SENATE COMMUNITY AFFAIRS REFERENCES COMMITTEE

INQUIRY INTO HEPATITIS C AND BLOOD SUPPLY IN AUSTRALIA

SUBMISSION

LUIGI PALOMBI

Introduction

This submission addresses two of the stated terms of reference. These are as follows:

(a) the history of post-transfusion Hepatitis in Australia, including when Non-A, Non-B Hepatitis (Hepatitis C) was first identified as a risk to the safety of blood supplies in Australia and internationally.

(b) the understanding of Hepatitis C by blood bankers, virologists, and liver specialists during the past 3 decades, including when Hepatitis C was first identified as a virus transmissible through blood.

In respect of the second term of reference, this submission addresses a issue which pertains to the transmission of HCV after the introduction in Australia of the first generation HCV immunoassays licensed by Chiron Corporation. This submission believes that this is relevant to this Inquiry because to focus on surrogate testing of non-A non-B Hepatitis (as HCV was known in the 1980's) without a consideration of the adequacy of the first generation HCV immunoassay gives an incomplete picture which may bias this Inquiry's findings.

The evidence which supports this submission was given before the Federal Court of Australia in the case of Murex Diagnostics Australia Pty Limited v Chiron Corporation and Ortho Diagnostics Systems, Inc (NSW District NG 106 of 1994) which was heard in Sydney before the Honourable Justice Burchett between June 24, 1996 and August 28, 1996. This case and issues relevant to it received media attention, e.g., ABC Lateline in 1994, Channel 9 Sunday in 1995, ABC

Qantum in 1996 and ABC The 7.30 Report in 1996. It was also featured in the Sydney Morning Herald in 1996. Evidence of expert witnesses filed in the proceedings included two of the named "inventors" of HCV, Drs Houghton and Kuo from Chiron, Dr. Bradley formerly of the Centers for Disease Control, U.S.A., Dr. Stephen Locarnini then the director of Victorian Infectious Diseases Reference Laboratory, Nobel Laureate Professor Baruch Blumberg of the Institute for Cancer Research, Fox Chase Cancer Center, U.S.A, Dr. David Kemp then of the Menzies School of Health Research, Professor Sir Gustav Nossal then the director of the Walter & Eliza Hall Institute for Medical Research, Professor John Shine of the Garvan Institute of Medical Research. There were other noted scientists and experts, but rather than mention them all individually at this point, it suffices for the purposes of this introduction if those mentioned here gives some indication of the high level of science considered throughout this case.

The issues which were germane to this case centred on the validity of Australian Patent 624,105 granted to Chiron Corporation. The patent AU 624,105 was granted by the Australian Patent Office in 1992 and is entitled "NANBH diagnostics and vaccines". Chiron cross-claimed that Murex infringed its patent by importing and selling in Australia an HCV immunoassay and an HCV serotyping assay. However, evidence was given relating to continuing, albeit reduced, spread of post-transfusion HCV and the immediate need for a variety of HCV immunoassays designed for Australia.

There was no judgment of the Federal Court of Australia because the proceedings came to a sudden halt when on August 28, 1996 Murex and Chiron settled their worldwide differences in confidence. Although Murex did succeed in obtaining a license to manufacture and sell its HCV immunoassay, this was a license that was restricted to certain areas of the world – fortunately Australia was one of those countries included within the license. However, Murex's HCV serotyping assay

became available on a worldwide basis. Within two years of this settlement, Murex was fully acquired by Abbott Laboratories (U.S.A.), one of the original Chiron licensees.

Why is this relevant? Any Inquiry that examines the spread of HCV based upon decisions not to implement surrogate testing of HCV in the 1980's without appreciating how this disease continued to spread after the introduction of HCV antibody testing is blinkered and may draw conclusions that are incomplete. As this submission will demonstrate, the failure of the Federal and State Departments of Heath to use powers reserved to them by the Patents Act facilitated the continued spread of post-transfusion HCV into the 1990's.

The simple and terrible truth is that the spread of post-transfusion HCV was not halted in Australia with the introduction of Chiron licensed HCV immunoassays in the early 1990's and there is evidence that people who received blood or blood products during the 1990's contracted HCV through that route.

The History Of Post-Transfusion Hepatitis In Australia, Including When Non-A, Non-B Hepatitis (Hepatitis C) Was First Identified As A Risk To The Safety Of Blood Supplies In Australia And Internationally.

The term Non-A Non-B Hepatitis (NANBH) first appeared in the mid-1970's. After the development of the hepatitis A test in Australia it seems that medical workers were able to identify patients that were exhibiting symptoms of hepatitis which were not hepatitis A and B infected.

The first affidavit of Professor Stephen Locarnini explains the situation.

2.5. My involvement in hepatitis research began in March 1974. I was a member of a research team at the Department of Microbiology, Monash Medical School, Monash University at the Alfred Hospital. This was the first research group in the world to detect the Hepatitis A virus in the faeces of patients with naturally acquired disease, and to prove conclusively that the particle was both disease-associated and serologically specific for naturally acquired Hepatitis A infection. Even though my Ph.D. was focused on hepatitis A, because my work was part of a prospective study of consecutive admissions of patients to Fairfield, it allowed me and my colleagues to define hepatitis as it was occurring in Melbourne at the time. **We were then able to break down patients into either hepatitis B, hepatitis A or non A, non B hepatitis (NANBH). Of the 118 consecutive patients admitted to Fairfield Hospital in 1975, 75 patients were hepatitis A, 31 patients were hepatitis B, 18 patients were non viral hepatitis, i.e., miscellaneous, and 16 patients were hepatitis of undetermined etiology which would be classified as NANBH.**

2.6. We collected both acute and convalescent sera as well as faecal specimens from all these patients. We used the standard approach (discussed below) to try to identify a new virus-like agent. This approach used convalescent antibody as the source of antibody, which reacted with the acute phase specimens, i.e. faeces or stools. We then tried to identify viral-like particles that could be the cause of their hepatitis, using the technique of immuno-electronmicroscopy. In 1976 when the study was completed we did not know whether these cases of hepatitis of undetermined aetiology

were parenterally transmitted or enterically transmitted. The studies focused exclusively on trying to identify enterically transmitted infectious agents. The hypothesis was that the enterically transmitted infectious agent would be in stools and be transmitted to the next person from the faecal-oral route. This was the approach we used to discover hepatitis A virus.

2.7. Part of the subsequent research conducted by the research team referred to at paragraph 2.5 above involved a prospective study which was carried out using patients admitted to Fairfield with hepatitis. During the course of the study, the serological responses following acute Hepatitis A infection were examined and a specific IgM (immunoglobulin M) response was discovered. This observation formed the basis of a rapid and routine diagnostic test we developed for hepatitis A. This test has since become commercially available throughout the world. I used some of the results of that research in my thesis for my Ph.D. entitled "*Studies on Viral Hepatitis Type A*". The results of that research were published in a paper entitled "*A Prospective Study of Acute Viral Hepatitis with Particular Reference To Hepatitis A*" in Bulletin of the World Health Organisation, Vol. 54, 1976.

At an international level, the affidavit of Professor Baruch Blumberg explains the discovery of hepatitis B virus (HBV) and the development of an HBV diagnostic assay in the late 1960's.

2.1. HBV was discovered in our laboratory at Fox Chase Cancer Center as a consequence of a general research program whose goal was to determine inherited biochemical and immunological differences in the blood which differentially affected susceptibility to disease. During the course of these studies we found, in 1967, an antibody to the surface antigen of HBV (abbreviated HBsAg) that is, the protein that coats the outside of the virus, in the serum of a patient who had developed the antibody after exposure to HBV as a consequence of multiple transfusions. During the course of these transfusions he had apparently received in the donor blood, HBV virus and also particles which contained only the surface antigen of HBV. The transfused patient had either developed an asymptomatic HBV infection, or an acute infection from which he recovered in a relatively short time. During this recovery period the

patients immune system had developed an antibody. The serum collected during this period which contained the antibody could be referred to as "convalescent serum" or "post exposure serum".

2.2. The antibody found in the transfused patients "convalescent serum" reacted in an immunodiffusion experiment (see below) with HBV which was present in the blood of another person. This person, an Aborigine from Western Australia, had been infected with HBV, probably as a consequence of maternal infection shortly after birth or in early childhood, or sexually later in life or by some other route. The Aborigine, rather than developing an antibody, became a chronic carrier of HBV and retained the virus as well as the surface antigen particles in his blood for many years. Hence, we could detect the virus because there was a striking difference in the way in which the two individuals had reacted to infection: the patient by developing antibody against the surface antigen, and the Aborigine by becoming an asymptomatic chronic carrier of HBV.

2.3. Immunodiffusion in agar gel is an immunological technique whereby the proteins and other chemicals contained in a serum (i.e. that of the transfused patient) are allowed to diffuse into agar gel from a small wells cut into the gel. Serum from another individual (i.e., the Aborigine), is allowed to diffuse into the gel from an adjacent well. If the sera from the transfused patient contains an antibody specific for a protein present in the Aborigine, then a precipitate will form in the gel between the two wells, and the protein can be detected. Since the surface antigen was originally discovered in an Australian we referred to it as "Australia antigen". It is now called HBsAg.

2.4. My colleagues and I recognized that the antigen we had discovered, HBsAg, was a protein (probably structural), which we now know is produced by one of the four reading frames (genes) of the virus. **The antibody against it, anti-HBs, allowed us to produce a diagnostic method for detecting HBV. This was particularly important because there were many cases of posttransfusion hepatitis caused by the use of blood obtained from donors who were asymptomatic carriers of HBV. Unknown to the donors themselves or to the blood banks these individuals could transmit the virus to others.** We began testing donor blood for a blood bank in Philadelphia. We did not seek to patent this antigen; rather we made the antigen and antibody freely available to investigators who requested them and

continued this service until it was taken over in the mid-1970's by the National Institutes of Health, a U.S. Government laboratory in Bethesda, Maryland. The distribution of these reagents accelerated research on HBV.

