

# THE SAFETY OF THE BLOOD SUPPLY

*Y. W. Ong and D. Teo*

Since the 1980s, widespread concern about AIDS has mobilised tremendous efforts to eliminate transfusion-transmitted disease (TTD). In spite of these global efforts, the ideal of a zero-risk blood supply is still not achievable. Meanwhile, a concerned public and the media demand that more be done to improve blood safety.

TTD has influenced the way in which transfusion medicine has been practiced. It has i) changed policies on blood collection (e.g., increased use of autologous blood and demand for directed donations); ii) imposed new and more stringent criteria for donor selection; and iii) mandated improved testing of donated blood with newer and more sensitive tests.

A multi-pronged approach is needed to improve the safety of the blood supply. This should include stringent donor selection criteria, pre-donation interviews designed to exclude donors at risk of transmitting infectious diseases, the use of appropriate technology for screening infectious agents in donated blood and education of both donor and public on the need for safe blood. Recruiting, educating and retaining safe blood donors is an important component to blood safety efforts. Emerging blood-borne infections that pose new threats to blood safety have to be considered. Organisations such as the World Health Organization (WHO) and the International Federation of Red Cross and Red Crescent Societies have assisted many countries in blood safety initiatives.

In developed countries, the residual risk of TTD has been reduced to very low levels. However, in a study carried out to define problems in and to formulate solutions for improving blood safety in developing countries,<sup>(1)</sup> it was reported that, despite improvements between 1988 and 1992, only 66% of developing countries (DGC) and 46% of the least developed countries (LDC) screen all blood donations for antibodies to HIV. Seventy-two percent of DGC and 35% of LDC test all donations for hepatitis B surface antigen. Syphilis testing was carried out in 71% of DGC and 48% of LDC. Many developing countries still rely heavily upon paid and replacement blood donors.

Thus, improvements that can be made to blood safety vary and depend on the socio-economic state of development of the country and the competing priorities for limited health care resources.

## **Human Immunodeficiency Virus (HIV)**

In 1982, the U.S. Transfusion Safety Study Group estimated the risk of contracting HIV through a blood transfusion in San Francisco as 1 in 90 products transfused. The current estimate of residual risk of HIV infection through blood transfusion in the U.S. is less than 1 in 450,000-660,000 donations. Between 18,000-27,000 donations per year would be infectious for HIV but remain undetected by the currently available screening tests.<sup>(2)</sup>

### **p24 HIV Antigen Testing**

There is still a small but identifiable risk of transfusion associated AIDS (TAA) due to the transmission of HIV virus by blood that has tested negative for HIV antibody. Most reported cases of TAA have occurred from screened blood donations collected during the early infectious window period from persons who had recently contracted HIV.<sup>(3)</sup> In countries where the rate of HIV infection is still rising rapidly, the pool of potential donors who are within the window period would be correspondingly larger. In the U.S. it is estimated that donor screening with HIV-1 antigen can be expected to prevent up to 25% of window period cases of TAA per year.<sup>(4)</sup>

The Food and Drug Administration (FDA) recommended in August 1995 that all donated blood and plasma be screened for HIV antigen within three months of the licensing of a test labeled for such use. The FDA recommended p24 antigen testing as an additional safety measure because (i) recent studies had shown that the p24 antigen test reduced the infectious window period by an average of 6 days;<sup>(5)</sup> (ii) it is expected to detect 5-10 HIV infected units (among the 12 million donations made in the U.S. each year) which would not be detected by current screening tests; (iii) it would further reduce the risk of HIV infection in the recipients of blood products, as antigen testing would result in the removal of an estimated 7-11 HIV-1 contaminated components per year; (iv) it would reduce the HIV-1 virus load in plasma pools for fractionation. p24 antigen testing should be regarded as an interim measure until the development of new technology that will further reduce the risk of HIV infection during the window period.<sup>(6)</sup>

