Contributions of BSRI to Transfusion Safety Research

Blood Research Systems Institute (BSRI) has an outstanding record over the past 20 years of successfully conducting laboratory research projects for NIH and CDC-funded multi-center programs related to transfusion safety and infectious diseases. BSRI/BSL has played a key role in many of the major blood safety research programs from the early 1980s to the present. These multicenter studies have resulted in over 150 publications. These manuscripts clearly document the breadth and depth of BSRI's laboratory capacity, including the ability to develop and apply innovative specimen processing and analytical methods that addressed critical questions posed by investigators in these programs.

Study History

Transfusion-Associated AIDS (1982 –1984): Irwin Memorial Blood Centers (IMBC), precursor to BSRI, identified the first possible case of transfusion-associated AIDS in the United States in an infant who received transfusions from 19 different donors as a newborn. The NIH funded IMBC researchers, working with the CDC and local San Francisco Bay Area health departments, to identify the infected donor thus supporting the hypothesis that HIV could be transmitted through the blood supply. IMBC pioneered the first look-back program for HIV. The look-back technique has been used in other new infections (HCV, West Nile virus) to determine if infectious agent could be transmitted through blood transfusion.

San Francisco Men's Health Study (1984-1992) Collaborated with the University of California Berkeley Public Health Department, Children's Hospital of San Francisco (now California Pacific Medical Center) and the University of California at San Francisco in a population-based study of AIDS funded by NIAID. The group looked at1,000 homosexuals and 200 heterosexual men from the Bay Area to determine the natural history of HIV infection/AIDS.

Transfusion Safety Study (1984-1996): IMBC was one of four major blood centers funded by NHLBI to establish a repository of ~200,000 donor specimens of the 9-month period preceding availability of anti-HIV-screened assays in 1985. This repository was later accessioned for testing at Irwin and the other centers for HIV and HTLV antibodies, and selected samples tested for HIV p24 antigen and nucleic acids [1-9] and Human Herpes Virus-8 (HHV-8) antibodies [10]. The TSS study established the seroprevalence of HIV and HTLV infections in the donor pool at that critical time, the rates and determinants of transmission of each virus, and the rates and correlates of disease progression in infected donors and recipients. [11-19] IMBC researchers worked on a number of TSS laboratory projects, including a large study to evaluate the utility of HIV-1 p24 antigen screening [2] and a series of studies to evaluate the performance and clinical utility of early generation HIV DNA and RNA PCR assays. [7-9, 17, 18]

Supplemental funding was received from NHLBI (an NHLBI R01 grant) to support additional research at IMBC/BCP related to evolution of HIV genetic diversity in infected donors and recipients enrolled in TSS. These studies capitalized on the unique existence in TSS of linked donor-recipient infection clusters, and established the role of viral and host immune factors in shaping quasispecies evolution, as well as the significance of viral diversity on HIV disease outcome. [14, 15, 20-26]

Transfusion-Associated Viral Infections and Immune Responses (1986-1991): This NHLBI-funded Program Project Grant supported studies to develop and evaluate methods for detection and quantitation of HIV infection as well as for measuring HIV-related immune responses. [27-36] The studies focused on serial specimens from cohorts of infected blood donors and recipients (and controls) identified at IMBC. All laboratory work was performed at IMBC, and included application of a number of what at the time were novel analytical methods, such as quantitative HIV cultures, capillary electrophoresis, PCR, and recombinant immunoblot assays. Key findings included the rate of persistent infection and disease in Western blot (WB)-confirmed positive donors, absence of infection in most donors with WB-indeterminate and -negative results, and analysis of sensitivity and specificity of viral culture and a variety of PCR assays.

Effectiveness of Screening for HIV-1 Antibody (1986-1993): This contract was funded by NHLBI in 1986, shortly after anti-HIV screening tests were implemented, in order to establish empirically the effectiveness of first generation donor screening tests. Two approaches were pursued in parallel. The first involved performing HIV co-cultures and DNA PCR on pooled mononuclear cells from 200,000 IMBC blood donors, using 50-donor pools and optimized culture and PCR assay systems developed by IMBC. [29, 37-39] This study, which detected one HIV infection missed by serological screening, was a prelude to what later evolved into routine minipool NAT screening. The other approach involved enrolling and following recipients of screened blood in San Francisco, followed by monitoring of pre- and post-transfusion specimens for evidence of all major bloodborne viruses known at the time. [36, 40-44] All testing of these specimens was performed at IMBC, which became Blood Centers of the Pacific (BCP) in 1997. The study yielded reassuring results as to the low rate of breakthrough transmission of transfusion-transmissible viruses.

