 trials that have been recently published.

25           There are a few selective trials that you see here

1 that have used various commercially available assays, and as  
2 you can see here, in the two largest treatment trials that  
3 were from combination therapy for hepatitis C, most patients  
4 had HCV RNA levels over 2 million copies.

5 So, even if we don't accept that the newer assays  
6 do control for hepatitis C genotypes, what you see here is  
7 that some of the studies here have shown where the range is  
8 available, the lower ranges of untreated patients is  
9 somewhere around 300,000 copies, at least 3 logs higher than  
10 the current assay that we are talking about today.

11 So, it is very unlikely that genotypic differences  
12 in an untreated patient would account for false negative  
13 results.

14 [Slide.]

15 Well, in the last few minutes, I would just like  
16 to provide some additional background concerning the studies  
17 that will be described in detail by Dr. Murray. I have  
18 participated in the design of the study and also served as  
19 the co-PI at my site where we were recruiting patients.

20 While we were designing the study, we were really  
21 in an odd position, almost a catch-22 of demonstrating that  
22 a PCR assay to detect viremia could diagnose hepatitis C  
23 without using the most common assay that we usually use to  
24 detect viremia, namely, the hepatitis C RNA assays.

25 Therefore, we used the combination of the

1 available tests, namely, the anti-HCV, the RIBA, the ALT,  
2 and the liver biopsy in order to make a diagnosis of  
3 hepatitis C infection, and we also used any additional  
4 medical records that were available at the time of the  
5 patient's enrollment to make a presumptive diagnosis of  
6 hepatitis C infection, and it is against all of these  
7 clinical parameters that the Amplicor assays were judged.

8 [Slide.]

9 We ended up with three main groups of patients  
10 shown here, that reflected the practices of the  
11 hepatologists and the institutions that were participating  
12 in this trial.

13 At my site, for example, hepatitis C infection is  
14 by far the most common reason for referral, and I spend most  
15 of my days dealing with hepatitis C-related issues.  
16 Patients were drawn from our general liver clinics, as well  
17 as clinics specific for viral hepatitis research, and I  
18 would like to stress that these are patients with chronic  
19 hepatitis C, not acute hepatitis C.

20 These were the kinds of patients who are  
21 frequently diagnosed through routine blood screening or  
22 through insurance physicals, and were found to be exposed to  
23 hepatitis C virus, and although they were only evaluated at  
24 one point in time for this study, their histories, clinical  
25 laboratory data, and their biopsies all were consistent with

1 chronic hepatitis C. In fact, I can really only recount a  
2 handful of patients that we see in our clinical practice  
3 over the last 10 year who really had the criteria for  
4 consideration as an acute hepatitis C patient, and I think  
5 that is the general experience of most of the hepatologists  
6 that we will talk to.

7           So, once again the prevalence of this disease in  
8 our patient populations and the integral role that HCV RNA  
9 testing plays in the diagnosis of our patients underscores  
10 the importance of a standardized assay.

11           [Slide.]

12           So, in summary, then, the diagnosis of chronic  
13 hepatitis C involves multiple modalities including the  
14 history, physical examination, laboratory tests, and the  
15 liver biopsy. Supplemental assays, such as RIBA, are most  
16 useful in the low risk patient populations that we see.

17           HCV RNA testing is required to determine the  
18 presence of viremia and in clinical practice, is really the  
19 second line test of choice for patients with suspected  
20 chronic hepatitis C infection.

21           I also believe that genotypic differences are  
22 unlikely to affect the diagnosis of hepatitis C given the  
23 levels of HCV RNA that we see in untreated patient  
24 populations.

25           Thanks very much.

1                                   **Review of Clinical Studies**

2                                   **Alison B. Murray, M.D.**

3                                   [Slide.]

4                                   DR. MURRAY: I am going to be reviewing the  
5 results of the clinical study that evaluated the performance  
6 of the two tests - the Amplicor and the Cobas Amplicor HCT  
7 test.

8                                   Should I hang on for the test of panel members to  
9 return? We have lost our quorum.

10                                  DR. WILSON: No, just go ahead.

11                                  DR. MURRAY: So, I will go ahead. Okay.

12                                  [Slide.]

13                                  The objectives of the clinical study were to  
14 assess the performance of the two tests that we are  
15 reviewing today in patients being investigated for hepatitis  
16 C virus infection. We evaluated the performance of these  
17 two assays across specimen matrices, that is, across serum,  
18 ACD and EDTA plasma, across patient groups and across the  
19 sites.

20                                  We have undertaken two sets of analyses looking at  
21 the performance of the assays. The first compared anti-HCV  
22 serology to the Cobas results, and the second, we compared  
23 the Cobas and Amplicor results to the anti-HCV serology, ALT  
24 levels, and liver histology data.

25                                  It is obviously important to mention that the

1 second analysis could only be conducted in a subset of  
2 patients for whom biopsies were available as we weren't able  
3 to evaluate biopsies in the proportion of patients who did  
4 not have biopsies. So, that is a subset analysis.