At least two large U.S. multi-centre evaluations of the HIV-1 p24 antigen assay for donor screening have concluded that the test would not significantly increase blood safety. In one study, Alter et al tested more than 500,000 donations at 13 U.S. blood centres for the HIV-1 p24 antigen. Five donors were found positive (0.001% of all donations), all of whom were also positive for anti-HIV and HIV-positive by PCR.<sup>(7)</sup> In another study, in 8,597 samples from male donors in the U.S. selected from demographic subgroups at higher risk for HIV infection (obtained from 200,000 stored serum samples collected in 1984-1985), Busch did not find any blood that tested positive for the p24 antigen and was HIV antibody negative.<sup>(8)</sup> Similar results have been reported from Germany and Austria where none of more than 600,000 samples were positive for the p24 antigen in the absence of HIV-antibodies.

Models have been designed to evaluate the use of the p24 antigen assay for donor screening recommended by the U.S. FDA and the likely incremental benefits of such testing. Using U.S. data, Mendelson et al have estimated that the probability of detecting an additional HIV-infected blood component is about 1 in 4.86 million. Adding HIV antigen testing would prevent approximately four cases of TAA per year in the U.S.<sup>(9)</sup>

In a French model which evaluated the risk of HIV transmission by repeat donors, the number of HIV contaminated units was 1-2 out of 7 which could have been identified by p24 antigen testing. Applying 1992 U.S. data to the French model, 20 contaminated components could be expected, of which 2/5 could have been detected by antigen testing.<sup>(10)</sup>

Another area of concern has been the rapid heterosexual spread of AIDS. Blood donors may not always tell the truth or may fail to recognise their HIV risk status. It would be impossible even with stringent predonation interviews to exclude all persons at risk from donating. The HIV Blood Donor Study Group have assessed the impact of

heterosexually acquired HIV infection on the U.S. blood supply. The Group concluded that the impact on the US blood supply was minimal.<sup>(11)</sup> In countries where the incidence of AIDS is still rising and where donor demographics differ from the U.S., the situation may need to be defined.

### **Transfusion-Associated Hepatitis**

The current residual risk for transfusion-associated hepatitis (TAH) is low. Studies indicate the incidence of TAH before 1985 to be in the range of 8-10%.<sup>(12)</sup> Improvements in donor selection, the exclusion of high risk groups from blood donation, availability of highly sensitive tests to screen for HBV, HCV and HIV and changes in physician blood ordering practices have combined to further limit the residual risk of TAH.

Since 1972, when the HbsAg test was introduced to screen donated blood, the incidence of post-transfusion hepatitis B (PTHB) has fallen to very low levels and accounts for 7-17 percent of PTH cases.<sup>(13)</sup> The fall in PTHB has been most marked following the onset of the AIDS epidemic, which brought about interventions to improve blood safety. The stringent donor criteria to exclude donors at risk from HIV also excluded donors at risk from HBV. Very sensitive tests further reduce the risk of HBV infection.

Despite stringent testing, PTHB is still reported in patients receiving blood that has screened negative for HBsAg but positive for HBV-DNA by PCR.<sup>(14)</sup> Donors with even subliminal levels of HbsAg can transmit infection. In Japan, fulminant PTHB has been reported due to a pre-core HBV mutant in recipients of HBsAg negative blood which had high antibody titres of antibody to hepatitis B core antigen (anti-HBc).<sup>(15)</sup>

### ***Role of Anti-HBc Testing***

Anti-HBc testing was instituted in the U.S. in 1986 as a surrogate marker for non-A, non-B (NANB) PTH.<sup>(16)</sup> However, there are now highly sensitive tests for screening donors for HCV, and the main role of this test is to further reduce the risk of PTHB. Anti-HBc testing is also important for detecting chronic carriers of HBV who have subliminal levels of HBsAg yet are infectious. In studies from high prevalence regions, HBV-DNA has been identified by PCR in nearly 5% of HBsAg-negative, anti-HBc-positive donors.<sup>(17)</sup> In a low prevalence population such as the U.S., HBV-DNA has not been detected in such donors.<sup>(18)</sup>

The U.S. National Institutes of Health invited a panel of experts to review infectious disease testing for blood transfusions.<sup>(19)</sup> The experts have recommended retaining anti-HBc testing to prevent PTHB and to prevent some cases of TAA from infectious window period donations. Anti-HBc can serve as a surrogate marker for HIV as there are overlapping risk factors such as parenteral and sexual transmission.