AIDS Epidemiologic Study of Blood Donors and Transfusion Recipients (1987-2003): This series of multicenter cooperative agreements with CDC were initially funded in 1987 to recall and interview HIV-infected blood donors in order to characterize the epidemiology of HIV in donors and understand behavioral factors that resulted in HIV-infected persons presenting to donate. Subsequently the studies were expanded to include enrollment and follow-up of HIV-infected transfusion recipients and their sexual partners. A number of laboratory projects were pursued at IMBC/BCP that employed serological, flow cytometric, viral culture, and NAT. [11, 25, 45-60] This program supported development at BCP of a novel “less-sensitive” or “detuned” HIV antibody assay for detection of recent HIV infection and projection of population incidence rates. [51, 53, 55, 56, 58, 60] IMBC/BCP was also responsible for Central Laboratory work related to surveillance for genetic subtypes of HIV-1 and HIV-2 in U.S. blood donors,

which led to a series of publications documenting a low but increasing rate of non-B clades in the U.S. [25, 45, 55, 59] Studies were also conducted in collaboration with the National Blood Service in South Africa, to investigate the value of HIV and HCV nucleic acid testing (NAT) in that developing country setting. [60]

Viral Activation Transfusion Study (1994-2000): VATS was an 11-center prospective clinical study, funded by NHLBI, that investigated the impact of non-leukoreduced versus leukoreduced blood transfusions given to HIV-infected patients. [61, 62] BCP (now BSRI) served as the Central Laboratory. We developed novel approaches for Clinical Center sample processing and shipping, and Central Laboratory analysis of frozen whole blood aliquots prepared from samples collected from donors and enrolled recipients. [63-65] These samples were tested, in serial sample batch mode, using quantitative nucleic acid amplification assays specific for multiple transfusion-associated viruses (HIV, CMV, HTLV-I/II, HBV, HCV, HHV-8). [62, 66-72] Quantitative PCR assays were also developed and applied to quality control and characterize leukoreduced blood components, [64, 73-75] and to investigate recipient samples for persistent donor leukocyte microchimerism. [76] Recipient samples were also studied for immunological markers, including lymphocyte immunophenotyping and activation studies by flow cytometry, and plasma cytokine activity by immunoassays. [62, 63, 65, 66] The study found no significant evidence that transfusions induce activation of pre-existing viral infections in recipients, and no significant differences in any parameters between recipients of leukoreduced and non-leukoreduced transfusions.

Specialized Center of Research in Transfusion Medicine (1996-2006): University of California at San Francisco's SCOR-TM grant, funded by NHLBI, includes a project based at BCP/BSRI that is investigating leukocyte microchimerism (MC) in transfusion recipients and other populations. A battery of highly sensitive and specific PCR assays targeting HLA alleles and other polymorphic genetic loci, have been developed to detect, quantitate and characterize extremely low concentrations of donor cells in transfusion recipients and other clinical settings. Studies in humans have included transfusion recipients in multiple U.S. hospital settings, as well as patients and pregnant women from Brazil and Japan. [65, 77-84] One important observation from these studies was the frequent development of persistent, multilineage MC and associated tolerance in multi-transfused trauma patients. [78, 84] A murine transfusion/transplantation model has also been established, which has allowed for controlled studies of blood component and recipient determinants of transfused leukocyte survival and tolerance induction. [82, 85-90] These studies have included transfusions into a wide variety of immunologically deficient "knockout" mice, followed by tracking of donor cell engraftment tolerance by allele-specific PCR and flow cytometry and skin graft studies. [82, 90] This project has also supported development of methods for detection and quantitation of genes encoding RBC and platelet polymorphisms for use in MC studies as well as for RBC and platelet compatibility studies in the setting of severe cytopenia or recent transfusion. [91]

Pathophysiology of HTLV-I/II Infections: HOST Cohort (1999-2004): The HOST study is a large cohort of HTLV-I and -II-infected and control donors that is supported by an

NHLBI-R01 grant to Dr. Edward Murphy, Director of Epidemiology at BSRI. BCP has served as the Central Laboratory for HOST since 1999 when it was funded to continue to follow this HTLV cohort first established through REDS. The laboratory work involves receipt and processing of plasma and PBMC from over 500 infected donors, sexual partners and controls seen annually at the REDS-I Blood Centers. [72, 92-95] In addition to managing the study repository, BSRI performs and coordinates routine testing of donor specimens, and has developed and applied novel, quantitative PCR assays for HTLV-I and HTLVI-II proviral load analyses.