2.5. The immunodiffusion method, although specific, is very insensitive. It helped to decrease the number of cases of posttransfusion hepatitis due to HBV, but it did not eliminate them. Therefore, we developed a radioimmunoassay method, which we did patent. Other scientists, using the "Australia antigen" we had discovered, subsequently developed radioimmunoassay and other methods which greatly increased the sensitivity of detecting HBV. Within a very short time, post-transfusion hepatitis due to HBV was eliminated from blood banks in countries where the facilities to do the screening were available (U.S., Europe, Japan, other locations in Asia, etc.).

2.6. I published a short essay describing my work after receiving the Noble Prize for Physiology or Medicine in Science 1977 Vol 197 pp 17-25 entitled *"Australia antigen and the biology of hepatitis B."*

Professor Blumberg proceeds to explain how NANBH was associated with patients exhibiting symptoms of hepatitis but not infected with HAV or HBV.

3.1. **It was soon recognized that, although a significant percentage of post-transfusion hepatitis cases had been eliminated, the problem persisted. At this time it became apparent that other blood-borne hepatitis viruses existed. Thus, the search for a non-A, non-B (NANB) hepatitis virus began**. Many scientists and companies who had worked on HBV as well as others focused their target on this unknown virus. The complete history of NANB hepatitis research is extensive, and the scientific papers which reflect that history are numerous. Over the years, intensive studies on NANB were conducted and the characteristics of the putative virus became better understood. By the mid-1980's, the combined knowledge gained from years of research was extensive and more importantly, several researchers had emerged as leaders in the field. Dr. Daniel Bradley was viewed by workers in the field including myself as one such leader, particularly in view of his characterization of the tubule-forming agent (TFA) which has subsequently been accepted to be the etiological agent of HCV.

The Understanding Of Hepatitis C By Blood Bankers, Virologists, And Liver Specialists During The Past 3 Decades, Including When Hepatitis C Was First Identified As A Virus Transmissible Through Blood.

(a) Prior to 1990

The first affidavit of Dr. Daniel Bradley explains,

4.1 By 1975, it was understood that a significant number (and perhaps a majority) of cases of post-transfusion hepatitis was not caused by any known human virus including hepatitis A virus (HAV), hepatitis B virus (HBV), cytomegalovirus (CMV) or Epstein-Barr virus (EBV). Rather, these cases appeared to be caused by one or more additional transmissible agents which, for lack of a better term, were known as "post-transfusion non-A, non-B hepatitis" agents (PT-NANBH), or, simply, NANBH agents. At about this time, it was generally agreed among scientists in the field that between 10% and 20% of patients transfused with blood or blood products developed PT-NANBH.

4.2 In 1977, I was contacted by Dr. Gary Dolana of Hyland Laboratories in Costa Mesa, California. Dr. Dolana's company manufactured and sold a Factor VIII product. Factor VIII is required by hemophiliacs to restore normal clotting activity. I learned from him that three lots of Hyland Laboratories' Factor VIII concentrate had been implicated in causing NANBH in several patients. Two patients had developed NANBH after receiving aliquots of Lot No. D0056A. Two other patients had been afflicted with NANBH after receiving Factor VIII from two other lots. I arranged to have all of the remaining supplies from each of these lots sent to my laboratory. I also arranged for convalescent phase sera from two of the three infected patients to be collected and sent to me.

4.3 After receiving the suspect Factor VIII materials, I attempted to visualize virus-like particles in both fractionated and unfractionated materials using standard electron microscopy (EM) and immune electron microscopy (IEM). Despite extensive efforts, no virus-like particles could be visualized with these techniques.

4.4 Since the presumed viral agent(s) could not be visualized, I designed a primate protocol to test the Factor VIII concentrates for the presence of a transmissible agent. In early 1978, four chimpanzees from CDC's primate facility were intravenously inoculated with the suspect Factor VIII

concentrates. Two chimpanzees received 30 mls each from suspect lot No. D0056A. The other two received the same amount from a pooled preparation from the other two suspect lots. Over a period ranging from 13 to 70 days, all four chimpanzees experienced an elevation of a liver enzyme called alanine aminotransferase (ALT) which was indicative of hepatitis. Light microscopic examination of liver biopsy materials obtained from all four animals also showed histopathological changes consistent with acute viral hepatitis. However, EM and IEM examination of liver tissue and plasma taken from each of the four animals did not reveal any disease-specific virus-like particles.

4.5 **Serological tests were performed to rule out the possibility that the ALT and histopathologic responses were caused by known viruses, i.e. HAV, B V, CMV and EBV. These tests were negative. I therefore concluded that the suspect Factor VIII concentrates indeed contained one or more transmissible agents capable of causing NANBH.**

Dr. Bradley was at that time an employee the Phoenix Laboratories of the Centers for Disease Control (CDC) which is a governmental agency of the US Department of Human Health Services.

Dr. Bradley then explains,

4.6 **The fact that the presumed viral agent(s) could not be visualized was a serious obstacle to developing an immunoassay for detection of virus-specific antigen or antibody**. I decided that a combination of steps would be required to identify and characterize the virus. First, I needed to find ways to increase the titer of the virus in whatever materials I was going to work with (i.e. liver, plasma, sera). In order for the virus to be detectable using IEM, I knew that the titer of the virus in any biological material would need to be 10^5 to 10^6 virus particles per milliliter; using electron microscopy of thin sections of liver biopsy tissues, I knew that the titer of virus would need to be on the order of 10^9 or 10^{10} virus particles per gram of tissue in order to be visualized. Second, I needed to develop a plan to physicochemically describe the virus.

4.7 I initiated passaging studies with chimpanzees at CDC in 1978 in an effort to increase the titer of virus to the point where it could be visualized by EM or IEM. These studies were done by intravenously inoculating chimpanzees with acute-phase liver homogenates, acute-phase plasma, and cesium chloride gradient fractions of selected acute-phase liver homogenates. The results of some of these studies were described in a paper called "Experimental Infection of Chimpanzees With Antihemophilic (Factor VIII) Materials: Recovery of Virus-Like Particles Associated with Non A, Non B Hepatitis" published in J. Med. Virol. 3: 253-269 (1979).

Dr. Bradley's research conclusively established a link between the agent present in the batch of Factor VIII from Hyland Laboratories and the infectivity of that agent to cause hepatitis that was neither HAV or HBV.

In terms of NANBH in Australia, Professor Locarnini in his first affidavit explains,

5.2 By 1980 we had been searching for an aetiological agent of non-A, non-B, hepatitis particularly amongst injecting drug users who were admitted to Fairfield from 1976. The initial studies were based on the premise that virus was present in the blood during the acute phase of the disease and that the patient developed antibodies during the convalescent period. The rationale of the research was based on the way we characterised hepatitis B and found hepatitis A. During the acute phase of the disease process, the virus accumulates in a patient's body fluid, be it in the serum, faeces or whatever; but in the convalescent phase, the patient should produce antibodies to the virus. We thought that we should be able to use those antibodies to seek out the aetiological agent.

5.3 At Fairfield, where we had the clinical facility, when a patient with acute hepatitis was admitted to the ward, the blood sisters would take an additional large bleed from the patient of about 20 mls of blood on admission. This blood specimen was then stored as the acute phase specimen. We anticipated that the virus would be present in the acute phase specimen. The patient would stay in hospital for one to two weeks and then return to the community once the ALT levels had normalised. The patient would subsequently return to the follow up clinic at Fairfield. The patient would be examined by one of the clinicians. The clinicians would take another large bleed from the patient of about 20 mls. This

blood specimen would be stored as the convalescent phase specimen. We tried to get this latter specimen at least three months after onset of dark urine. A difficulty with many of these patients related to the fact that they were injecting drug users and unreliable. Accordingly, they tended not to come back to the follow up clinics. The clinician's role was, consequently, very important as they were able to persuade the patients to return, which many did. Thus we accumulated a large panel of very valuable acute phase and convalescent phase serum.

5.4 **We constructed our own "in-house" assays trying to find the NANBH virus.** We worked on the assumption that antibodies present in the convalescent phase would react with the acute phase virus in the serum. We made an additional assumption that the disease would have resolved in about half of the patients whilst half of them would develop chronic hepatitis. Nevertheless, we processed all "convalescent" sera as a source of antibody.

5.5 The purpose of the research was to try to identify directly the antigen or aetiological agent of non-A, non-B, hepatitis using electron microscopy and solid-phase immunoassay.

5.6 I believed that a patient would produce an antibody response within three months of a viral infection. Therefore, the way to improve the level of detection of the virus was to use antibodies present in convalescent phase serum of a patient with NANBH, irrespective of whether the patient was chronically infected or not. The antibodies created an immune reaction or complex with the virus, which resulted in particles clustering together in aggregates which could then be seen by electron microscopy.

5.7 An examination of the electron microscopy plates containing the blood samples revealed that there were a number of structures of viral origin. Electron microscopy showed enveloped viral-like particles, which were non specific. The results of this research was published in a paper by Dr. Hui Zhuang et. al., entitled "*Characterization of a Precipitating Antigen Detected in the Serum of Patients With Viral Hepatitis*" published in Journal of Medical Virology 11: 267-276 (1983).

5.8 Even though we saw viral particles in both sera and in stools, we were unable to demonstrate a serological relationship between those particles and the infection. The conclusion that my colleagues and I reached in about 1980 was that the amount of virus in clinical specimens was low. My

colleagues and I concluded that a method was needed to improve the level of detection of the NANBH antigen or virus.

5.9 My colleagues and I also considered the possibility that antibodies were not produced to the aetiological agent of NANBH. If this were the case, then one possibility was that the agent was a viroid. A viroid is a piece of nucleic acid with no protein coat. It is usually the protein coat that stimulates an antibody response. As far as I am aware there is no case in which a virus does not stimulate the production of antibodies. My colleagues and I, with Dr. Carl Reingarnum, a senior lecturer in plant virology, then developed a strategy to look for viroids in acute phase sera. Dr. Reingarnum had done viroid work in the early 70's. We took about one dozen acute phase plasma specimens from patients with NANBH. We extracted them for viroids but they were all negative. So we concluded that the aetiological agent of NANBH was not a viroid.