The main disadvantage of routine testing for anti-HBc is the large number of false-positive tests. This results in a large number of discarded blood units and unnecessary anxiety to the donor, leading to additional medical costs for unwarranted investigations.

### ***NANB Hepatitis and HCV***

The worldwide distribution of HCV is consistent with seroprevalence rates of around 0-1% in populations studied. Epidemiology studies have shown that HCV is the cause of > 90% of post-transfusion NANBH. HCV is a major public health problem as 40% of patients will eventually develop chronic active hepatitis and there is an associated higher risk of hepatocellular carcinoma. People at risk for HCV infection include those with a history of multiple transfusions, haemophiliacs, intravenous drug users and renal dialysis patients. Besides the well-established mode of transmission through blood products, other modes of transmission are less well defined. Sporadic community cases of NANBH occur, and 40% of patients have no identifiable risk factors. HCV can spread by sexual transmission but is uncommon unless there is co-infection with HIV. Unlike HBV, vertical transmission from mother to newborn is uncommon.<sup>(20)</sup>

Surrogate testing was not implemented in Canada until 1987. In a recently completed Canadian study,<sup>(21)</sup> the overall reduction in the transfusion-associated PTH rate was 40% for the recipient group that was transfused with blood from which units positive for surrogate markers were withheld. The HCV infection rate in this group fell by 70%. The other group received blood which had not been tested for surrogate markers. The PTH rate was 20.2 per 1,000 recipients of blood which was not tested for surrogate markers versus 5 per 1,000 recipients of blood from which units positive for surrogate markers had been withheld.<sup>(21)</sup>

### ***Hepatitis A Virus***

Hepatitis A virus (HAV) contamination of blood products is rare, with very few reports of HAV infection in those exposed frequently to blood products, such as hemophiliacs and those receiving multiple transfusions. However, in 1992, HAV transmission through processed plasma products was reported in Italian haemophiliacs receiving Factor VIII concentrates prepared by ion-exchange chromatography and solvent detergent treatment.<sup>(22)</sup> Similar reports followed from Germany,<sup>(23)</sup> Ireland<sup>(24)</sup> and Belgium.<sup>(25)</sup> HAV nucleotide sequences were identical in the Factor VIII concentrates and in infected recipients.

### ***Other Hepatitis Agents***

Previously identified strains of hepatitis virus are A, B, C, D and E. Speculation on the possibility that there may be a non-A, non-B, non-C agent for hepatitis comes from various observations. Ten to 20% of NANBH cases are negative for anti-HCV by second generation tests and are HCV-DNA negative by PCR.<sup>(12)</sup> In studies of individuals documented to have multiple episodes of NANBH,<sup>(26)</sup> most cases of fulminant hepatitis are categorised as non-A, non-B, non-C.<sup>(27)</sup> Evidence for multiple NANB agents comes from cross-challenge studies in chimpanzees which have demonstrated two different types of NANBH.<sup>(28)</sup>

### ***GB Virus (GBV) and Hepatitis G Virus (HGV)***

Some recently discovered hepatitis viruses are known to be transmissible by blood. They belong to the flavivirus family, which includes HCV, yellow fever and dengue. Researchers from Abbott Laboratories were able to identify two distinct

flaviviruses in the infectious serum of a tamarin (small monkey).<sup>(29)</sup> The original agent came from a Chicago surgeon (GB) who developed acute hepatitis in the early 1960s. Subsequent studies identified a third virus, GBV-C. GeneLabs also recently identified another flavivirus called hepatitis G virus (HGV). Antibodies to HGV have been found in intravenous drug users, dialysis patients, haemophiliacs, multiply transfused patients and some NANBH cases.