Transfusion-Related Infections Prospective Study (2000-present): TRIPS is a prospective study recently launched by Dr. Harvey Alter that is enrolling adult and pediatric transfusion recipients and monitoring them for a large number of transfusion-transmitted infectious agents as well as other complications such as occult graft-vs-host disease (GvHD). Unlike the REDS RADAR study, TRIPS acquires frequent specimens over the initial months following transfusion, enabling detection of transient infections and examination of acute markers of viral infections. In addition to broader involvement in study design and laboratory protocol development, BSRI is responsible for laboratory work to detect transmission of the 3 major potential transfusion-transmitted Herpes viruses (CMV, EBV, HHV-8). We are also applying MC assays to pre- and serial post-transfusion specimens from enrolled subjects.

Non-Transfusion-Related Central Laboratory activities at BSRI: BSRI has served a major Central Laboratory function for numerous other PHS-funded multicenter studies. In the 1980s and early 1990s BSRI (IMBC at that time) was the central repository and core testing laboratory for the San Francisco Men's Health Study, a large cohort study of gay men in the Bay Area. Peripheral blood mononuclear cells (PBMC), serum, and plasma repositories were established and maintained at BSRI, and a variety of flow cytometry, serological, and molecular assays performed on study specimens. [8, 15, 27, 29, 37, 42, 46, 48, 51, 96-103] The National Marrow Donor Program (NMDP) research repository has been based at BSRI since its inception 14 years ago. This large-scale repository includes a variety of specimen preparations, including EBV-transformed cell lines from donors and recipients who have undergone marrow or peripheral blood stem cell transplants through NMDP. [104-107] BSRI has served as Central Laboratory for a number of collaborative studies with UCSF scientists, including the Urban Health Study, UFO Study, HCV in California Prisons Study, HCV Heterosexual Transmission Study, and the Immunologic and Virologic Features of Early HIV Infection Study. [39, 44, 48, 55-57, 60, 70, 108-114] In each of these programs we manage specimen receipt and processing, establish and maintain the study repository, and perform or coordinate routine and special testing.

Retrovirus Epidemiology in Donors Study (REDS)

REDS is the major NHLBI funded research program focused on blood donor epidemiology and safety. REDS-I began in 1989 and continued through 2004; REDS-II launched in 2004 with BSRI participating as both a clinical site and the Central

Laboratory. BSRI played a major role in all aspects of REDS-I, with particular responsibility for and contributions to laboratory-based research. REDS publications that involved. The text below reviews these major studies, divided into four categories based on study objective:

Prevalence/incidence of infectious agents in donor populations. These studies involved testing specimens in existing REDS and other NHLBI donor repositories, or accumulating data prospectively on new donor populations, in order to establish the frequency of detection of nucleic acid or immunological markers of infectious agents in U.S. blood donors. These studies required knowledge of the structure and characteristics of existing NHLBI donor-recipient repositories and routine donor screening practices, and the ability to retrieve and test samples and transmit data to the Westat, the REDS-I Coordinating Center. One example from early in the REDS-I contract period involved idiopathic CD4 T lymphocytopenia (ICL), which at the time was thought to relate to an occult virus inducing immunodeficiency similar to HIV. A study was launched to evaluate rapid CD4 screening methods, and to apply one of these to determine the distribution of CD4 cell counts in healthy donors. [100] A series of studies in which PCR assays were developed and applied to investigate the frequency of viral nucleic acids in selected REDS donor samples. These included studies to determine if donors with negative or indeterminate results for established transfusion-transmitted agents such as HIV, HTLV, HBV, HCV and CMV, are actually infected with these agents. [69, 115-123] Several projects were also launched to look for recently discovered or emerging viruses, including Primate T-lymphotropic Viruses (PTLVs) and Human Herpes Virus, type 8 (HHV-8). [124, 125] During REDS-II such studies are likely to be needed both to investigate the donor prevalence of currently known agents for which screening is not conducted in the U.S. (e.g., SARS, vCJD, T *cruzi*, other parasites) as well as in response to newly discovered agents. The proposed long-term project is designed to enhance this rapid response capacity.