5.10 By 1981 I had concluded that the aetiological agent of NANBH was most likely a rubella-like virus, which is a similar virus to a flavivirus. This conclusion was reached with the assistance of the work of Dr. Bradley of the Centers for Disease Control in the USA. Dr. Bradley had published two papers which I had read and considered at the time. They were "*Experimental Infection of Chimpanzees With Antihemophilic (Factor VIII) Materials: Recovery of Virus-Like Particles Associated With Non-A, Non-B Hepatitis*" in Journal of Medical Virology 3: 253-269 (1979) and "*Non-A/Non-B Hepatitis in Experimentally Infected Chimpanzees: Cross-Challenge and Electron Microscopic Studies*" in Journal of Medical Virology 6: 185-201 (1980). The importance to me of Dr. Bradley's work was his finding that once the NANBH agent was inside a cell, the cell displayed certain characteristics, i.e. the proliferation of smooth membranes with tubule formation. This made me think that the NANBH agent was like the rubella virus (i.e. an RNA virus similar to a flavivirus) because rubella virus also causes cytoplasmic proliferation. Further, rubella virus was the only RNA virus I knew, at that time, to cause persistent infection.

5.12 In about 1981 I gave further consideration for additional research to be conducted in the search for the aetiological agent of NANBH. This involved the possibility of cloning that agent, which as stated above was likely to be an RNA plus stranded virus. The idea of cloning the aetiological agent of NANBH was suggested to me by Professor Ian Gust. He suggested that I

speak with Dr. David Kemp, a molecular biologist at the Walter & Eliza Hall Institute in Melbourne. ...

5.13 We also discussed pedigreeing the source material in which the virus was present. I concluded at that time that we could take the work to the stage described by Professor Kemp (however, if we could not find the potential agent, then that was as far as we could go without our own chimpanzees). The problem was that the clinical material at Fairfield was not the optimum source for cloning because it had not been pedigreed to the level required to give me the degree of confidence that the clinical material actually contained the NANBH virus. There was no other source of starting material available to Fairfield. The only way to obtain pedigreed source material for cloning in Australia was (and is) to use chimpanzee animal models. As it was (and still is) unacceptable to use humans as a source of pedigreed material for cloning, the only alternative was to use chimpanzees. There was (and is) no other animal model that could be used for this purpose. Unfortunately, at that time (and to the best of my knowledge, the same is true today) there was no chimpanzee colony in Australia for use by infectious disease researchers. The cost of establishing such a colony was prohibitive for Fairfield.

5.14 An alternative to obtaining our own pedigreed source material was to send infectious material to an organisation, such as the NIH, that did have a chimpanzee colony and try to arrange for this organisation to inoculate a chimpanzee for Fairfield. The logistical problems in respect of this option, in my opinion, made it impractical for Fairfield to pursue this as a realistic option. It was (and is) difficult to send infectious material around the world from centre to centre because of the difficulty in getting couriers to transport infectious material. First, infectious material is hazardous and secondly, requires specific care in order to survive the long journey.

5.15 Another real problem facing Fairfield was funding the research. It is extremely expensive to establish and maintain chimpanzee colonies. I did not believe that the NH&MRC would have approved funding because we did not have any positive results from the previous research into NANBH. The funding system in Australia is such that you must have a proven track record and the papers on NANBH produced at Fairfield did not, in my opinion, amount to a proven track record, to a level which would attract the amount of funding required to advance the research work.

Professor Terukatsu Arima, of the Second Department of Internal Medicine at the Faculty of Medicine of Kagoshima University in Japan explains,

22. **By the late 1970's I was aware of the existence of NANBH through the clinical treatment of patients suffering from hepatic liver disease. Hepatitis A virus (HAV) infections were excluded by the absence of infection in the intestinal-oral route. Hepatitis B virus (HBV) infections were excluded by diagnostic testing using test kits commercially available at that time. I was aware of many patients that were suspected of being infected with the putative agent for NANBH.** In Japan, about 60% of chronic Hepatitis patients (about 720,000 patients), about 40% of Liver cirrhosis patients (about 100,000 patients) and about 40% of liver cancer patients (about 7,000) were patients having NANBH. Further, the mortality rate attributed to NANBH reached 16 thousand per year. Nearly 1 to 6% of all blood donors in Japan were NANBH carriers.

23. In 1984 I read many publications relating to NANBH. In particular, I recall reading publications discussing the research of Dr. Bradley and, separately, Dr. Feinstone. Those publications being: Bradley et al (1979) Journal of Medical Virology 3 : 253-269; Shimizu Y.K., Feinstone S.M., Purcell R.H. et al (1979) Science 205 : 197-200; and Alter, H.J. Purcell R.H., Feinstone S.M. et al (1978), in Vyas GN, Cohen SN, Schmidt T. eds *"Viral Hepatitis: aaaetiology, epidemiology, pathogenesis and prevention",* Franklin Institute Press, Philadelphia, 359-369. Each of these researchers was, in my opinion, an excellent scientific investigator. Their research was scientifically credible and reputable. I placed a great deal of weight on the findings of Dr. Bradley, in particular. I had not met either of these researchers at this time. My conclusions were drawn purely from their published papers.

24. With the exception of the research conducted by Drs Bradley and Feinstone, I had serious reservations about the repeatability of many of the published results relating to NANBH research. In 1984, my colleagues and I attempted to identify reverse transcriptase activity in the serum samples of NANBH infected patients. We failed to identify any enzymic activity that could be attributed to this enzyme. The results of this research were presented at the 21st meeting held by the Japanese Society of Hepatology, but were never published. In contrast, Seto et al (1984)

"Detection of Reverse Transcriptase Activity in Association with the Non-A-Non-B Hepatitis Agent(s)" Lancet 2:941-943 reported finding such activity. Since I had total confidence in my own results I did not pursue this finding further.

25. I recall at that time that a number of researchers suggested that NANBH might be caused by something other than a virus. I saw many patients infected with NANBH and was of the opinion that the aetiological agent of NANBH was a virus. This opinion was supported by Dr. Bradley's transmission studies of NANBH which I was aware at that time: Bradley DW et al (1979) *"Experimental Infection of Chimpanzees with Antihemophilic (Factor VIII) Materials: Recovery of Virus-Like Particles Associated with Non-A-Non-B Hepatitis"* Journal of Medical Virology 3:253-269. It was also supported by my own experience of witnessing transmission of NANBH between patients suffering from the disease at the First Department of Internal Medicine, Okayama University Medical School.

26. Further, my colleagues and I conducted clinical experiments in late 1984 and throughout 1985 to identify the effect, if any, that human Beta Interferon had on NANBH patients. We postulated that if NANBH was caused by a virus then interferons (i.e. chemical substances that interfere with viral replication) would be expected to have an effect on controlling disease activity. Seventeen patients received human Beta Interferon by slow intravenous infusion at a dosage of 2 to 3 million units daily for 4 or 8 weeks. The results suggested that human Beta Interferon therapy is beneficial in controlling disease activity in some patients with chronic NANBH. This confirmed our opinion that the agent of NANBH was a virus. The results of our research were published in a paper entitled *"Treatment of Chronic Non-A-Non-B Hepatitis with Human Beta-Interferont"* in Viral Hepatitis and Liver Disease 898-901 (1988) Alan R Liss Inc (A J Zuckerman ed.)

27. From my review of the NANBH literature I realised that classical virological methods for the isolation of the aetiological agent for NANBH had failed. Researchers were unable to grow the agent in tissue culture, unable to visualise the virus by electron microscopy and unable to detect an antigen-antibody complex by using serum from patients with the disease and from patients recovering from the disease, which was specific for NANBH. The apparent absence of detectable quantities of viral particles in an infected patient suggested to me that we needed to adopt a new approach that

would allow us to amplify the amount of NANBH-specific antigen. It was clear to me that the way forward was to adopt a molecular biological approach for isolating the nucleotides coding for epitopes of the aetiological agent of NANBH.

28. By the mid 1980's I was aware through articles authored by Dr. Bradley that there was evidence of two causative agents of NANBH, the major one being known as the tubule forming agent (TFA). Early in 1985 Bradley's research showed the following about the putative agent for NANBH:

It was highly infective and present in low titre.

It was small (i.e. ranged in diameter from 22 to 70 nm).

It was not infective after treatment with chloroform ($ChCL_3$) (i.e. it was sensitive to $ChCL_3$ treatment and was therefore most probably an enveloped or lipid-containing virus).

It had chronicity to the health of adults.

29. In 1985, Bradley summarised the physiochemical and pathogenic properties of the tubule-forming post transfusion agent in an article entitled *"The Agent of Non-A-Non-B Hepatitis"* Journal of Virological Methods, 10 307-319 (1985). These properties were part of my everyday working knowledge by the end of 1985. I will set out the properties for convenience.

29.1 Physicochemical Properties

Infectivity is destroyed by:

formalin 1:1,000, 37°C, 96L;

Heat 100°C, 5 minutes; for 60°C, 10h;

Treatment with 20% v/v $ChCL_3$.

Agent contains essential lipid (enveloped).

Diameter of infectious agent is < 80nm.

Can be pelleted from plasma (assumes agent has an S_{20W} of > 200S).

Agent can be recovered from chronic-phase plasma by a multi-step procedure used for the purification of small enveloped RNA viruses.

Approximate buoyant density of 1.24 g/cm^3 in cesium chloride.

29.2 Pathogenetic Properties

Agent normally causes persistent infection and/or slowly-resolving disease.

Agent interferes with the replication of other hepatotrophic viruses (HAV, HBV).

Specific ultrastructural changes associated with replication in Chimpanzees are confined to hepatocyte cytoplasm.

Ultrastructural changes in Chimpanzee hepatocytes are most similar to those induced by some enveloped mammalian RNA viruses.

Titre of virus in majority of inocula reported to be $< 1 \times 10^3$ CID/ml.

Recrudescence of disease may spontaneously occur > 3 years after the initial infection.

30. Bradley concluded that the TFA was a kind of togavirus or toga-like virus, which I knew at the time to be a small RNA virus with a lipid envelope. By comparing the aforementioned properties with the characteristics of the then known viruses, I hypothesised that the TFA might be a flavivirus similar to Japanese encephalitis virus, and likewise a small blood borne RNA virus.

31. In 1985 to investigate this hypothesis further, I discussed with Dr. A. Igarashi, Professor of Virology at the Institute of Tropical Medicine, Nagasaki University, whether the aetiological agent of NANBH was a flavivirus or flavi-like virus. I explained to him the characteristics that were known about NANBH. He agreed that my hypothesis was reasonable.