5 [Slide.]

6 These are the sites that were enrolled in our  
7 study, and I would like to take this opportunity to thank  
8 all the staff at the sites for all the hard work they have  
9 put into this study.

10 As you will note, we had a site in Atlanta. Dr.  
11 Fried was actually working in Atlanta at the time that the  
12 study was conducted. He doesn't work in two places. He has  
13 moved.

14 A site in Miami, Eugene Schiff site in Miami;  
15 Mitch Schiffman at University College of Virginia, and  
16 University of Washington, Dave Gretch and Steve Polyak.

17 The important think to note here is that we do  
18 have a reasonable geographical spread across the United  
19 States. We have got sites covering a wide distribution of  
20 geography within the U.S.

21 [Slide.]

22 Now, the design of the clinical study. The tests  
23 being evaluated were each evaluated against a number of  
24 reference methods. We used the base methods that we had  
25 available to us. Anti-HCV EIA was performed using a third-

1 generation EIA test. Not all sites used the same EIA test,  
2 but they were all third-generation tests.

3           Recombinant immunoblot assay was used with a  
4 second-generation test. Alanine transaminase was performed  
5 at the local study sites, and liver histology reports were  
6 collected where these were available.

7           [Slide.]

8           Now, we have looked at a number of patient groups  
9 in the study. The largest of the groups is the general  
10 patient group, as I will be referring to them from now on.  
11 These are essentially patients who are being referred in for  
12 investigation of hepatitis C, and this group of patients had  
13 not previously been treated for hepatitis C, and were not  
14 post-transplant.

15           Also included in this group of patients who were  
16 attending clinics for investigation of other liver diseases,  
17 so they weren't all being investigated specifically for  
18 hepatitis C.

19           The second group of patients, and the second in  
20 terms of size, was a previously treated group of patients.  
21 These had to have completed anti-HCV therapy at least six  
22 months prior to enrolling in the study.

23           The third and smallest group was the post-  
24 transplant group who were being investigated for clinically  
25 suspected post-transplant hepatitis C.

1 [Slide.]

2 Now, this is an important slide really just  
3 outlining the number of patients that we had in the study  
4 overall and the numbers in each group, and this is actually  
5 an issue that I am sure John Ticehurst, when he does his  
6 presentation, will refer to.

7 We actually included over 870 patients in the  
8 analyses for the study. The same set of patient were  
9 evaluated for both assays, so the numbers that you see for  
10 Amplicor and Cobas Amplicor are essentially the same people.

11 The populations across the two assays differed by  
12 only four patients across the two tests.

13 The majority of patients by far were in the  
14 general patient group where just under 80 percent of  
15 patients were in the general category.

16 The next size group was the previously treated  
17 patients, which accounted for approximately 20 percent of  
18 the patient population, and then we had a very small group,  
19 of 4 percent, of post-transplant patients.

20 Because of the sizes of these groups, for the  
21 discussion where I am looking at the performance of the  
22 assays across matrices, I will be concentrating on the  
23 general group of patients. You do have data on all groups  
24 of patients looking at all matrices in your packages, but  
25 because of time, I can't go into all the analyses. I will

1 focus on the general group.

2           Then, looking at the proportion of patients who  
3 had biopsies available, we had biopsy reports available in  
4 approximately 60 percent of the patients.

5           Looking at the patient and sample disposition, I  
6 think it is important for us to distinguish patients from  
7 samples in this study, because otherwise you have an  
8 enormous trouble trying to add up all the numbers.

9           We screened, in fact, 948 patients for the study.  
10 We excluded 70 patients who did not have all the lab tests  
11 available, missing CRF data, or patients who had been  
12 treated with the previous six months, with approximately 878  
13 and 876 patients going into the Cobas and the Amplicor parts  
14 of the study respectively.

15           The first number in each of these block is for the  
16 Cobas assay, and the second number is for the Amplicor  
17 assay.

18           Now, looking at the number of samples that were  
19 assessed, the majority of the samples were indeed serum  
20 samples. There were over 900 samples available for testing  
21 on Amplicor and just under 900 on Cobas. We had 27 ACD  
22 samples and 161 EDTA samples available.

23           Eleven samples were excluded on the Cobas assay  
24 and 4 on the Amplicor assay for samples that were either  
25 potentially inhibitory or came within the grey zone and

1 there was insufficient sample available for retesting.

2 So, in total, across the study in the two assays,  
3 we have evaluated over 2,000 samples in the study.

4 Now, the results that I am going to show you of  
5 the PCR data have all been interpreted after the addition of  
6 a grey zone to the interpretation of the results. As Karen  
7 mentioned in her talk, following the non-clinical evaluation  
8 of the assay, the cutoff was set at 0.15 for the Cobas test.