Determinants of transmissibility of transfusion-transmitted agents: There are viral, blood component and donor and recipient genetic and immunolgical factors that influence the probability that a donation harboring an infectious agent will transmit that agent to a recipient. These issues were investigated in REDS-I on several occasions. For example, specimens from the REDS HTLV-I/-II cohort were accessed to establish the relationship between viral load in the donor and secondary transmission to infected donors' sexual partners. [126] A collaboration was established to investigate the prevalence in REDS donors of a genetic polymorphism in the CCR5 gene that protects from HIV infection; a parallel study with TSS established that several recipients of HIV infected blood products were protected as a result of this polymorphism. [127] Several studies were also published that examined the relationship between very low HIV and HCV RNA load in infected donations and transmission to recipients. [128-130] These studies, and several projects still in progress at BSRI/Westat under REDS-I auspices focused on HIV and HCV NAT performance versus chimp infectivity using plasma donor seroconversion panels, addressing the critical question of the relationship between infectious dose and level of detectable viral nucleic acids in donor units. The recipient and donor allogeneic repository (RADAR) collected under the REDS-I contract, and the

other NHLBI donor-recipient repositories discussed above, will be available to REDS-II to extend this line of work. An example of a RADAR based study that could be done in REDS-II would be to assess the correlation of Parvovirus B19 virus concentration in blood components with transfusion-transmission of this agent. Such a study could be important in helping determine the sensitivity of Parvovirus B19 assays that would be required if national policy dictated that such testing is needed to enhance blood safety.

Viral Dynamics of Acute HIV, HBV and HCV Infections. One of the major contributions of REDS-I was development and refinement of the incidence-window period model, which has proven critical for estimating transfusion risk and projecting yield of enhanced assays targeting window-phase units. [117, 118, 121] An improved understanding of the viral load kinetics following infection, and the time course to development of serological markers, was critical to this endeavor. To achieve this, BSRI researchers identified and acquired large numbers of plasma donor panels composed of serial units collected during the pre-seroconversion phases of HIV, HBV, HCV, and CMV infections. These panels were studied in great detail at BSRI/BSL using NAT, viral load and serological assays to generate datasets that were transmitted to and analyzed by Westat statisticians. This work yielded precise estimates (with confidence intervals) for key acute infection parameters, such as the duration and characteristics of viral "blips" during the pre ramp-up viremic phase, the viral load doubling-time during the ramp-up phase (which allowed projection of the duration of infectious window periods preceding specified viral load thresholds), and the window periods from detection of viremia by NAT assays to detection by various serological markers. [57, 121, 131-135] Similar studies are proposed in our short-term project for REDS-II, with the goal of refining our understanding of the acute infection dynamics of WNV. As other established and emerging infections are addressed during REDS-II, it will be crucial that the Central Laboratory has the expertise and capacity to similarly characterize the dynamics of acute infection in order to inform policy decisions over optimal screening strategies and corresponding risk projections.

Evaluation of Laboratory Tests and Algorithms. As tests for new infectious agents are considered or implemented in the blood donor screening setting, it is important that the performance characteristics of assays and related testing algorithms are established. This was important for REDS-I both to assure that viral marker data used in epidemiological analyses were accurate, and to guide policy related to the value of these assays and their interpretation for donor counseling and recipient lookback. During REDS-I, BSRI/BSL conducted many laboratory studies and directed numerous analyses in order to define the sensitivity, specificity and predictive values of relevant assays and algorithms. These included published studies of 1) HTLV screening and supplemental assay performance, [136, 137] 2) rapid CD4 lymphocyte counting methods, [103] 3) hepatitis surrogate markers (ALT and anti-HBc), [138-140] 4) HIV-1 p24 Ag and Western blot assays, [56, 132, 141, 142] 5) HCV antibody screening and confirmatory tests, [143-146] and 6) NAT assays for HIV, HBV, HCV and CMV. [67, 132, 147] Recently completed studies under REDS-I auspices, now in the manuscript development phase, focused on refined comparisons of HIV, HCV and WNV NAT assays, as well as a comparison of WNV serological assays. Such studies will

undoubtedly continue to be a key function of the Central Laboratory for REDS-II. There are several candidate pathogens that may spark such studies in the next five years: these include T. *cruzi*, B. *microti*, and the agent of vCJD.