Dr. Bradley's work in the mid to late 1970's was central to demonstrating an association between elevated ALT's and the "tubule forming agent" or TFA as the primary candidate, causative of NANBH. His research with chimpanzees prior to 1980 and papers "Experimental Infection of Chimpanzees With Antihemophilic (Factor VIII) Materials: Recovery of Virus-Like Particles Associated with Non A, Non B Hepatitis" J. Med. Virol. 3: 253-269 (1979) and Non-A /Non-B Hepatitis in Experimentally Infected Chimpanzees: Cross Challenge and Electron Microscopic Studies. J. Med. Virol 6: 185-201 (1980) that describes some of his research was one of the reasons that prompted a meeting of U.S. blood diseases experts in Washington D.C. on January 9, 1981 (See attachment A). Dr. Bradley explains,

4.7 I initiated passaging studies with chimpanzees at CDC in 1978 in an effort to increase the titer of virus to the point where it could be visualized by EM

or IEM. These studies were done by intravenously inoculating chimpanzees with acute-phase liver homogenates, acute-phase plasma, and cesium chloride gradient fractions of selected acute-phase liver homogenates. The results of some of these studies were described in a paper called "Experimental Infection of Chimpanzees With Antihemophilic (Factor VIII) Materials: Recovery of Virus-Like Particles Associated with Non A, Non B Hepatitis" published in J. Med. Virol. 3: 253-269 (1979).

4.8 I also studied the effect of immunosuppression on a chimpanzee experimentally infected with NANBH. A chimpanzee named Alfred was immunosuppressed to the point that his circulating white cell counts approached zero. He was then inoculated with known infectious NANBH material. Electron microscopy of a liver biopsy sample taken from him at the time he died (at day 35 after inoculation) showed massive accumulations of convoluted membranes (Type II structures)indicative of vigorous viral replication. I surmised that Alfred's liver had an unusually high titer of virus.

4.9 I inoculated another chimpanzee ("Sparky") with a homogenate from Alfred's liver. Sparky exhibited a very short incubation period (i.e. he had obvious ALT elevations at just 7 to 9 days post-inoculation) accompanied by the early appearance of characteristic ultrastructural changes in liver biopsies viewed by electron microscopy. I concluded from these studies that the length of the incubation period for the disease was inversely related to the amount of virus with which a chimpanzee was inoculated.

4.10 During the course of these studies, **I observed that significant increases in ALT activity were paralleled by, and strongly correlated with electron microscopic and histologic evidence of increased hepatocellular degeneration or cytopathology. The most obvious ultrastructural changes observed were curved membranes (Type II structures). Other ultrastructural changes included dense fibrillar masses and tubules.** I coordinated an extensive series of cross-challenge studies in chimpanzees that included a variety of inocula. These studies strongly suggested that there was a single agent (or class of agents) associated with NANBH. I surmised that the ultrastructural changes described above were all induced by the major causative agent of NANBH. I termed that agent the "tubule forming agent" or "TFA". Based on the correlation between high ALT values and ultrastructural changes during the chronic phase of the disease, **I believed that elevated ALT values**

during the chronic phase of the disease could be used as a basis for predicting relatively high titers of the TFA in infected chimpanzees.

4.11 As noted above, my early studies indicated that chronic-phase plasma might be a richer source of virus than acute-phase plasma, since disease in many animals appeared to worsen with time after the initial infection. Accordingly, I directed technicians to plasmapherese certain chronically infected chimpanzees at specific times when I predicted that the titer of virus was the highest. These plasma collections were based, in part, on my hypothesis that the agent(s) responsible for NANBH was (were) to some extent cytopathic and that there was a positive correlation between elevated ALT values and the titer of virus in chimpanzee plasma. **I therefore set out to determine statistically significant ALT elevations for each chimpanzee involved**. Since I had noted that individual chimpanzees may have a different range and mean values for baseline ALT activity (i.e. normal for that individual), I statistically analyzed 8 to 16 (or more) individual ALT values taken 1 to 4 months prior to inoculation to determine a baseline for each animal. I determined the 95% and 99% confidence levels for the upper level of normal ALT activity for each chimpanzee used in the studies. **This enabled me to establish an accurate ALT cutoff value against which elevations in ALT activity could be measured for each chimpanzee**. By using statistically significant ALT values to predict increased titer of virus, I was able to avoid the necessity of conducting frequent liver biopsies to assess the ultrastructural evidence for increased viral replication. This also enabled me to avoid extensive biopsies which are invasive procedures that can jeopardize the health of chimpanzees. Significant delays in obtaining data from EM and histologic analysis of liver biopsy tissues can also occur.

4.12 Two chimpanzees were of particular interest and value in these studies. Chimpanzees “Don” and “Rodney” exhibited relatively more frequent and higher ALT values (as well as concurrent EM and histologic evidence of disease exacerbation indicative of increased viral replication) than other chronically infected chimpanzees . I prospectively collected plasma from these two animals during periods of significantly increased ALT activity over a substantial period of time. Nearly 8,000 mls of plasma were collected from Rodney over nearly six years. Approximately 15,000 milliliters of plasma were collected from Don over an eleven year period.

This plasma (and that from other chimpanzees) was carefully inventoried and stored under controlled conditions at CDC at my direction and under my control.

4.13 I personally devised every protocol used in these studies. I interacted regularly with the primate handlers to ensure that the studies were performed in accordance with my protocols. The chimpanzees required extensive hands-on care by skilled and experienced handlers. Chimpanzees are extraordinarily demanding and expensive to use and every factor in their care is important to the results obtained. **Chimpanzees are genetically quite similar to humans and they are every bit as complex physiologically and behaviorally.**

Incidentally, the plasma pool that Dr. Bradley developed from plasma collected from chimpanzee "Rodney" was exclusively used in a collaboration with Chiron to clone the causative agent of NANBH or HCV. From this plasma pool Chiron scientists produced a lambda gt-11 library of clones one of which was clone 5-1-1 which ultimately lead to the sequencing of the virus now known as HCV and which is the subject of AU 624,105 and many other patents throughout the world.

After this meeting Dr. Katz of the American Red Cross wrote to a colleague, Dr. Keating, also of the American Red Cross. In his letter of January 15, 1981 he explained some of his concerns regarding the conclusions drawn from that meeting. In particular he stated that "[e]ven if there were a substantial number of false positives, with implications for donors, the imperic association with recipient increase in ALT seems pretty solid. ... [It was] recognized that [the introduction of ALT testing] ... would [have] an impact [on the national blood supply] although its extent and timing is unknown. If as many as 3% of donations were lost, this would require increased recruitment effort nationwide ... This concern did not outweigh the medical, scientific, ethical, legal and public relations judgement that it was incumbent upon us to prepare to implement ALT

as a donor screening procedure in order to decrease NAB hepatitis in recipients." (See Attachment B)

Despite that meeting, ALT testing was not made mandatory in the United States until 1986. The reasons for the about-turn decision were explained in an internal American Red Cross report dated March 31, 1982 from Dr. Roger Dodd, Assistant Director Blood Services Head, Transmissible Diseases and Immunology Laboratory (See Attachment C). In his report, Dr. Dodd concluded,

> "In summary, post-transfusion NANB hepatitis has been perceived as a significant problem. However, the true magnitude of this problem cannot be properly estimated at this time. Nevertheless, it has been suggested that rejection of donor blood with high ALT levels would reduce the incidence of this infection. This proposal has not yet been tested by controlled studies. Evaluation of the expected effects of ALT testing upon the blood delivery system has identified a host of real and potential problems. The magnitude of these problems is such that a full analysis of all advantages and disadvantages of ALT testing must be performed before policy decisions are made. *Therefore, it is recommended that Blood Services should not implement donor ALT testing at this time.*" (emphasis added)

(b) After 1990

The first and second generation Chiron licensed HCV immunoassays in Australia were not as accurate as they should have been in detecting HCV in donated blood. Dr. Locarnini explains,

> 6.6. An important issue that is emerging relates to the geographical distribution of hepatitis C genotypes. The most important study on the various genotypes of HCV to date comes from the Edinburgh University. A research team supervised by Dr. Peter Simmonds, published the results of their research in McOmish et al.,*Geographical Distribution of Hepatitis C Virus Genotypes in Blood Donors: An International Collaborative Survey*

Journal of Clinical Microbiology, April 1994, 884-892. ... This ... shows that there are at least 6 genotypes of hepatitis C, classified by the McOmish Simmonds system. Genotype 1 is a common one. The original Chiron clone 5-1-1, is from genotype 1a. All these six genotypes, show strong reactivity to at least the core protein, which is the c22-3. The next most important protein in terms of antibody response is the c33c (the helicase protein). Clearly it is important in respect to genotype 1, genotype 2, 4 , 5 and 6, but is weak for genotype 3, i.e. it is only 80% reactive. The next most important protein is the c100-3. However, it is only modestly reactive even against its own strain, i.e., genotype 1a, and it is extremely weak against genotype 3. The least important is 5-1-1. In this context it is completely non reactive against genotype 5 and has extremely low reactivity against genotype 3.

6.7. The problem I have as a medical virologist in setting up hepatitis C testing in Victoria is that **only approximately 45% of persons infected with hepatitis C are genotype 1a; 5-10% are genotype 1b; and 45% are genotype 3a.** There are two sources which support these statistics. One source is the data being generated at the moment from my own laboratory at Fairfield. This is not yet available in a published format. The other source has been published in a report prepared by the Hepatitis C Task Force of which I am the chairman, entitled "*Report On The Epidemiology, Natural History And Control Of Hepatitis C*" and tabled with the NH & MRC in November 1993. This report refers to studies from Western Australia which show that of the 23 Australians in one study, 13 of 23 (56%) were genotype 1, 3 of 23 (13%) were genotype 2; and 7 of 23 (31%) were genotype 3. The study conducted at Fairfield in Victoria showed that 45% were genotype 1a; 10% were 1b; and 45% were 3a. The significant point is that genotype 3 is an important strain in Australia.