9 Retrospectively, during the evaluation of the  
10 clinical studies, we did evaluate a grey zone for samples  
11 that fell between 0.15 and 1 on the Cobas OD reading were  
12 retested in duplicate in order to assess whether the results  
13 changed after retesting if they were within this grey zone.

14 What we have here is a frequency count of the  
15 number of samples within each of these optical density  
16 categories for the samples that were tested on the Cobas  
17 assay in the study, and as you will see, the majority of the  
18 positive samples had an OD reading of greater than 3, which  
19 is the cutoff for the instrument reading on this instrument,  
20 and the majority of negative samples actually had an OD  
21 reading of below 1.

22 It is probably quite difficult for you to see it,  
23 but there are indeed three cases here within the grey zone  
24 that underwent repeat testing, and on retesting the sample  
25 down here, resolved to a very clear negative within an OD of

1 less than 1, on retesting, these two samples resolved to  
2 clear positive with OD's of greater than 2 when tested in  
3 duplicate on retesting.

4 We have had some discussion with the FDA as to  
5 whether the grey zone has, in fact, been set at the right  
6 level, and so we have, in fact, undertaken retesting of all  
7 samples in the area between OD's of 1 and 2, as well, and,  
8 in fact, on retesting, all of the samples that were between  
9 1 and 2 on the original testing, all came out as greater  
10 than 3 on retesting, very clearly positive.

11 So, in fact, although these samples were retested,  
12 the results did not change on retesting. So, the results  
13 that I am going to show you have included the grey zone  
14 data, but would not change if the grey zone was, in fact,  
15 moved on subsequent discussions with the agency.

16 [Slide.]

17 This is where the Cobas Amplicor and Amplicor  
18 tests were compared to anti-HCV serology. This is how we  
19 interpreted the serology. EIA tests were performed on all  
20 patients. If EIA was repeatedly reactive, a RIBA test was  
21 performed.

22 As is normal practice, RIBA testing was not  
23 conducted on EIA-negative samples. So, what we ended up  
24 with was actually a number of categories of serology to  
25 evaluate. We had EIA negative and EIA positive samples, and

1 within the EIA-positive category, we had RIBA positive, RIBA  
2 indeterminate, and RIBA-negative cases.

3 In fact, out of the 2,000 samples tested, only one  
4 of the samples was indeed a RIBA-negative sample. That one  
5 was PCR negative, and clearly fitted in very nicely with the  
6 way we interpret serology today.

7 This is the set of data where we are comparing the  
8 performance of the Cobas Amplicor test, and I am going to  
9 concentrate on the Cobas Amplicor test throughout my  
10 presentation. I do have one slide showing you the  
11 performance of the Amplicor against the Cobas Amplicor, but  
12 I will focus on the Cobas Amplicor because the results were  
13 essentially the same for both assays.

14 Now, looking at this graph here, this the general  
15 patient group, looking at the results across sample  
16 matrices. These are results for the serology positive  
17 patients.

18 So, what we have here are the serology positive  
19 patients and the proportion of those in whom HCV RNA was  
20 detected on the Cobas assay for serum, ACD plasma, and EDTA  
21 plasma. As you can see, we have a very high degree of  
22 agreement between the Cobas Amplicor and the serology data  
23 with between 90 and 95 percent of patients that were  
24 serology positive being positive on the Cobas Amplicor test.

25 [Slide.]

1           Looking at the serology negative samples, these  
2 were all serum and plasma that were serology negative, and,  
3 in fact, PCR was negative in between 94 and 100 percent of  
4 these cases, so again, very good agreement between serology  
5 and the PCR data.

6           Now, although those numbers are actually very  
7 impressive, we actually think that the ones that don't match  
8 are probably the more interesting of the cases. So, I am  
9 going to go through the discrepant samples in a little bit  
10 more detail for you now.

11           So, looking at the serum samples from the general  
12 patient group where there was a serology positive result  
13 confirmed by RIBA, and the PCR was negative, we used some of  
14 the other clinical information available to us to try and  
15 evaluate which was the real result.

16           In fact, in 76 percent of the cases that were PCR  
17 negative, the ALT result was normal, indicating that there  
18 was no large degree of liver injury occurring in these  
19 patients. There was a small number of patients who had an  
20 elevated ALT. One of them had a history of upper GI  
21 bleeding and no history of hepatitis.

22           One of them had cirrhosis, but the biopsy was  
23 inadequate and there was no pathological diagnosis possible,  
24 and certainly no mention of hepatitis on the biopsy.

25           There were two cases that indeed had hepatitis on

1 biopsy, but the hepatitis in these two cases was so mild  
2 that the HAI score was zero for the necrosis component for  
3 both of those with both having low scores for inflammation  
4 too.

5           Then, there was one patient who had a history of  
6 chronic HCV, but no biopsy available. We did attempt to  
7 perform some genotyping in these patients, but obviously,  
8 genotyping was not possible because you need hepatitis C RNA  
9 to undertake genotyping, and so we weren't able to genotype  
10 any of these samples.