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LITERATURE CITED

1. Operskalski, E.A., et al., *Epidemiologic background of blood donors with antibody to human T-cell lymphotropic virus. Transfusion Safety Study Group.* Transfusion, 1989. **29**(8): p. 746-8.
2. Busch, M.P., et al., *Screening of selected male blood donors for p24 antigen of human immunodeficiency virus type 1. The Transfusion Safety Study Group.* N Engl J Med, 1990. **323**(19): p. 1308-12.
3. Alter, H.J., et al., *Prevalence of human immunodeficiency virus type 1 p24 antigen in U.S. blood donors--an assessment of the efficacy of testing in donor screening. The HIV-Antigen Study Group.* N Engl J Med, 1990. **323**(19): p. 1312-7.
4. Donegan, E., et al., *Transfusion of blood components from a donor with human T-lymphotropic virus type II (HTLV-II) infection. The Transfusion Safety Study Group.* Ann Intern Med, 1990. **113**(7): p. 555-6.
5. Busch, M.P., et al., *Donor HIV-1 p24 antigenaemia and course of infection in recipients Transfusion Safety Study Group.* Lancet, 1990. **335**(8701): p. 1342.
6. Busch, M.P., et al., *Risk of human immunodeficiency virus (HIV) transmission by blood transfusions before the implementation of HIV-1 antibody screening. The Transfusion Safety Study Group.* Transfusion, 1991. **31**(1): p. 4-11.
7. Busch, M.P., et al., *Poor sensitivity, specificity, and reproducibility of detection of HIV- 1 DNA in serum by polymerase chain reaction. The Transfusion Safety Study Group.* J Acquir Immune Defic Syndr, 1992. **5**(9): p. 872-7.
8. Lee, T.H., et al., *Absence of HIV-1 DNA in high-risk seronegative individuals using high-input polymerase chain reaction.* Aids, 1991. **5**(10): p. 1201-7.
9. Adams, M., et al., *Rapid freezing of whole blood or buffy coat samples for polymerase chain reaction and cell culture analysis: application to detection of human immunodeficiency virus in blood donor and recipient repositories. The Transfusion Safety Study Group.* Transfusion, 1993. **33**(6): p. 504-8.
10. Kedes, D.H., et al., *The seroepidemiology of human herpesvirus 8 (Kaposi's sarcoma-associated herpesvirus): distribution of infection in KS risk groups and evidence for sexual transmission.* Nat Med, 1996. **2**(8): p. 918-24.
11. O'Brien, T.R., et al., *Heterosexual transmission of human immunodeficiency virus type 1 from transfusion recipients to their sex partners.* J Acquir Immune Defic Syndr, 1994. **7**(7): p. 705-10.
12. Donegan, E., et al., *Transfusion transmission of retroviruses: human T-lymphotropic virus types I and II compared with human immunodeficiency virus type 1.* Transfusion, 1994. **34**(6): p. 478-83.