6.8. The Hepatitis C Task Force recommendations contained in the November 1993 report focus on the laboratory diagnosis of hepatitis C , case definitions, epidemiology and control mechanisms of hepatitis C in Australia. The Hepatitis C Task Force, after receiving submissions and reviewing the literature, indicated that a number of important public health issues had come to light. The Hepatitis C Task Force found that Australian strains of hepatitis C are probably different from strains circulating in the northern hemisphere. Consequently, the first recommendation of the report was that Australian research laboratories "*be encouraged to*

undertake full nucleotide sequence studies on Australian strains of hepatitis C virus". The reason for this recommendation was that cases of post-transfusion hepatitis C were occurring in the community that were being missed by the existing, "second generation" screening tests. The reason for this is still unknown today. The Ortho/Abbott second generation screening kits were introduced in Australia in May 1991. The Abbott "third generation" screening kits are presently being introduced in Australia.

6.9. **The concern of the Hepatitis C Task Force is the strong and unequivocal evidence indicating that, despite the use of second generation anti-HCV screening assays as supplied in Australia, there were antibody negative HCV infectious blood donors in Australia. So in the opinion of the Hepatitis C Task Force there is sufficient evidence to indicate that there are genotypes of hepatitis C in Australia which may not be detected by the current anti-HCV screening assays.**

6.10. In my view which is supported by the published literature, **the anti-HCV screening assay that is produced by Ortho or licensed by Chiron to Abbott Laboratories in Australia, is only 90% sensitive in relation to genotype 1a and probably much less so with respect to genotypes 3 or 5.** I have written a paper with Dr. A. Breshkin entitled *"Comparison of Three Second Generation Immunoassays for Detection of Hepatitis C Virus Antibody"* published in Australian Journal of Medical Science, Vol. 14, February 1993. The papers which support my view are as follows:-

Aach, R.D., et. al., 1991 N. Engl. J Med. 325 : 1325;
Alter, H. J., et. al., 1992 N. Engl. J Med. 327 : 1899;
Sugitani, M., et. al., 1992 Lancet 339; 1018 - 1019;
Wang, Y., et. al., 1994 J. Hepatol 21; 634-640.

6.11. **I am aware, from my position as chairman of the Hepatitis C Task Force, that the level of sensitivity referred to in paragraph 6.10 is not acceptable to blood banks in Australia.** What is acceptable is the benchmark set for HIV which is 99.4% sensitive and specific. The current Chiron anti-HCV screening assay which is no more than 90% sensitive, is clearly unacceptable and must be improved upon. Sensitivity is defined as the ability of a diagnostic test to actually find those who are truly infected to be positive, whereas specificity is to find those that are truly

not infected to be negative, so when the test is evaluated for sensitivity one finds a high risk group that are likely to be infected. For specificity, one finds a low prevalence group.

6.12. **Apart from the differences in genotypes there are many factors that affect the performance of diagnostic kits**. When I first came into this field, in the mid 1970's, I spent six months collecting hepatitis A and hepatitis B specimens from patients and purifying local reagents so that the laboratory had local strains of hepatitis B as reference material. The laboratory also had reference material from the National Institutes of Health ("NIH") in the United States of America. The laboratory ran the NIH reference material and our own reference material in parallel. That is a very important function of a reference laboratory; by obtaining all its own local material the reference laboratory can produce its own diagnostic test.

6.13. With such a diagnostic test, whether for hepatitis A or hepatitis B, it was then possible to assess how that test performed against the NIH reference panel and the local laboratory reference panel. Once the results had been obtained and interpreted it was then possible to calculate sensitivity and specificity of the diagnostic tests. In order to make hepatitis B reagents, the laboratory obtained a unit of blood from a person infected with hepatitis B and purified the surface antigens. It would then use that material as a source of antigen for the subsequent tests. For hepatitis A the laboratory would collect material from hepatitis A patients and purify that. The material had two characteristics; (a) it contained the local viral genotype and (b) it contained native viral antigen as presented to the infected host. Accordingly, because of the availability of local reagents the locally produced diagnostic kits had very high sensitivity and very high specificity.

6.14. Before molecular biology was used for the production of diagnostic kits, the diagnostic kits used clinical material that contained the whole virus. It was common knowledge in Australia in the 1970's that Abbott Laboratories used livers of marmosets infected with hepatitis A to produce HAV screening assays/diagnostic kits. This was a very simple procedure because, with hepatitis A and hepatitis B, the clinical material was abundant. With a hepatitis B carrier I could just draw out a unit of blood and I would have enough antigen to last me for six months. I could set up thousands of diagnostic kits with a unit of that blood. So laboratories used

this clinical material, from humans or laboratory animals, for diagnostic reagents.

6.15. These kits were, however, hazardous because they contained the whole virus. They carried warnings such as "this kit contains potentially infectious material". The clinical material was infectious. With human immuno-deficiency virus ("HIV") in the early 1980s this hazard became a real concern to health authorities all over the world. The diagnostic manufacturers could not draw upon HIV positive clinical material in massive batches as had been done for hepatitis B and hepatitis A. Moreover, it was too dangerous to grow HIV in the laboratory to very high titres in large volumes. In the early 1980s the first generation HIV kits used native antigen. As the laboratories had experience with native antigen from cell culture with HIV, the immune response could be adequately defined. As the laboratories found indeterminants, i.e., false positives and false negatives, the laboratories communicated their findings to the kit manufacturers such as Abbott Laboratories or Wellcome. The kit manufacturers then further developed and refined the kits and improved their performance. That feedback was a very important part of the evolution of the HIV kits. By the end of the first generation of HIV kits, they were about 98.4% sensitive and specific.

6.16. Subsequently, the introduction of molecular biology in the production technology of diagnostic kits saw kit manufacturers mainly using *E.coli* and yeast systems to express HIV antigens. It was found that these antigens worked even better than the native antigens for HIV. These kits do not use native antigens, i.e. clinical material. They used fusion proteins. The *E.coli* produces a protein that is fused with HIV proteins. A protein is a three dimensional molecule, so that when a person becomes infected by a virus, a person's immune response is to the whole native antigen and antibodies are produced to the epitopes contained on the native antigen. With *E.coli* or a yeast expressed protein, the fused protein that is produced is not necessarily conformational as it is in its native original form. A fusion protein is unlikely to retain the original native conformational epitope. As has been found with HIV this may not necessarily affect the performance of the screening assay/ diagnostic kit, since linear epitopes that are part of the fusion protein used in HIV kits are adequate in producing an antibody-antigen reaction. This is probably due to the form in which HIV epitopes exist *in vivo* (the natural setting).

6.17. For hepatitis C, however, the same may not be true. **With hepatitis C it is now suspected, due to the fact that anti-HCV screening assays/diagnostic kits have not achieved sensitivity above 90% and the numbers of indeterminants that have been recorded, that many of the immune responses are conformation dependent.** In other words there are nuances of the three-dimensional folding which are critical to antibody detection. It is extremely difficult to reproduce the natural three dimensional folding of a protein containing an epitope in *E.coli* or yeast fusion protein systems. With such systems all that is produced is a linear epitope of one small fragment of the genome of a strain of HCV inserted in the *E.coli* or yeast expression protein. The difficulty with hepatitis C is that a person is not infected with *E.coli* containing clone 5.1.1 proteins, or c-100-3 proteins; that person is infected with the whole native hepatitis C virus of a particular genotype.

6.18. There is also evidence emerging that some third generation kits such as the Abbott Laboratories anti-HCV diagnostic kits have no greater sensitivity than the second generation kits, even though the manufacturer has included longer fusion protein inserts (i.e., more genetic material) into the kits.

6.19 In other words, improvement to kit performance is dependent to some degree on epitope mimicking and with conformational dependent viruses, such as the hepatitis C virus, simply inserting more genetic material into the kits is not the answer.

6.20. What is becoming apparent with hepatitis C is that an epitope from the core region of the genome of the hepatitis C virus is almost certainly a linear epitope since all genotypes of HCV produce a strong immune response to that region. In other words, it is not genotype specific. It is noteworthy that this region is highly conserved between different Hepatitis C isolates. By contrast the evidence suggests that the envelope proteins and, the c100-3 and the 5.1.1 proteins, which are not derived from conserved regions, are clearly strain dependent or genotype dependent and are also probably conformation dependent. This is a very important principle of serological diagnosis.

7.1. Fairfield uses the Murex anti-HCV diagnostic kit as a secondary kit. Any reference laboratory has to have a number of kits or a number of testing ranges, not just one or two for a particular disease. For HIV for example, we have to use up to ten tests on the one sample to confirm its status as

truly positive. We have two screening tests, one supplementary and four confirmatory plus culture. With hepatitis C, as with HIV, all initial repeat reactors detected in the screening are tested in another test and a positive result must be obtained with the other tests before the result is classified as positive i.e., "antibody detected by EIA". Otherwise the result is classified as an indeterminant.

7.2. In my opinion it is a necessary policy for a reference laboratory like Fairfield to have more than one diagnostic kit available. This is in the best interests of public health. This is especially true for hepatitis C where, as discussed above, there are present in Australia at least three genotypes of HCV. Genotype differences are more likely to cause false negatives. This means that persons with HCV infection may be missed and infected blood may be transmitted to other persons.

In his second affidavit Dr. Locarnini answered a criticism suggesting that his complaints about the adequacy of the Chiron licensed HCV immunoassays were unfair since this test was better than no test as was the situation during the 1980's. Dr. Locarnini explains,

4. The question which Dr Beal poses is this: Is a test better than no test? In my opinion, this is not the right question. There is no doubt that, prior to the first generation anti-HCV test kits, the risk of being infected with PT-NANBV as a consequence of receiving a blood transfusion was much higher than today. However, in my opinion, there remains an unacceptable risk, even with the use of subsequent generation anti-HCV test kits, of hepatitis C being transmitted through blood transfusions i.e., false negatives. A more important issue for blood banks in Australia, however, is the number of blood donors permanently stood down by blood banks of blood donors as a consequence of indeterminate results or false positive results from the present anti-HCV test kits. Blood donors are a low risk group for HCV.