### ***Cytomegalovirus (CMV)***

CMV infection is ubiquitous. CMV is a member of the herpes family. It generally causes a mild or asymptomatic infection in healthy adults. However, CMV infection causes serious morbidity and mortality in immunocompromised individuals. Other groups at risk include low-birth weight infants and patients who are CMV-seronegative awaiting bone marrow or organ transplants.

The usual method of providing CMV-negative blood products is to screen donors for CMV antibodies. However, a recent approach is to provide leukodepleted blood products, as the latest generation of leukodepletion filters remove in the order of 99.99% of leucocytes and are thus highly effective.

### ***Human T-Lymphotropic Virus (HTLV) Types I and II***

HTLV occurs worldwide and is endemic in certain parts of Africa, Japan and the Caribbean. HTLV-I-associated leukemia/lymphoma has been found mainly in Japan and Africa. The neurological disorders - tropical spastic paraparesis (TSP) or HTLV-I-associated myelopathy (HAM) - occur mainly in Japan and the Caribbean. HTLV-II may be less pathogenic, and associated disease is less well proven, although it may be linked to a neuromuscular illness similar to HTLV-I-associated TSP, HAM and some forms of hairy cell leukaemia.<sup>(30)</sup>

HTLV-I and HTLV-II are closely related and it is difficult to differentiate serologically between the antibodies. The seroprevalence of HTLV-I in U.S. volunteer blood donors is about 0.025-0.03%.<sup>(31)</sup> Testing in the U.S. has shown that HTLV-II is more prevalent than HTLV-I in the Southwest region, in Native Americans and in intravenous drug users.

### ***Parvovirus B19***

Parvovirus B19 is a non-enveloped DNA virus isolated during studies of post-transfusion hepatitis. It is the causal agent of Fifth's disease or erythema infectiosum - "slapped cheek" syndrome in children. The virus preferentially infects replicates and kills dividing erythroid cells. In patients with chronic hemolytic anaemias such as sickle cell disease, thalassaemia, and hereditary spherocytosis, parvovirus B19 infection can cause an aplastic crisis. In healthy persons, it causes a mild self-limiting reduction in erythropoiesis. Recurrent granulocytic aplasia as a manifestation of persistent parvovirus B19 infection has been reported.<sup>(32)</sup>

About 30-60% of adults in the U.S. are seropositive. A mass donor screening test is not available, and the risk of transmission by seropositive donors is uncertain. Parvovirus B19 does not have an envelope, and viral inactivation steps to eliminate HIV, HTLV, HBV and HCV in the preparation of safe plasma derivatives may not be effective.

## *Syphilis*

Transfusion-transmitted syphilis was first described in 1915.<sup>(33)</sup> It is now exceedingly rare. In 1985, an advisory panel of the FDA proposed that serological testing for syphilis (STS) be stopped. The proposal was not acted upon because STS was considered a possible surrogate marker for HIV. STS was deleted from the Standards of the American Association of Blood Banks (AABB) in 1987; however, the AABB has made STS mandatory again.<sup>(34)</sup> In the U.S., TPHA tests occur in fewer than 1 in 1,000 donors, compared to 6 per 1,000 in Germany and 0.5 per 1000 in the United Kingdom.<sup>(35)</sup> In developing countries, there are very few reports of transfusion-transmitted syphilis because the problem has hardly been defined.

*Treponema pallidum* loses viability within a few days in blood stored at 4°C. Data indicate that some spirochetes may survive up to 96 hours, but many units of blood are often stored for shorter periods. Platelets are stored at room temperature and there is no data on *T. pallidum* survival under such conditions.

Recently, STS has become a controversial issue in blood transfusion once more. The FDA convened a panel of experts, which has recommended that testing donors for syphilis should continue.<sup>(19)</sup>

## **Parasites**

### *Malaria*

Malaria is a worldwide public health problem associated in many countries with a high morbidity and mortality. *Plasmodium falciparum*, *vivax*, *ovale* and *malariae* may all be transmitted by blood transfusion. International travel, tourism and migration have been factors that make the continued surveillance of transfusion-transmitted malaria necessary. In the U.S., malaria is the most common parasite transmitted through transfusion. About three cases a year are reported.<sup>(36)</sup>

Malaria parasites survive in whole blood and in red cells that have been stored up to one week at 4°C. Parasites survive in platelet products stored at room temperature and they survive cryopreservation in glycerol and subsequent thawing.