13. Busch, M.P., et al., *Epidemiologic background and long-term course of disease in human immunodeficiency virus type 1-infected blood donors identified before routine laboratory screening. Transfusion Safety Study Group.* Transfusion, 1994. **34**(10): p. 858-64.
14. Diaz, R.S., et al., *Dual human immunodeficiency virus type 1 infection and recombination in a dually exposed transfusion recipient. The Transfusion Safety Study Group.* J Virol, 1995. **69**(6): p. 3273-81.
15. Delwart, E.L., et al., *Rapid molecular epidemiology of human immunodeficiency virus transmission.* AIDS Res Hum Retroviruses, 1995. **11**(9): p. 1081-93.
16. Operskalski, E.A., et al., *Human immunodeficiency virus type 1 infection: relationship of risk group and age to rate of progression to AIDS. Transfusion Safety Study Group.* J Infect Dis, 1995. **172**(3): p. 648-55.
17. Busch, M.P., et al., *Factors influencing human immunodeficiency virus type 1 transmission by blood transfusion. Transfusion Safety Study Group.* J Infect Dis, 1996. **174**(1): p. 26-33.
18. Operskalski, E.A., et al., *Role of viral load in heterosexual transmission of human immunodeficiency virus type 1 by blood transfusion recipients. Transfusion Safety Study Group.* Am J Epidemiol, 1997. **146**(8): p. 655-61.
19. Operskalski, E.A., et al., *Comparative rates of disease progression among persons infected with the same or different HIV-1 strains. The Transfusion Safety Study Group.* J Acquir Immune Defic Syndr Hum Retrovirol, 1997. **15**(2): p. 145-50.
20. Diaz, R.S., et al., *Lack of dual HIV infection in a transfusion recipient exposed to two seropositive blood components.* AIDS Res Hum Retroviruses, 1996. **12**(13): p. 1291-5.
21. Diaz, R.S., et al., *Divergence of HIV-1 quasispecies in an epidemiologic cluster.* Aids, 1997. **11**(4): p. 415-22.
22. Zhang, L., et al., *Host-specific driving force in human immunodeficiency virus type 1 evolution in vivo.* J Virol, 1997. **71**(3): p. 2555-61.
23. Wilkinson, D.A., et al., *A 32-bp deletion within the CCR5 locus protects against transmission of parenterally acquired human immunodeficiency virus but does not affect progression to AIDS-defining illness.* J Infect Dis, 1998. **178**(4): p. 1163-6.
24. Diaz, R.S., et al., *HIV type 1 tat gene heteroduplex mobility assay as a tool to establish epidemiologic relationships among HIV type 1-infected individuals.* AIDS Res Hum Retroviruses, 1999. **15**(13): p. 1151-6.
25. de Oliveira, C.F., et al., *Surveillance of HIV-1 genetic subtypesand diversity in the US blood supply.* Transfusion, 2000. **40**(11): p. 1399-406.
26. Costa, L.J., *Evidence for selection of more adapted human immunodeficiency virus type 1 recombinant strains in a dually infected transfusion recipient.* Virus Genes, 2004.
27. Busch, M.P., et al., *In situ hybridization and immunocytochemistry for improved assessment of human immunodeficiency virus cultures.* Am J Clin Pathol, 1987. **88**(6): p. 673-80.
28. Calman, A.F., et al., *Transcription and replication of human immunodeficiency virus-1 in B lymphocytes in vitro.* Aids, 1988. **2**(3): p. 185-93.

29. Ulrich, P.P., et al., *Assessment of human immunodeficiency virus expression in cocultures of peripheral blood mononuclear cells from healthy seropositive subjects.* J Med Virol, 1988. **25**(1): p. 1-10.
30. McHugh, T.M., et al., *Relation of circulating levels of human immunodeficiency virus (HIV) antigen, antibody to p24, and HIV-containing immune complexes in HIV-infected patients.* J Infect Dis, 1988. **158**(5): p. 1088-91.
31. Busch, M.P., *Laboratory diagnosis of HIV infection.* Transfus Med Rev, 1988. **2**(4): p. 250-63.
32. Busch, M.P., *Future Trends in Retrovirus Testing.* J Clin Immunoassay, 1988. **11**: p. 126-129.
33. Brownlee, R.G., F.J. Sunzeri, and M.P. Busch, *Application of capillary DNA chromatography to detect AIDS virus (HIV-1) DNA in blood.* J Chromatogr, 1990. **533**: p. 87-96.
34. Rawal, B.D., et al., *Evaluation of leukocyte removal filters modelled by use of HIV-infected cells and DNA amplification.* Blood, 1990. **76**(10): p. 2159-61.
35. Rawal, B.D., et al., *Dual reduction in the immunologic and infectious complications of transfusion by filtration/removal of leukocytes from donor blood soon after collection.* Transfus Med Rev, 1990. **4**(4 Suppl 1): p. 36-41.
36. Busch, M.P., et al., *Reliable confirmation and quantitation of human immunodeficiency virus type 1 antibody using a recombinant-antigen immunoblot assay.* Transfusion, 1991. **31**(2): p. 129-37.
37. Sheppard, H.W., et al., *A multicenter proficiency trial of gene amplification (PCR) for the detection of HIV-1.* J Acq Immun Def Synd, 1991. **4**: p. 277-283.
38. Busch, M.P., et al., *Evaluation of screened blood donations for human immunodeficiency virus type 1 infection by culture and DNA amplification of pooled cells.* N Engl J Med, 1991. **325**(1): p. 1-5.
39. Sankary, T.M., et al., *Rare detection of hepatitis B and hepatitis C virus genomes by polymerase chain reaction in seronegative donors with elevated alanine aminotransferase.* Transfusion, 1994. **34**(8): p. 656-60.
40. Hwang, L.Y., et al., *Human immunodeficiency virus seroprevalence among blood product recipients in San Francisco before transfusion.* Transfusion, 1989. **29**(2): p. 113-8.
41. Scillian, J.J., et al., *Early detection of antibodies against rDNA-produced HIV proteins with a flow cytometric assay.* Blood, 1989. **73**(7): p. 2041-8.
42. Roy, S., et al., *Persistent immune complexes and abnormal CD4/CD8 ratios in HIV infection.* J Acquir Immune Defic Syndr, 1990. **3**(2): p. 134-8.
43. Eble, B.E., et al., *Determination of human T lymphotropic virus type by polymerase chain reaction and correlation with risk factors in northern California blood donors.* J Infect Dis, 1993. **167**(4): p. 954-7.
44. Murphy, E.L., et al., *A prospective study of the risk of transfusion-acquired viral infections.* Transfus Med, 1998. **8**(3): p. 173-8.
45. Busch, M.P., et al., *Monitoring blood donors for HIV-2 infection by testing anti-HIV-1 reactive sera.* Transfusion, 1990. **30**(2): p. 184-7.
46. Gadol, N., G.J. Crutcher, and M.P. Busch, *Detection of intracellular HIV in lymphocytes by flow cytometry.* Cytometry, 1994. **15**(4): p. 359-70.