5. In a letter recently published in the Medical Journal of Australia Vol. 163, 2 October 1995 entitled "A positive hepatitis C enzyme immunoassay antibody test in a low risk population: what does it mean" the authors state "*The introduction of screening of all blood donations for antibodies to the hepatitis C virus (anti-HCV) by enzyme immunoassay (EIA) has reduced the number of cases of post transfusion hepatitis C. Current third*

generation EIAs typically include antigens from the structural region (capsid) as well as one or more antigens from the non-structural region of the virus (NS3, NS4 or NS5). Such assays are highly reliable among individuals with risk factors for or symptoms and signs of hepatitis C virus infection, but the false positive rate remains a significant problem when a low risk population (such as blood donors) is screened....A definitive diagnosis cannot be made from a positive anti-HCV EIA test result in a healthy asymptomatic individual with no risk factors for HCV infection and a normal ALT." (emphasis added)

6. **A significant finding by the authors of the said letter was that with third generation anti-HCV EIA a repeatedly reactive test result was "*interpreted as false positive reactions in approximately 75% of cases*".**

7. Blood banks in Australia and elsewhere are losing blood donors permanently. This means that the source of blood needed on a daily basis by the Australian community and other communities, is being seriously threatened. Once a blood donor is labelled as an HCV-indeterminate or HCV positive, their blood is excluded from the blood supply, even though they maybe truly negative for HCV. In other words, blood donors are being falsely labelled as "HCV positive" when in fact they are not because of the inadequacies of the present anti-HCV test kits.

8. The fact that third generation anti-HCV test kits are giving such results is really saying something: it means in a low risk group such as blood donors, the present generation anti-HCV tests are detecting something other than HCV and giving false positive results in up to 75% of cases. **It has been five years since the first anti-HCV test kits were first used in Australia and the manufacturers of these kits have not yet produced a kit which is as sensitive and specific as the test kits for HIV.** This is clearly unsatisfactory.

9. What must be understood is that the test results from anti-HCV test kits need to be interpreted before a final conclusion can be reached as to whether a person does or does not truly have HCV. It is not simply a matter of testing a person's blood with a test kit. The background of that person is relevant. If that person comes from a high risk group, such as injecting drug users and a positive result is obtained, then one can conclude with a high degree of certainty that it is a true positive result, but if a person is from a low risk group, the same is not true.

10. A positive diagnosis drastically affects peoples lives. Once people are labelled HCV positive, their blood is lost to the community if they are a blood donor; they are referred to a liver clinic; their private lives are affected; their relationships are affected; their insurance policies are affected; their quality of life is affected. In the case of a false positive, this to me is unacceptable and a great deal of research must be undertaken to encourage improvement in the specificity of these tests.

11. When you contrast the developmental history of HIV tests with HCV tests the reasons for my concern are more readily apparent. With HIV the specificity and sensitivity was in the high 90s very quickly together with a confirmatory strategy that worked with the Western Blot. That has not happened with HCV, so clinical laboratories have had to struggle with the false positives issue.

12. **In my opinion the current anti-HCV tests are better than no test, but that is not the point. Once you have a test, the test needs to be highly sensitive and specific and the current tests are not as sensitive nor specific as they need to be.**

13. On 8 November 1995, at the 12th National Workshop on Retrovirus Testing my team at the Victorian Infectious Diseases Reference Laboratory presented a paper about the results of our research. What my colleagues and I did was to prepare a panel of 180 samples which were tested by the Roche PCR Amplicor as well as our in house PCR in order to determine the true HCV status of those serum samples. All samples were repeatedly positive for HCV RNA in both assays. In other words they were all truly HCV positive. My colleagues and I then tested these samples against nine anti-HCV assays available in Australia in accordance with the manufacturers instructions. What was interesting was that none of the nine anti-HCV assays detected all 180 samples as reactive. One sample was non reactive in all nine anti-HCV assays. When we genotype tested this particular sample we found that the strain was HCV genotype 3A. Another sample was non-reactive in six of the assays and this sample was HCV genotype 1b. One of the assays gave non-reactive results for eight samples.

14. **In my opinion, the sensitivity of these tests can be improved if the kit manufacturers would include reagents from genotypes other than from strains HCV 1a and 1b. In the Australian community HCV strains 1a, 1b, 3, 4 and 6 are present because of ethnic**

diversity of the population. In my opinion, the kit manufacturers need to develop and include better conformational antigens for all strains of HCV in their kits. Specificity is the real problem with HCV test kits and much more research is required before I am satisfied that anti-HCV test kits are sufficiently reliable in Australia.

22. In a paper "Study on Reliability of Commercially Available Hepatitis C Virus Antibody Tests" Feucht et al published in the Journal of Clinical Microbiology, March 1995, p 620-624. The authors state, *"First generation HCV enzyme immunoassays (EIA) detected only antibodies against nonstructural region 4 (NS 4) with recombinant antigen c100-3. The tests of the second generation, the Abbott HCV 2.0, used additional antigens of the core region (c22-3), the NS 3 region (c33c), and (especially the Ortho RIBA 2.0) a part of the c100-3 (named 5-1-1) from the NS 4 region."*

23. The Abbott HCV 2.0 assay is the assay marketed by Abbott Laboratories in Australia under licence from Ortho and Chiron. These assays included antigens of the core c22-3 region. Despite the inclusion of the c22-3 core antigen in the assay the authors reported, *"In daily diagnostic work, the commercially available Abbott second generation HCV EIA and the supplementary Ortho RIBA 2.0 often do not yield clear indications of whether an HCV infection exists."*

24. The authors describe the study which they conducted and their results. They then concluded at page 623: *"One of the major problems in daily routine work is to decide whether a patient is HCV infected. In a number of cases, the commercial tests did not help us to decide whether an HCV infection existed...Until now, the absence of a "gold standard" for HCV antibody tests has made it difficult to compare the sensitivities and specificity's of diagnostic assays.* ***The lack of detectable specific antibodies against HCV does not exclude the possibility of an infection, especially during the acute phase of infection or of patients who are immunosuppressed because of transplantations, cancer, human immunodeficiency virus infection, or chronic hemodialysis, for example....A worldwide standard for HCV antibody testing should be established. Our results show that the commercially available HCV antibody tests need to be improved."*** I agree with this conclusion. In my opinion, the results of this research apply in Australia.

25. In a paper by Mayumi et al entitled "Hepatitis C virus antibodies among blood donors in Beijing" published in Journal of Hepatology, 1994; 21: 634 - 640 the authors describe a study that they conducted among blood donors in Beijing using the second generation EIA's. They stated in their paper that: *"Although EIA-1 has been useful for the diagnosis of acute and chronic hepatitis C and for excluding blood units contaminated with HCV, it is neither very sensitive nor specific. EIA-2 has overcome most of these shortcomings, but is still limited. In particular, the sensitivity of EIA-2 in detecting viremia is not satisfactory in the low-risk populations typified by blood donors.* ***Approximately 10% of post-transfusion non-A, non-B hepatitis cases cannot be prevented by screening blood units by EIA-2.*** *These patterns require a more direct method to identify viremia, by detecting either viral RNA or viral proteins, like hepatitis B virus DNA or hepatitis B surface antigen used for the diagnosis of ongoing HBV infection."*

26. **After more than five years since Chiron/Ortho/Abbott first released their HCV EIA, the lack of a "gold standard" for HCV EIAs is a significant public health issue throughout the world.** This is in sharp contrast to my experience with the development of HIV tests. With HIV there were many more diagnostic manufacturers competing to make HIV tests. The dialogue between clinical laboratories and reference laboratories and diagnostic kit manufacturers was such that the sensitivity and specificity of HIV testing reached 99.9% very quickly. My experience with HCV has been to the contrary.

27. The only diagnostic kit manufacturer that has used antigens from a different strain of HCV is Murex Diagnostics. All other kits use antigens from HCV strain 1a. Murex uses HCV strain 1b. In Australia, we have a diverse ethnic population in which HCV strains 1, 3, and 6 exist. The only diagnostic kit manufacturer that has produced a serotyping assay is Murex Diagnostics.

28. **In my opinion, without competition amongst diagnostic manufacturers it is unlikely that this real public health issue will be addressed in Australia or in other parts of the world with different genotypes, for example, Egypt with genotype 4 or South Africa with genotype 5. There is a real need to encourage further research and development in the HCV diagnostics market both in Australia and the world.** The existence of the patent in suit and the

manner in which Chiron has restricted licensing has had a detrimental effect on the development of more sensitive and specific HCV diagnostic assays.

Dr. Simmonds from the University of Edinburgh explains,

3.5 In about 1992 I began to realise that there was a significant problem in the nomenclature used to name different isolates of HCV. Different laboratories throughout the world used different classification systems to identify their isolates. This made a comparison of different HCV research results difficult. In collaboration with Dr. Michael Urdea of Chiron Corporation, we proposed a nomenclature that could be used to classify all HCV isolates. That nomenclature was subsequently published in 1994 in a letter to the editor of the Journal Hepatology. The letter was joint authored by 22 other leading researchers in the HCV field, which Dr. Urdea and I consulted [Simmonds. P., et al., (1994) "A proposed system for the nomenclature of Hepatitis C Viral Genotypes" Hepatology, 19 (1), 1321-1324].

3.6 Research effort for my HCV research group is now concentrated on the exploration of sequence variability of HCV and its effects on pathogenesis, diagnosis and treatment. The level of sequence variability in certain regions of the HCV genome is extremely high. All currently known variants can be classified into six major phylogenetic groups corresponding to genotypes 1 to 6 in the proposed nomenclature with sub-types within them.

3.7 The Department of Medical Microbiology at the University of Edinburgh where I work carries out a range of diagnostic tests on clinical specimens from the Royal Infirmary of Edinburgh, and elsewhere. My colleagues and I currently receive approximately 16,0000 samples for HIV, HBV and HCV testing/year, 12,000 samples for virus culture and/or direct virus detection, and 5,000 samples for other virus serology. The laboratory in which I work is a regional reference center for chlamydia, hepatitis and HIV-1 testing and confirmation. Working in such a clinical laboratory has enabled me to gain research experience in all aspects of routine diagnostic virology, including cell culture and virus isolation, immunofluorescence, electron microscopy, ELISA and other serological techniques, and result interpretation.