Although a battery of efficient laboratory tests are available for the clinical diagnosis of malaria, none are suitable for mass donor screening. Transfusion-transmitted malaria can be prevented by careful questions in the donor interview on travel to, or residence in, malaria endemic areas. The current criteria used to exclude donors at risk are very effective.<sup>(37)</sup>

### Chagas' Disease

The causal agent for Chagas' disease is *Trypanosoma cruzi*, which is transmitted to humans through bites from infected reduvid bugs. The disease is endemic in South America where it is a major cause of morbidity and mortality. The parasite is found in the myocardium and smooth muscle cells of the gut in the infected patient. In endemic areas, infection through blood transfusion is common.<sup>(38)</sup> The disease is of growing concern in the U.S. because of immigration and travel trends from endemic areas. In the U.S., persons with a history of Chagas' disease are permanently deferred from blood donation.

## **Creutzfeld-Jakob Disease**

Creutzfeld-Jakob disease (CJD) is a rare and fatal form of degenerative encephalopathy characterised by dementia and ataxia. The majority of cases are sporadic with no family history. In about 10% of patients the disease is familial.<sup>(39)</sup>

CJD is related to several other spongiform encephalopathies including Gerstmann-Strussler-Scheiker disease, fatal familial insomnia, kuru, scrapie (in sheep) and bovine spongiform encephalopathy (BSE; mad cow disease).<sup>(40)</sup>

Since 1985, CJD has been reported in some recipients of human pituitary derived growth hormone,<sup>(41)</sup> human dura mater transplants,<sup>(42)</sup> and corneal transplants. The causal agent is an infectious protein called a prion.<sup>(39)</sup> CJD has never been documented to be transmitted through blood or blood products. A number of recent events have linked CJD to the epidemic of BSE in the U.K.

A variant form of CJD has been reported in the U.K. in 10 cases. The disease occurred at younger ages than is usual for CJD.<sup>(43)</sup> An expert panel set up in the U.K. to investigate whether there is a link between BSE and CJD concluded that there is no definite link between the variant CJD and BSE, but the evidence suggests that exposure to BSE is the most likely explanation. In April 1996, WHO invited an international panel of experts to review the public health issues related to BSE and the new variant CJD. The panel concluded that there is no definite link between BSE and variant CJD but that evidence suggests exposure to BSE may be the most likely explanation.<sup>(44)</sup>

In 1994, a frequent blood donor in the U.S. died of CJD. Plasma from his donation had been processed into plasma derivatives. The American Red Cross and manufacturers initiated a voluntary withdrawal of implicated products including intravenous gammaglobulin, factor VIII, albumin and plasma protein fraction. A similar event occurred in Canada in 1995 when the death of a blood donor due to CJD forced the Canadian Red Cross to withdraw plasma derivatives manufactured from the donor's plasma.

Epidemiological studies of CJD in the U.K. have identified 21 CJD patients who received blood and 29 who were blood donors out of 202 cases. This frequency of blood transfusion and donation did not differ from that of age- and sex-matched controls. Clinical features in patients with a history of blood transfusion were similar to those of classical CJD and clearly distinct from CJD in recipients of human growth hormone. The evidence does not suggest that blood transfusion is a major risk factor.<sup>(45)</sup>

## **Bacterial Contamination**

Bacterial contamination of transfused blood products is a well-recognised source of sepsis, but reactions to infected blood are difficult to distinguish from acute hemolytic reactions. Organisms documented to be important in transfusion-related infection differ among the various blood products. Bacteria contaminating RBC transfusions are more likely to grow at 4°C, the storage temperature for RBC.