11           [Slide.]

12           Looking at the serum, again the group of patients,  
13 but these are the EIA negatives, serology negative, PCR  
14 positive patients. We only had five patients in this  
15 category. In 2 out of the 5, the ALT was elevated in  
16 association with this positive HCV RNA test.

17           These are the clinical diagnoses in these  
18 patients. We were indeed able to genotype two of the  
19 patients and one in each of these categories, and two of  
20 them were type 1a, and one was type 1b.

21           [Slide.]

22           Looking at the plasma samples where there were  
23 discrepant results in the general population, we had only 7  
24 cases where there was positive serology and a negative PCR  
25 result. The ALT was normal in all 7 of the PCR negative

1 cases. None of them had a biopsy, which was a bit  
2 frustrating when I was trying to evaluate the cases, but it  
3 is really understandable in the setting of a normal ALT, it  
4 is pretty difficult to justify doing a biopsy.

5 Most of them had a clinical diagnosis of HCV  
6 probably based on the serology results, and again because  
7 HCV RNA was not available, we weren't able to undertake  
8 genotyping in this group of patients.

9 Then, we had my favorite patient. We had one  
10 patient that was EIA negative and HCV RNA positive. This  
11 patient had a normal ALT, a clinical diagnosis of chronic  
12 hepatitis C. I am not sure where that diagnosis had come  
13 from.

14 On the biopsy, there was a clear diagnosis of  
15 primary biliary cirrhosis, and the patient actually had no  
16 HCV RNA detectable when the genotyping was performed, so I  
17 think this is a very clear false positive case.

18 [Slide.]

19 So, summarizing the discrepant cases in the  
20 general patient group, essentially, what we have is a very  
21 small proportion of discrepant samples with the data being  
22 backed up in some way by some of the other tests. The data  
23 for the PCR was only 4 percent of patients actually having a  
24 discrepant result, so a very low proportion of discrepant  
25 results with supportive clinical information, in fact, in

1 quite a lot of the cases.

2 [Slide.]

3 Now, this is actually probably the most  
4 interesting group in the study, and these are the patients  
5 who were in the general group, who had RIBA indeterminate  
6 results on serology.

7 The reason why this group is actually so important  
8 to us is that this group is a clinically difficult group of  
9 patients to evaluate because the indeterminate RIBA results  
10 essentially leaves a clinician high and dry in terms of  
11 knowing what the diagnosis is in this case. An  
12 indeterminate result actually tells you nothing about the  
13 patient's status, and so the PCR data in this case is  
14 actually quite useful to look at in detail.

15 We had approximately half of the patients with PCR  
16 negative, half were PCR positive, and, in fact, this is  
17 really very much in line with what Mike Fried is using in  
18 his clinical practice, whereas, he mentioned earlier  
19 approximately 50 percent of patients who have liver disease,  
20 who are RIBA indeterminate, in fact, are PCR positive. So,  
21 this is not inconsistent with what is published in the  
22 literature.

23 In the PCR positive cases, we had an elevated ALT,  
24 and the majority of them, in fact, 80 percent of them had an  
25 elevated ALT in association with the detection of HCV RNA.

1           Six biopsies were available in this group of  
2 patients, and, in fact, there was mild or moderate chronic  
3 hepatitis on all six biopsies, and there was fibrosis or  
4 cirrhosis in three of the six biopsies. This is actually  
5 quite an important finding here because one of the debates  
6 that we always have about indeterminate RIBAs, you know, is  
7 this a seroconversion event that we are seeing, and if we  
8 follow the patient up long enough, will the RIBA become  
9 positive.

10           Well, clearly, at least three of these patients  
11 have had their hepatitis C for long enough to establish a  
12 cirrhosis. In fact, one patient had quite clearly  
13 established cirrhosis, so the RIBA indeterminate case is  
14 occurring in a setting of what is clearly a chronic  
15 hepatitis.

16           Thirteen out of 18 cases we were able to get HCV  
17 RNA out for genotyping, and the majority of these were type  
18 1a or 1b, as we would expect in the U.S. In fact, the  
19 patients where we weren't able to genotype them, three of  
20 them actually were the biopsy cases above, so where we  
21 weren't able to genotype, we actually had biopsy evidence  
22 suggesting that there was hepatitis C in these patients.

23           Looking at the HCV RNA negative cases, there were  
24 12 of these, and again the ALT matched our PCR data, and the  
25 majority of cases was 80 percent-plus having a normal ALT.

1 Again, in patients with normal ALTs, we had a dearth of  
2 biopsies, so the biopsies didn't help us out here.

3 When we attempted genotyping in this population,  
4 genotyping was not possible because HCV RNA could not be  
5 obtained from any of these specimens.

6 So, again, looking at the very small proportion of  
7 RIBA indeterminate cases, and the PCR results, the clinical  
8 data actually was on the side of the PCR resulting in the  
9 vast majority of cases.