5.2 The greatest sequence variability amongst all HCV genotypes arises in the envelope region. In that region there is only about one-half predicted amino acid sequence identity between the different genotypes. The core and 5' non-coding region (5' NCR) are the most highly conserved. Elsewhere, sequence variability is approximately evenly distributed throughout the remaining viral genes. Antibodies elicited by infection with one HCV genotype as the extent of variation observed could possibly fail to neutralise other HCV genotypes. The extent of variation observed within HCV is comparable to that between serotypes of other RNA viruses. HCV differs from most RNA viruses in its ability to establish chronic infection and progressive disease in a large proportion of those exposed to it. The mechanism for this remains uncertain.

5.3 Some genotypes of HCV (types 1a, 2a, 2b) show a broad worldwide distribution. Others such as type 5a and type 6a are only found in specific geographical regions. Understanding the current distribution of HCV requires knowledge of routes of transmission, historical data on the prevalence and risk groups for HCV, and the time of the divergence of the major genotypes and sub-types. We know very little about any of these factors. It is difficult to draw conclusions from existing information. The problem is compounded by the scarcity of information concerning genotype distribution in many geographical regions such as Africa.

5.4 In the United States of America there is predominantly HCV type 1a and 1b. In Europe (including the United Kingdom) there is predominantly HCV types 1a, 1b, 2 and 3. In the Middle East there is predominantly types 3 and 4. Individuals from countries like Pakistan, India and Bangladesh are most frequently infected with and other subtypes of type 3a. Type 4 is frequent in central Africa, type 5 is frequent in South Africa and type 6 is frequent in South East Asia (including Vietnam). In Australia there are basically 3 HCV types, type 1, type 2, and type 3. I believe type 5 and 6 have also been reported in Australia. There are no strict geographical boundaries limiting the various HCV types.

6.1 Different immunological screening assays use antigenic material from different sources. The Murex HCV assays uses antigenic material derived from a human donor in the UK and is of HCV genotype now known as type 1b. In the United Kingdom genotype 1b is one of the most prevalent genotypes. That is the sequence that Murex first isolated and that Murex antigens are based on. **The Chiron based assays use antigenic**

material derived from a chimpanzee in the USA and is of HCV genotype type 1a, which is the most predominant type in the USA.

6.2 Part of the research we have conducted on the distribution of HCV throughout the world has focused on determining the efficiency of the early immunoassay kits. We found that antigens that were used in first generation immunoassay kits exhibited variable degrees of efficiency in identifying HCV, depending on the genotypes present. In a scientific paper written by my colleagues and I entitled: "Mapping of Serotype-Specific, Immunodominant Epitopes in the NS-4 Region of Hepatitis C Virus (HCV): Use of Type Specific Peptides to Serologically Differentiate Infections with HCV Types 1, 2 and 3". Simmonds P., *et al.*, Journal of Clinical Microbiology (1993), 31(6), 1493-1503 we observed *".... the consensus subtype 1a sequence of the first antigenic region (residues 1691 to 1708) differed from subtype 2b at 9 of the 18 amino acid residues and from type 3 by 6 residues. In the second antigenic region (1710 to 1728), sequences of types 1a, 2b and 3 differed from each other by 7 to 12 of 19 residues.* ***This specific difference in antigenicity between peptides corresponding to the two regions is consistent with the absence of serological reactivity of most sera from HCV type 2- and 3- infected individuals to the recombinant protein 5-1-1 (8,33), which extends*** *from residues 1694 to 1736 and therefore includes part of the first antigenic region and all of the second (2).* ***Similar type-specific reactivity has been also observed with the longer c100-3 protein, which includes all of these residues, those upstream in NS-3 and those downstream towards the end of NS-4..."*** (Emphasis added)

6.3 **Thus by using polypeptides derived from clone 5-1-1 and c100-3 sequence information, disclosed in the Chiron patent specification, in an immunoassay kit we failed to identify many type 2 and 3 infected individuals.**

6.4 My colleagues and I found that a large proportion of infected individuals were missed by the early assays (based on the Chiron's sequence data) because they contained antibodies generated against HCV genotypes other than type 1. If you incubate a type 2 serum against type 1 peptides containing 5-1-1 epitopes, recognition of the serum is generally very weak or may not be observed at all.

6.5 Subsequent research conducted by my colleagues and I examined the variability of nucleotides and amino acids in the NS-4 protein around the

5-1-1 region in six different genotypes of HCV. Amongst the findings made in those studies we showed substantial amino acid variability between the six HCV types in the NS-4 protein region. From this information we prepared a phylogenetic analysis of a 342 nucleotide region of NS-4 which summarised the degree of variability observed within the 6 HCV genotypes. The results of this research, including our phylogenetic analysis, were published in a paper entitled "Use of NS-4 peptides to identify type-specific antibody to hepatitis C virus genotypes 1, 2, 3, 4, 5 and 6*". Bhattacherjee V., *et al*, (1995) Journal of General Virology, 76, 1737-1748. This research showed that substantial amino acid diversity exists between the HCV genotypes in the region corresponding to clone 5-1-1 (amino acids 1694 to 1736).

6.6 The phylogenetic differences observed in the NS-4 region of the isolates examined in Bhattacherjee V. *et al.,* (supra) are, in fact, mirrored in other subgenomic regions of the subgenomic structure of different HCV genotypes. NS4, NS5, E1 all produce the same phylogenetic tree. This observation reflects the degree of nucleotide variation that exists between the different genotypes of HCV.

6.7 The results from this type of research largely reflect the practical results that various users of the early generation testing kits found. When the first generation assays were released the problem observed by my colleagues and I when using the kits was not their sensitivity for type 1a HCV genotypes but their inability to reliably detect HCV infections by genotypes other than type 1a.

6.8 In essence these observations were the stimulus for some of my current research. That is to develop immunoassays that are sensitive to all HCV genotypes and immunoassays that are capable of distinguishing between the various HCV genotypes.

6.9 My colleagues and I started this research by examining the extent to which antigenic variation between genotypes affects the sensitivity of two 3rd generation enzyme immunoassays (Ortho EIA 3.0 and Murex VK48). We investigated the influence of viraemia status, HCV genotype and host factors such as age, gender and risk group upon antibody levels in a consecutive series of 117 anti-HCV positive volunteer blood donors. The results are discussed in a paper entitled "Influence of Viraemia and Genotype upon Serological Reactivity in Screening Assays for Antibody to Hepatitis C Virus" Dhaliwal S.K. *et al.,* Journal of Medical Virology (1996)

48,184-190. Our results showed lower serological reactivity to the type 1a (Ortho EIA 3.0) or 1b (Murex VK48) antigens used in the immunoassay kits with samples from donors infected with non-type 1 genotypes. In particular we observed the following *"By multivariate analysis no evidence was found for any significant effect of age, risk group or gender upon antibody reactivity, while the genotype differences were shown to be independent of the differences in antibody level between PCR-positive and negative samples (see above). The magnitude of the difference in levels provides an estimate of the relative amounts of genotype-specific and cross reactive antibody to the core, NS-3, NS-4 and NS-5 antigens. For* ***example the 4-4.5 fold difference between type 1 with types 2 and 3 infected donors could be interpreted as indicating that a major proportion of antigenic determinants in the EIA's are genotype-specific. This conclusion is consistent with the finding of type-specific epitopes in core, NS-4 and NS-5 regions*** *(Machida et al., 1992; Simmonds et al., 1993, Tanaka et al., 1994; Bhattacherjee et al., 1995; Zhang et al., 1995)"* (Emphasis added).

6.10 **This research provides grounds for concern that antigenic variability of HCV impairs the performance of current assays. It also shows that antigenic variability is a problem, dictated by genotype and phenotype (i.e. nucleotide and amino acid) differences amongst HCV's**.

6.11 The significance of our serology data and our examination of the kits is really placed in perspective when one considers countries such as Australia and South East Asia which do not predominantly contain HCV type 1 infected individuals. **A high proportion of the blood donors in Australia are infected with HCV type 3**. I am aware from discussions with my colleagues in Australia that there is a clinical suspicion that the present diagnostic assays are actually missing many HCV type 3 infected individuals. **This is not a major issue in the United States, most of Southern and Western Europe and Japan, where the screening assays appear to be most extensively used because these countries generally only have type 1 HCV infected individuals.**

Dr. Nicholas Crofts then of the Macfarlane Burnet Centre for Medical Research in Melbourne explains,

21. During discussions I had with Dr John Barbara, Head of the North London Blood Transfusion Service, Dr Phillip Mortimer, Director of Virology at the Central Public Health Laboratories and Professor Richard Tedder I was told that in **the UK, the Health Department made a decision, that was a carefully discussed and calculated decision, not to use the first generation HCV assays when they were first released by Ortho in 1990. The UK blood banks did not start screening for HCV until the second generation assays were introduced, some eighteen months later. This meant that during that period there were a number of people that were potentially infected with HCV as a result of receiving blood transfusions.** The justification for this decision was based on the concern of the UK blood banks that too many blood donors would be lost from the already over stretched blood supply. **They recognised that the first generation tests would produce too many false positives and that would place enormous pressures on the UK blood supply to keep up with demand for blood.** There was also concern about the potential misunderstanding that could occur in explaining to HCV positive blood donors that they may not in fact be truly HCV positive. The social consequences of positive results that could not be confirmed also influenced their decision.

22. The reality is that most blood banks around the world have less blood on hand than is actually needed at any point in time. Blood banks cultivate donors who regularly donate blood for many years. These donors are a very valuable resource. The UK blood banks realised in 1990 that once a positive HCV assay result was obtained (irrespective of the fact that the first generation HCV assay lacked specificity), those donors would be lost forever as it was not be possible to determine which donors were truly false positives.

23. The statistical reality is demonstrated by this example. If I take a high risk person such as a drug user and test for HCV with an assay that is 99% specific and a positive result is obtained then I would believe that positive result. If I apply that same assay to a low risk person such as a blood donor and the result is positive the chance that the result is a true positive is in the order of 1 in 10 to 1 in 100.

24. The Australian blood banks, however, did not hesitate in 1990 to introduce HCV screening because of the potential for litigation. Most Australian

blood banks at that time were being sued with respect to HIV transmissions in the 1980's.

25. Nevertheless, the Australian blood banks cannot afford to lose regular blood donors unnecessarily. The Melbourne Blood Bank has blood reserves of one day. It obviously very important to remove real HCV positive donors from the blood supply but the cost of achieving this is that for every real positive blood donor that is removed at least nine blood donors are removed that are not HCV positive.