Platelets are currently stored for up to 5 days at 22°C. One in 14,000 platelet transfusions stored for less than 2 days resulted in sepsis, whereas 4 in 1,000 units stored

for 5 days resulted in sepsis. Platelet storage increased from 3 to 5 days in 1982 and to 7 days in 1983. Bacterial sepsis occurred in a large number of platelets stored for more than 5 days. In 1986, platelet storage was again reduced to 5 days.<sup>(46)</sup>

Sources of bacterial contamination include organisms from the donor's skin during venepuncture due to inadequate skin preparation or from undetected transient bacteremia. Contamination can also occur due to improper storage of blood and during component processing. The majority of contaminating microorganisms are found as part of the normal skin flora, as water-borne agents, or they circulate in the blood undetected. In the 1980s, the first reports of fatal *Yersinia enterocolitica* infections from RBC transfusions were documented.<sup>(47)</sup> *Y. enterocolitica* grows preferentially at 4°C. Nineteen cases (59%) of 32 contaminations were fatal.<sup>(48)</sup> In 1991 there were reports from Scandinavia<sup>(49)</sup> of *Serratia marcescens* septicemia attributed to faulty manufacturing of blood bags. There have been several reports of *Pseudomonas* species infection, including a death from *P. cepacia* contamination of cryoprecipitate.<sup>(50)</sup>

Bacterial contamination may be overlooked. Any patient developing fever, chills and hypotension soon after transfusion should be evaluated for a transfusion-related bacterial infection along with a work-up for an acute hemolytic transfusion reaction.

### ***Emerging Infections***

Migration trends, travel, and changes in eco-systems have brought about a greater awareness of the concept of emerging infections, which may be defined as any infectious diseases that have increased over the past two decades or that threaten to increase in the near future.<sup>(51)</sup> Surveillance of such emerging infections should be an integral part of efforts to prevent new infectious agents from contaminating the blood supply. It would be necessary to i) evaluate whether the disease is potentially transmissible through blood; ii) review donor selection testing and component processing; iii) examine the public health implications, including the possible need for "look back" programmes; and iv) provide donor and public education.

### **Conclusion**

It is important to regularly review measures to protect the blood supply against infectious risks to ensure that procedures are adequate and effective. It is also necessary to recognise the new threats that emerging infections may pose to blood safety. While the development of strategies and interventions to prevent the transmission of infectious diseases must always be based on the best epidemiological and scientific evidence of potential risks, community attitudes and perceptions of these risks can influence the implementation of policies.