10 [Slide.]

11 Now, I am going on to show you some of the data in  
12 serum across the different patient groups. What we have  
13 here are serology positive cases once again in the general,  
14 the treated, and the transplant group.

15 As you can see, again, we have very good agreement  
16 between the PCR data and serology data with well over 90  
17 percent of patients being both PCR and serology positive in  
18 all patient categories.

19 [Slide.]

20 The next slide shows us the serology negative  
21 cases, and you will notice a big black hole in the middle of  
22 the slide here where we, in fact had no treated EIA negative  
23 cases. So, the patients who did not have hepatitis C on  
24 serology had not been treated, which indicated to us that we  
25 had chosen the right sites to perform the study, because

1 they know how to diagnose hepatitis C before treating their  
2 patients.

3           Again, what we saw in the general group and the  
4 transplanted group is very good agreement between the  
5 serology and the PCR, with 98 and 100 percent of samples  
6 respectively being PCR negative in the serology negative  
7 category.

8           [Slide.]

9           Now, if you put all the data together, looking  
10 across patient groups and across sample matrices, in serum,  
11 in all three patient groups, very good agreement between the  
12 results between serology and PCR.

13           [Slide.]

14           In plasma, again very good agreement when you look  
15 at the proportions. Those of you who know anything about  
16 statistics will notice the very, very large confidence  
17 interval in the transplant patients and in this group of ACD  
18 plasma patients.

19           Essentially, what we have here is just one patient  
20 driving those confidence intervals. We were very fortunate  
21 in that that one patient had serology and PCR in agreement,  
22 but we really do need to be very cautious in interpreting  
23 the data for the plasma samples in the transplant category,  
24 because of the very small numbers of patients in that group.

25           [Slide.]

1           This is the data looking at the performance across  
2 sites. This is to test whether they were all doing their  
3 job as we had hoped they would do, and, in fact, they all  
4 performed very similar to one another. There was consistent  
5 results in the--this is the serology positive group looking  
6 at serum data--across sites, very consistent results.

7           In the serology negative cases, we had almost a  
8 perfect score.

9           [Slide.]

10           So, having spent quite a lot of time showing you  
11 some of the information across matrices and across patient  
12 groups with the Cobas AmpliCor data, I thought it was worth  
13 just taking a very brief look at the AmpliCor data to show  
14 you how these tests perform relative to one another.

15           In fact, this is the serology positive group of  
16 patients, and you can see that the Cobas AmpliCor and  
17 AmpliCor results give us very, very similar results. The  
18 numbers are almost identical across these two tests.

19           In the serology negative cases, the results are  
20 very consistent for serum and EDTA plasma with very similar  
21 proportions being PCR negative in both of the tests and very  
22 similar confidence intervals except in one case here, the  
23 ACD plasma case where you will notice that the proportion  
24 who were PCR negative dropped way down to 75 percent, and  
25 the confidence interval matched it.

1           What we had actually happen here is that we only  
2 had four samples for ACD plasma samples that were EIA  
3 negative, and in the Cobas Amplicor assay, all four of the  
4 samples were negative, and in the Amplicor assay we had a  
5 false positive, so we had three out of four being negative.

6           So, I think the difference is due to one sample  
7 difference in the two datasets.

8           [Slide.]

9           Now, this is the second analysis which I think is  
10 clinically the more interesting analysis even though it is  
11 under the subset of patients, and these are on the patients  
12 for whom we had histology data available.

13           Here we have compared the performance of the tests  
14 to not only serology, but we have also taken the ALT and the  
15 liver histology data into account.

16           [Slide.]

17           This is how we have interpreted the data, and ALT  
18 was evaluated as either normal or elevated based on the  
19 local site's normal range.

20           The histology data was evaluated by looking at  
21 histology reports, and the histology reports were separated  
22 interview two categories - those with hepatitis and those  
23 not having hepatitis, and it is important to stress that the  
24 biopsy reports where there was no hepatitis available, there  
25 was, in fact, pathology on those reports, but it was just

1 not hepatitis.

2           In order to qualify as hepatitis cases, the  
3 reports had to describe histological features of hepatitis.  
4 The diagnosis hepatitis, hepatitis had to be mentioned  
5 either in the diagnosis or it had to be described in the  
6 body of the report.

7           But what is also important to note about the  
8 biopsies is that liver histology is not specific for  
9 hepatitis C. You cannot look at a liver biopsy and say that  
10 this patient definitively has hepatitis C. So, included in  
11 the category of hepatitis, we, in fact, have cases that are  
12 either due to viral hepatitis--some of them were, in fact,  
13 hepatitis B--or auto-immune hepatitis, and we had some cases  
14 of non-alcoholic steatohepatitis, as well.

15           [Slide.]

16           We have actually divided the patient data now into  
17 two separate categories. The format of this graph is  
18 slightly different to the format that I have been using up  
19 until now, so I think it is probably worth me just pointing  
20 out the differences.