26. The numbers are small but because the margins in blood bank reserves are also small it is imperative that these numbers be further reduced. The only feasible way that this can be achieved is through the implementation of the standard adopted for HIV testing with respect to HCV. One of the significant deficiencies in HCV testing in Australia at the moment is that all of the anti-HCV assays except for assays manufactured by and supplied by Murex use exactly the same genotype (1a) proteins. I have been informed by my colleagues that these assays are manufactured or supplied by Chiron licencees. Murex uses proteins from strain 1b. However, we also need in Australia, HCV assays that use proteins from other strains such as genotypes 2, 3 and 6 because we have a very diverse ethnic population base. HCV 1 and 3 are the most prevelant. HCV 6 is mostly in the Asian community. No firm conclusions can yet be reached but there is no doubt in my mind that much more research is needed to be done about the genetic deversity of HCV and how this impacts on the specificity and sensitivity of current HCV testing. There are still many unknowns about HCV; much more than with HIV.

27. Despite recent reports that attempt to show that HCV assays in Australia are 100% specific and sensitive, in my opinion it is impossible to achieve this result. The HIV assays are the most specific and sensitive assays in the world today. There is no doubt that the HCV assay manufacturers should work to achieve the same levels, but the answer to the problems that I have already discussed are in the standards that have adopted for positive HIV results from low risk populations that is, it is necessary to have a confirmatory assay or assays.

28. Sensitivity is not solely characteristic of the assay because the same assay will work differently with different sera because of cross reactivity with contaminants in the sera. Accordingly it would be undesirable to use only one type of assay.

29. Then there is the effect of different antibody window periods. Between the time a person is infected and the time that detectable antibodies are produced to infection there is a window when these types of assays cannot be used to detect infection.
30. The only way to overcome the problems of detection during window periods is to use an antigen test i.e., a test that detects antigen and not antibodies to the antigen. Ideally this is what we should be testing for with HCV. However, to produce an antigen test assay manufacturers must be able to grow the virus in commercial quantities *in vitro*. This is not yet possible with HCV.
31. It is important to note that even with HIV assays that have achieved a sensitivity of 99%, a positive blood screening result is not the only test that is conducted on positive blood samples. Once the blood bank has a positive HIV result from a donor, that sample is subjected to further tests to confirm the initial blood screening test result, such as a Western Blot HIV test. The same needs to apply with respect to HCV.
32. In Australia today there are at least 100,000 people infected with HCV. Those infected people can live for another twenty or thirty years and not all of them will die of liver failure, but the same sort of numbers will die from HCV as will die from HIV infection. The period of illness with HCV is much longer and some people will need very expensive medical treatment, such as a liver transplant to keep them alive. The impact on the community in terms of social and economic cost is, in my opinion, at least equal to HIV and yet we know much less about HCV than what was known about HIV for the corresponding period. There are many reasons for this including the level of government intervention and the political priority given to HIV as compared to HCV, but in my opinion, there has been a significantly lower level of cooperation with HCV assay manufacturers than there was with HIV in all respects. The consequences of this are now being understood. While with HIV the increase in infections has fallen with HCV it is still rising. At the present moment in Australia that is rising at a level of 8,000 to 10,000 people per year. When you extrapolate this into social and economic cost terms, the cost is very significant.
33. In my opinion it is against the best interests of the Australian community that only one type of anti-HCV assay be permitted to be manufactured and supplied using the same antigen produced in the same way. We need

many different HCV tests made in different ways for the reason that I have given.

34. Despite the fact that the highest risk group for HCV infection is injecting drug users, it is worth noting that HCV can be spread in ways that we are still finding out. As an example, recently Italian scientists published the results of a study which found that when they took acute cases of hepatitis C and investigated the risk factors within the previous six months from infection that about 9% of those infected with HCV had no other risk factors other than a history of dental work. Another group of Italian scientists followed this study up by taking about 37 HCV PCR positive people who were about to have dental work. After their dental work this team went into the dental surgery and swabbs all surgery surfaces. They found HCV on about 15% of the swabbs. Dentists do not disinfect a surgery between patients. HCV contaminates dental surgeries by the action of high pressure water sprays on gums that are bloodied. The blood is aerosolised in this way and the contaminated blood then goes every where. Handles on the light that the dentist touches between patients is a source of contamination. Dentists may wear gloves, but light handles and some other surfaces are often not disinfected between patients.

35. In 1995 the Public Health Branch of the Victorian Government of Health & Community Services published a report entitled *"Management, Control and Prevention of Hepatitis C. A Strategy for Victoria"*. I wrote the section entitled "Prevention and Control of HCV Infection". At page 25 of this report I wrote about the fact that HCV can and is being spread to populations other than injecting drug users. I stated in this report *"Of concern is the evidence of continued HCV transmission in populations and settings where harm reduction strategies are in place and where HIV is not spreading, such as clients of needle and syringe exchange programs and methadone maintenance patients. All people who are currently injecting are at risk of further infection. Even among those who are already infected, there is some evidence that they may be at risk of further infection with different genotypes of HCV. Hence, strategies, must also address the prevention of reinfection of individuals already exposed to HCV. Other target groups include tatooists, people having tattoos outside registered agencies, other engaged in skin piercing procedures (such as ear piercing and acupuncture), heath care workers (in relation to their own*

risks and to the risks of transmission in the health care setting from patient to patient) people engaging in sports where bleeding may occur, and recipients of blood products. Young people should be targeted in order to discourage risk behaviour."

36. I have also co-authored a paper, with my colleague Dr Kaye Brown, that has been submitted for publication to the Medical Journal of Australia concerning economic costs to Medicare of the continuing HCV epidemic among injecting drug users. This paper forms the basis of a report to the Commonwealth Department of Health. Dr Brown is an economist at the School of Public Health at the Queensland University of Technology. The objective of our study was to estimate the direct health care costs to Medicare of a continuing epidemic of HCV infection among injecting drug users in Australia. We concluded from our study [that] *"For every 1,000 chronic carriers of HCV there is an implied $14.32 million in health care spending over the years as sequelae become manifest, with cumulative total costs of approximately half a billion 1994 dollars after 60 years. If the estimated 10,000 new HCV infections in injecting drug users in Australia per year continue for the next 60 years, total health care costs will be around $4 billion over that period."*

37. This report is not a definitive statement on the total costs to Medicare of the continuing HCV epidemic in Australia because it focuses on injecting drug users. This is merely a first step in that process. I am not able to exhibit this paper to my affidavit as it has been submitted for publication.

38. Even though the greatest increase in HCV infection is occurring among injecting drug users, we as a community, should not be complacent about the transmission of this disease throughout the community generally. Although HCV is different to HIV in the manner in which it is spread i.e., sexually (although it is possible for HCV to be spread sexually it is far more unlikely than with HIV) it is important to realise that there is evidence that HCV can be spread ways that HIV is not spread i.e., HCV contaminated surfaces in dental surgeries. It is also important to realise that the political response to HIV has been greatly influenced by a very vocal lobby group that is politically sophisticated and by the immediacy of AIDS after infection from HIV. The lack of a political response to the spread of HCV among this community is a reflection of the fact that injecting drug users are politically disenfranchised as a group and that the consequences from infection to HCV are not as swift or apparent as with

HIV. However the lack of a political response cannot be regarded as an accurate measure of the affects of the HCV epidemic to Australian society today and in the future.

39. Dealing with the HCV epidemic requires the coordination of many fields in disease control at both a political and medical level. It also requires that we learn from our experience with HIV. **In my opinion, the HCV diagnostic process will be considerably harmed if the only anti-HCV assays available to Australian laboratories are those which use the same antigens. It is absolutely essential that confirmatory assays be available to Australian laboratories.**

This submission demonstrates that despite the fact that since the late 1970's the scientific means have been available in Australian and internationally to detect NANBH or HCV as it is now known in donated blood that blood banks and governments in Australia and internationally failed to act in the best interests of public health. This is evident both prior to and after the identification of the causative agent of HCV, a virus in 1987. Despite the grant of a patent to Chiron and the introduction of Chiron licensed HCV immunoassays in 1990, post-transfusion HCV continued to be spread through the blood supply in Australia. The full extent of the damage caused unnecessarily to innocent people will not be known until the Federal Government calls for a Royal Commission which will have the appropriate powers to demand the production of all relevant documents and witnesses. Without doubt, the failure of governmental agencies to use power reserved to them under the Patents Act 1990 contributed to the situation. Moreover, Chiron's failure and refusal to license the manufacturer of HCV immunoassys that did not use the Chiron HCV polypeptide also contributed to this situation.

The fact that the first and second generation Chiron licensed HCV immunoassays failed to detect 99.4% of infected HCV blood and human body-organ donations was confirmed in a US House of Representatives Committee Report entitled

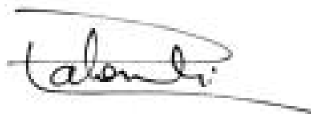
"Hepatitis C: Silent Epidemic, Mute Public Health Response" dated October 15, 1998 (See Attachment D). At page 11 of this Report the Committee reported,

> "The sensitivity of the first generation test was 84-89 percent, while the sensitivity of the second generation test was 93-95 percent. The specificity of the first generation test was 22 percent, while the specificity of the second generation test was 30%."

It is clear from this Report that the failure of Governmental Health Agencies to act appropriately with respect to reducing the spread of HCV infection has not been isolated to Australia.

Although this Report suggests that the inaccuracy of the first and second generation Chiron licensed HCV immunoassays were responsible for high numbers of false-positives, implying that the problem with the tests was not so much in increasing the risk of spreading HCV infection, but in inappropriately removing uninfected but test positive donors from the blood supply, this submission suggests that the evidence presented both in the Murex v Chiron case and in this submission prove otherwise. The problems associated with the accuracy and therefore predictive value of these tests have resulted not only in high numbers of false positives, but also in an inappropriate and completely unnecessary incidence of HCV infection spread after 1990 through the receipt of blood, blood products and human donor organs.

Signed in Sydney on December 8, 2003



Luigi Palombi LL.B., B.Ec.
PhD Candidate,
Faculty of Law,
The University of New South Wales,
Kensington, N.S.W., Australia