### **References**

1. Gibbs WN, Corcoran P. Blood safety in developing countries. *Vox Sang* 1994;67:377-381.

2. Lackritz EM, Satten GA, Aberle-Grasse J et al. Estimated risk of transmission of the human immunodeficiency virus by screened blood in the United States. *N Engl J Med* 1995;333:1721-5.
3. Ward JW, Holmberg SD, Allen JR, et al. Transmission of human immunodeficiency virus (HIV) by blood transfusion screened as negative for HIV antibody. *N Engl J Med* 1988;318:473-8.
4. Busch MP, Alter HJ. Will human immunodeficiency virus p24 antigen screening increase the safety of the blood supply and, if so, at what cost? (editorial) *Transfusion* 1995;35:536-9.
5. Busch MP, Lee LLJ, Satten GA, et al. Time course of detection of viral and serologic markers preceding human immunodeficiency virus type 1 seroconversion: implications for screening of blood and tissue donors. *Transfusion* 1995;35:91-7.
6. Centers for Disease Control and Prevention. U.S. Public Health Service Guidelines for testing and counselling blood and plasma donors for human immunodeficiency virus type 1 antigen. *MMWR* 1996;45(No.RR-2):1-9.
7. Alter HJ, Epstein JS, Swenson SG, et al and the HIV-Antigen Study Group. Prevalence of human immunodeficiency virus type 1 p24 antigen in US blood donors - an assessment of the efficacy of testing in donor screening. *N Engl J Med* 1990; 323:1312-1318.
8. Busch MP, Taylor PE, Lenes BA, et al and the Transfusion Safety Study Group. Screening of selected male donors for p24 antigen of human immunodeficiency virus type 1. *N Engl J Med* 1990; 323, 1308-1312.
9. Mendelson DN, Sandler SG. A model for estimating incremental benefits and costs of testing donated blood for human immunodeficiency virus antigen (HIV-Ag). *Transfusion* 1990;30:73-5.
10. Pont FL, Costagliola D, Rouzioux C, Valleron AJ. How much would the safety of blood transfusion be improved by including p24 antigen in the battery of tests? *Transfusion* 1995;35:542-547.
11. Petersen LR, Doll LS, White CR et al and the HIV Blood Donor Study Group. Heterosexually acquired human immunodeficiency virus infection and the United States blood supply: Considerations for screening of potential blood donors. *Transfusion* 1993;33:552-557.
12. Alter HJ. Transfusion transmitted hepatitis C and non-A, non-B, non-C. *Vox Sang* 1994;67(suppl);19-24.
13. Hoofnagle JH. Post-transfusion hepatitis B (editorial). *Transfusion* 1990;30:384.
14. Thiers V, Nakajima E, Kremsdorf D, et al. Transmission of hepatitis B from hepatitis B-seronegative subjects. *Lancet* 1988;1273-1276.
15. Kojima M, Shimizu M, Tsuchimochi T, et al. Post-transfusion fulminant hepatitis B associated with pre-core defective HBV mutants. *Vox Sang* 1991;60:34-9.
16. Koziol DE, Holland PV, Alling DW et al. Antibody to hepatitis B core antigen as a paradoxical marker for non-A, non-B hepatitis agents in donated blood. *Ann Intern Med* 1986;104:488-95.
17. Wang JT, Wang TH, Shue JC, et al. Detection of hepatitis B virus DNA by polymerase chain reaction in plasma of volunteer blood donors negative for hepatitis B surface antigen. *J Infect Dis* 1991;163:397-9.

18. Douglas DD, Taswell HF, Rakela J, Rabe D. Absence of hepatitis B virus DNA detected by polymerase chain reaction in blood donors who are hepatitis B surface antigen negative and antibody to hepatitis B core antigen positive from a United States population with a low prevalence of hepatitis B serologic markers. *Transfusion* 1993;33:212-6.
19. Infectious Disease Testing for Blood Transfusions. NIH-Consensus Statement 1995 Jan 9-11;13(1):1-29.
20. Dienstag JL. Non-A, non-B hepatitis: I. Recognition, epidemiology and clinical features. *Gastroenterology* 1983; 85:439-462.
21. Blajchman MA, Bull SB, Feinman SV. Post-transfusion hepatitis: Impact of non-A, non-B hepatitis surrogate tests. Canadian Post Transfusion Hepatitis Prevention Study Group. *Lancet* 1995;345:21-25.
22. Mannucci PM. Outbreak of hepatitis A among Italian patients with haemophilia. *Lancet* 1992;339:819.
23. Gerritzen A, Schneweis KE, Brackmann HH, et al. Acute hepatitis A in haemophiliacs. *Lancet* 1992;340:1231-2.
24. Temperly JJ, Cotter KP, Walsh TJ, et al. Clotting factors and hepatitis A. *Lancet* 1992;340:1466.
25. Peerlinch K, Vermeylen J. Acute hepatitis A in patients with haemophilia A. *Lancet* 1992;341:379.
26. Mosley JW, Redeker AG, Feinstone SM, Purcell RH. Multiple hepatitis viruses in multiple attacks of acute viral hepatitis. *NEJM* 1977;296:75-80.
27. Gimson AES, White YS, Eddlestone ALWS, Williams R. Clinical and prognostic differences in fulminant hepatitis type A, B, and non-A, non-B. *GUT* 1983;24:1194-8.
28. Yoshizawa H, Itoh Y, Iwakiri S, et al. Demonstration of two different types of non-A, non-B hepatitis by reinjection and cross-challenge studies in chimpanzees. *Gastroenterology* 1981;81:107-113.
29. Simons JN, Pilot-Matias TJ, Leary TP, et al. Identification of two flaviviruses-like genomes in the GB hepatitis agent. *Proc Natl Acad Sci USA* 1995;92:3401-3405.
30. Loughran TPJr, Coyle T, Sherman MP, et al: Detection of human T-cell leukaemia/lymphoma virus type II in a patient with large granular lymphocyte leukaemia. *Blood* 1992;80:1116-1119.
31. Williams AE, Fang CT, Slamon DJ, et al. Seroprevalence and epidemiological correlates of HTLV-1 infection in US blood donors. *Science* 1988;249:643-646.
32. Pont J, Puchhammer-Stockl E, Chott A, et al. Recurrent granulocytic aplasia as clinical presentation of a persistent parvovirus B19 infection. *B J Haem* 1992;80:160-165.
33. Schryver AD, Meheus A. Syphilis and blood transfusion: a global perspective. *Transfusion* 1990;30:844-847.
34. Standards for Blood Banks and Transfusion Services. AABB, 14th Edition, 1991;17.
35. International Forum. Does it make sense for blood transfusion services to continue the time-honored syphilis screening with cardiolipin antigen? *Vox Sang* 1981;41:183-192.