21           What we have here in bright yellow are the  
22 serology positive cases, and in blue are the serology  
23 negative cases. Again, on this axis are the proportion of  
24 cases in which the serology data and the PCR data were in  
25 agreement.

1           Looking at all of the hepatitis patients, we have  
2 excellent agreement between serology and PCR. It was almost  
3 100 percent agree in both normal ALT and the elevated ALT  
4 category.

5           What we have here is essentially 99 percent of the  
6 serology positive cases being PCR positive, and all of the  
7 serology negative cases being PCR negative. You will notice  
8 from the numbers at the bottom here that, in fact, the  
9 majority of these cases were indeed serology positive.

10           Now, the more interesting category are the ones  
11 who do not have hepatitis, and this is on the slide, and  
12 what you will notice here, that in the patients who had  
13 liver disease that was not hepatitis, we had no cases that  
14 were PCR positive and serology positive.

15           We, in fact, had one serology positive case in  
16 each of these groups, but neither of them had hepatitis C  
17 RNA detectable. All of the cases that were serology  
18 negative were PCR negative, as well, in the elevated ALT  
19 category, and in the normal ALT category we had our one  
20 false positive patient that I mentioned earlier on accounted  
21 for us not reaching 100 percent in that category.

22           [Slide.]

23           Just jumping to the histology data in a little bit  
24 more detail, overall, looking at all the cases with  
25 hepatitis, so that is combining the ALT normals and ALT

1 elevated together, 99 percent of patients who had hepatitis  
2 on histology, and who were serology positive, were also HCV  
3 RNA positive.

4           Only 1 percent of cases, in fact, it is just two  
5 samples were HCV RNA negative, and those patients you have  
6 seen before in this presentation, as well. Those were the  
7 two patients who had very, very mild hepatitis on biopsy  
8 with necrosis scores of zero in both cases.

9           The EIA negative results, we had 100 percent match  
10 with the serology, and none of these patients, in fact, had  
11 histological features that were typical of hepatitis C on  
12 biopsy, and what we saw on the histology in the serology PCR  
13 negative cases was hepatitis that was auto-immune, in fact,  
14 in the majority of cases we had a few cases of hepatitis B,  
15 some non-alcoholic steatohepatitis.

16           We did have 9 cases that had non-specific  
17 inflammation, but it was clear that the pathologists were  
18 very uncomfortable calling this viral hepatitis because the  
19 inflammatory response was atypical in some way in all 9 of  
20 those cases.

21           There was one very interesting case that had  
22 inflammation on the biopsy with some features of hepatitis,  
23 but they were non-caseating granuloma, suggesting that this  
24 might, in fact, be sarcoidosis, but they were all PCR  
25 negative in this category.

1 [Slide.]

2 Looking at the other set of patients, the ones who  
3 did not have hepatitis on histology, if you add all the ALT  
4 normals and the ALT elevated together, 98 percent of them  
5 were, in fact, serology negative and PCR negative.

6 We had only one case that was PCR positive and  
7 serology negative in this category, and that was our false  
8 positive that I mentioned earlier on, and that the liver  
9 disease in these cases was due to other causes, most of them  
10 biliary tract pathology.

11 [Slide.]

12 In conclusion, we feel that we have demonstrated  
13 that the assays have performed, both of them, very  
14 consistently across sample matrices, across patient groups,  
15 and across sites.

16 In the majority of cases, in fact, the vast  
17 majority of cases, the Cobas Amplicor and the Amplicor  
18 results were supported by the serology data, and in the  
19 small proportion of cases where the serology data did not  
20 support the PCR results, there was other clinical evidence  
21 that points to the fact that the PCR data may, in fact, be  
22 either more viable results.

23 [Slide.]

24 The subsets of patients who had histology data  
25 were particularly interesting where we had very good

1 agreement between serology and PCR, and data that was  
2 appropriate for the clinical state of disease as defined in  
3 the biopsies in well over 99 percent of cases.

4 So, we feel that this data overall supports the  
5 clinical utility of these assays in patients with liver  
6 disease and antibodies to hepatitis C.

7 [Slide.]

8 In the package overall, we feel that we have  
9 demonstrated that both tests are very specific and sensitive  
10 down to a level of 50 International Units per mL against the  
11 WHO standard.

12 We have comparable detection of genotypes that are  
13 listed in the "Consensus Classification of HCV." We have  
14 looked at the majority of genotypes in that classification.

15 Comparable detection of hepatitis C virus in  
16 serum, ACD and EDTA plasma in the non-clinical and in the  
17 clinical studies.

18 Very good agreement between our results in  
19 serology, and where the results weren't in agreement with  
20 serology, the data werē supported by the other clinical  
21 information that we had available to us.