36. Ture JM. Malaria and blood transfusion. In: Westphal RG, Carlson KB, Ture JM, eds. *Emerging Global Patterns in Transfusion Transmitted Infections*. Arlington, Va : American Association of Blood Banks;1990;31-43.
37. Schulman IA. Parasitic infections and their impact on blood donor selection and testing. *Arch Pathol Lab Med* 1994;118:366-370.
38. Wendel S, Gonzaga AL. Chagas' disease and blood transfusion: a new world problem? *Vox Sang* 1993;64:1.
39. Masters CL, Harris JO, Gajdusek DC, Gibbs CJ Jr, et al. Creutzfeldt-Jakob disease: Patterns of worldwide occurrence and the significance of familial and sporadic clustering. *Ann Neurol* 1979;5:177-188.
40. Dormant D. Natural history of human transmissible subacute spongiform encephalopathies. *Transfus Clin Biol* 1994;1(5):319-31.
41. Fradkin JE, Schonberger LB, Mills JL, et al. Creutzfeldt-Jakob disease in pituitary growth hormone recipients in the United States. *JAMA* 1991;265:880.
42. Martinez-Lage JF, Poza M, Tortosa JD. Creutzfeldt-Jakob disease in patients who received a cadaveric dura mater graft—Spain, 1985–1992. *MMWR* 1993;42:561.
43. Ironside JW, Zeibler M, et al. A variant of Creutzfeldt-Jakob disease in the UK. *Lancet* 1996;347:921-5.
44. WHO consultation on public health issues related to bovine spongiform encephalopathy and the emergence of a new variant of Creutzfeldt-Jakob disease. *MMWR* 1996;45(14):296-303.
45. Esmonde TGF, Will RG, Slattery JM, et al. Creutzfeldt-Jakob disease and blood transfusion. *Lancet* 1993 341;205
46. Morrow JF, Braine HG, Kickler TS, et al. Septic reactions to platelet transfusions: A persistent problem. *JAMA* 1991;266:555-558.
47. Centers for Disease Control. *Yersinia enterocolitica* bacteremia and endotoxin shock associated with red blood cell transfusions—United States, 1991. *MMWR* 1991;40:176-178.
48. Sazama K. Bacteria in blood for transfusion. *Arch Pathol Lab Med* 1994;118:350-365.
49. Hogman CF, Fritz H. *Serratia marcescens* septicemia. *Transfusion* 1993;33:189-191.
50. Centers for Disease Control. Follow-up on nosocomial *Pseudomonas cepacia* infection. *MMWR* 1979;28:409-410.
51. Centers for Disease Control and Prevention. *Addressing emerging infectious disease threats*. Atlanta, GA, 1994.