22 So, we feel that this data supports the proposed  
23 indication that was mentioned at the beginning of this talk.

24 Thank you.

25 DR. WILSON: Thank you.

1           At this point I would like to invite the panel  
2 members to ask questions of the speakers. Dr. Specter.

3           DR. SPECTER: I guess my question would be for  
4 either Dr. Gutekunst or Murray, and that is, in the studies  
5 that were done, especially those in which you looked at  
6 specificity and exclusivity of other organisms, I would just  
7 like to get some insight into the rationale that was used in  
8 the specimen selection, specifically relating to whether  
9 you, in fact, did check gene banks for any cross-reactive  
10 organisms, and then why you chose not to test any other  
11 Flaviviruses.

12           DR. GUTEKUNST: That is an excellent question. We  
13 did do gene bank searches. Unfortunately, we didn't have  
14 those completed in time to include them in the submission,  
15 but we did those. We did not see any cross-reactivity.

16           The difficulty that we had in obtaining some  
17 clinical isolates representative of other Flaviviruses just  
18 precluded us from doing that testing directly. Then, of  
19 course, the cross-reactivity, the clinical specimens with  
20 other viral hepatitis that was non-hep C, and then the other  
21 specimens that were chosen were really based on availability  
22 that we had at the time.

23           DR. WILSON: Dr. Tuazon.

24           DR. TUAZON: I have two questions. It seems like  
25 your Cobas Amplicor and Amplicor perform equally well except

1 for that one setting where the ACD plasma was not detected  
2 by the Amplicor. Are there any specific clinical settings  
3 where you would refer Cobas versus Amplicor?

4 DR. MURRAY: I think we need to be careful making  
5 the interpretation that the test performed differently  
6 because that data is based on a single sample. So, you  
7 know, these tests were designed to perform in exactly the  
8 same way, and so the tests are essentially interchangeable.

9 The setting that would probably drive whether you  
10 choose one or the other would probably relate to the  
11 throughput of samples within your laboratory. The microwell  
12 plate has a much higher throughput than the Cobas has, so we  
13 tend to find that the busier labs that are having more  
14 requests for testing on a daily basis would use the  
15 microwell plate format because you can put many more samples  
16 per run through that format than the Corbas.

17 It is actually really driven by volume of testing  
18 rather than performance of the assay.

19 DR. TUAZON: The second question I have is for  
20 practical purposes in terms of clinical setting, I mean if  
21 you look at the algorithm that you have, most of the time if  
22 EIA is positive, you go to PCR anyway regardless of the RIBA  
23 test. What specific testing would you have to go from EIA  
24 to, to RIBA?

25 DR. FRIED: I think that is true, and as I

1 mentioned, most of the time we do go to PCR, and I think it  
2 has to do with the patient populations that we are seeing.  
3 If we have a volunteer blood donor who just comes to us  
4 saying I was told I was anti-HCV positive, but I have no  
5 risk factors whatsoever for hepatitis C, and I am very  
6 surprised by this diagnosis, the best test for that patient  
7 is probably a RIBA test, because of the RIBA is negative, we  
8 can send the patient on their way and be comfortable that  
9 that was a false positive anti-HCV test.

10 In contrast, if we did a PCR assay, and the PCR is  
11 negative in that patient, then, we would still be in the  
12 position of saying, well, does this patient have  
13 intermittent viremia as rarely happens, or extremely low  
14 levels for whatever reason, and we would recommend that they  
15 get another PCR test in about six months.

16 But in the rest of our clinical practice where the  
17 patients who we are seeing most of the time with the risk  
18 factors, et cetera, we would go preferentially to PCR assay.

19 DR. TUAZON: Other than the clinical setting, I  
20 think that's the only use for the RIBA, because for  
21 management purposes, you still need your PCR.

22 DR. FRIED: Exactly, exactly. You would still be  
23 in the position of needing a PCR test and viremia to show  
24 that the patient is viremic before you would contemplate  
25 liver biopsy or even treatment for sure.

1 DR. TUAZON: Thank you.

2 DR. WILSON: Dr. Durack.

3 DR. DURACK: I have question about inhibitory  
4 samples. You have included the internal control, it was  
5 well described, and I infer from looking at the numbers that  
6 the number of inhibitory samples must have been very low,  
7 but I didn't see a summary statement about either the  
8 observed or expected number of inhibitory samples. Maybe I  
9 missed it, but could you just say a word about that?

10 MR. THOMAS: Are you requesting data from the  
11 clinical setting?

12 DR. DURACK: Either.

13 MR. THOMAS: Well, it gives us a denominator. As  
14 you know, there is about 2,000 samples. I believe the  
15 number of inhibitory samples that were then retested were  
16 12.

17 DR. DURACK: But it is extremely low.

18 MR. THOMAS: Yes.

19 DR. WILSON: Dr. Hollinger.

20 DR. HOLLINGER: I actually have lots of questions.

21 Let's start with the interfering substances first.

22 Can you tell us what these interfering substances are?

23 Let's just start with the interfering substances and whether

24 these are reproducible and how often they are reproducible

25 when you find something that looks like it is interfering.

1           And along the same line and whether or not these  
2 are really interfering substances or whether or not--you  
3 know, one of the real problems initially with the assay is  
4 not being able to see that pellet on centrifugation, the  
5 first pellet, and this is where it gets sucked up and lost,  
6 and therefore, the internal control looks negative. I mean  
7 that is a real major problem, and I will talk about that in  
8 just a minute or ask you a question about that.

9           But first of all, let me hear about these  
10 interfering substances.

11           DR. GUTEKUNST: Would you like to actually see  
12 some data?

13           DR. HOLLINGER: Sure.

14           DR. GUTEKUNST: We have some overheads that  
15 include the interfering substances that we have evaluated.

16           So, first, we looked at endogenous substances. We  
17 obtained specimens from individuals. We purchased these  
18 commercially that had elevated levels of albumin,  
19 hemoglobin, bilirubin, triglycerides, immunoglobulins.

20           What we did was we tested those specimens as we  
21 received them. They were anti-HCV negative, so we tested  
22 them to see if they would generate false positive results,  
23 and they did not.

24           Then, we took a high-titered HCV positive specimen  
25 and spiked it into those samples and then analyzed them to

1 see whether we had inhibition of HCV RNA detection, and we  
2 did not observe any inhibition. I think my colleague is  
3 trying to put those data up there.

4 DR. HOLLINGER: No, I have seen all of these, all  
5 this data, so I know which ones don't do that. What I am  
6 asking the question is which ones cause--

7 DR. GUTEKUNST: Actually, data that we didn't  
8 present, which is somewhat interesting, we do have data to  
9 suggest that if we were to spike these substances directly  
10 into a PCR reaction, they, in fact, do inhibit the PCR  
11 reaction, but when you take it through the specimen  
12 processing, then, you potentially remove that material.

13 So, we haven't actually been able to find anything  
14 that reproducibly inhibits PCR other than heparin. We know  
15 that specimens that are collected in heparin will inhibit  
16 PCR, and we can't remove that. Presumably, the heparin  
17 binds to the nucleic acid.

18 DR. HOLLINGER: With this in mind, have you  
19 tested--I didn't see any data that was presented in patients  
20 who are on Lovenox--

21 DR. GUTEKUNST: No, sir.

22 DR. HOLLINGER: --or who were heparinized,  
23 particularly hemodialysis patients who often have some  
24 heparin prior to their--there are a lot of patients in the  
25 hospitals who are being heparinized, and I didn't see that

1 you looked at any data on patients who had been heparinized  
2 and whether or not this really interferes in any way with  
3 the assay.

4 DR. GUTEKUNST: We didn't do a specific study to  
5 look at that, but we have occasionally had reports, people  
6 call in and say, you know, we have this patient, and we see  
7 some funny results. They are a hemodialysis patient.

8 We recommend that they get a new specimen, make  
9 sure that that specimen is taken well before the dialysis  
10 procedure is conducted, and generally, that's an  
11 intermittent inhibition in those types of specimens, it's  
12 not reproducible.

13 DR. HOLLINGER: Do you plan to put that into some  
14 sort of either a warning or something like this, because I  
15 don't think clinicians understand this basically, you know,  
16 that this is a potential--it's a potential problem for a lot  
17 of hepatitis tests, not only this, but HBS antigen, and so  
18 on. But is there something that is going to be placed in  
19 there about this?

20 I know you stated about heparin, but I think these  
21 other specifics might be useful.

22 DR. GUTEKUNST: I am assuming that the agency will  
23 ask the panel to make recommendations about things like this  
24 that you feel are relevant that we haven't demonstrated.

25 DR. HOLLINGER: Again, on the interfering

1 substances, if you have something that has an interfering  
2 substance, let's say, the internal control is negative, can  
3 this be diluted out, and if it's diluted out, and you have a  
4 positive sample, what happens to that positive sample in  
5 there?

6 For example, if you have an inhibitory substance  
7 and you say dilute it out in normal serum or something like  
8 this, so that it becomes negative basically or it becomes  
9 absent, what happens to a positive sample that apparently  
10 came out negative and then ultimately became positive, it  
11 would be diluted out.

12 DR. GUTEKUNST: I don't think we have directly  
13 tested that, what you are asking, so I can't answer that. I  
14 don't believe we have data.

15 DR. HOLLINGER: Let me look at another item, if I  
16 could.

17 DR. MURRAY: Can I make a comment on that just to  
18 explain what we did in the clinical study?

19 DR. HOLLINGER: Please.

20 DR. MURRAY: Samples that came up as inhibitory  
21 with a negative internal control and a negative PCR result  
22 were retested. The same sample was retested and, in fact,  
23 in the majority of cases we got a clear result out just on  
24 retesting of the same sample.

25 What we would recommend is if you get a repeatedly