47. Petersen, L.R., et al., *Duration of time from onset of human immunodeficiency virus type 1 infectiousness to development of detectable antibody. The HIV Seroconversion Study Group.* Transfusion, 1994. **34**(4): p. 283-9.
48. Busch, M.P., 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**(2): p. 91-7.
49. Pau, C.P., et al., *Surveillance for human immunodeficiency virus type 1 group O infections in the United States.* Transfusion, 1996. **36**(5): p. 398-400.
50. Busch, M.P., et al., *Value and cost-effectiveness of screening blood donors for antibody to hepatitis B core antigen as a way of detecting window-phase human immunodeficiency virus type 1 infections. The HIV Blood Donor Study Group.* Transfusion, 1997. **37**(10): p. 1003-11.
51. Janssen, R.S., et al., *New testing strategy to detect early HIV-1 infection for use in incidence estimates and for clinical and prevention purposes.* Jama, 1998. **280**(1): p. 42-8.
52. Garcia Lerma, J.G., et al., *Measurement of human immunodeficiency virus type 1 plasma virus load based on reverse transcriptase (RT) activity: evidence of variabilities in levels of virion-associated RT.* J Infect Dis, 1998. **177**(5): p. 1221-9.
53. McFarland, W., et al., *Detection of early HIV infection and estimation of incidence using a sensitive/less-sensitive enzyme immunoassay testing strategy at anonymous counseling and testing sites in San Francisco.* J Acquir Immune Defic Syndr, 1999. **22**(5): p. 484-9.
54. Ling, A.E., et al., *Failure of routine HIV-1 tests in a case involving transmission with preseroconversion blood components during the infectious window period.* Jama, 2000. **284**(2): p. 210-4.
55. Machado, D.M., et al., *Use of the sensitive/less-sensitive (detuned) EIA strategy for targeting genetic analysis of HIV-1 to recently infected blood donors.* Aids, 2002. **16**(1): p. 113-9.
56. Rawal, B.D., et al., *Development of a new less-sensitive enzyme immunoassay for detection of early HIV-1 infection.* J Acquir Immune Defic Syndr, 2003. **33**(3): p. 349-55.
57. Fiebig, E.W., et al., *Dynamics of HIV viremia and antibody seroconversion in plasma donors: implications for diagnosis and staging of primary HIV infection.* Aids, 2003. **17**(13): p. 1871-9.
58. Kral, A.H., et al., *Trends in human immunodeficiency virus seroincidence among street-recruited injection drug users in San Francisco, 1987-1998.* Am J Epidemiol, 2003. **157**(10): p. 915-22.
59. Delwart, E.L., et al., *Two percent of HIV-positive U.S. blood donors are infected with non-subtype B strains.* AIDS Res Hum Retroviruses, 2003. **19**(12): p. 1065-70.
60. Fang, C.T., et al., *Human immunodeficiency virus-1 and hepatitis C virus RNA among South African blood donors: estimation of residual transfusion risk and yield of nucleic acid testing.* Vox Sang, 2003. **85**(1): p. 9-19.
61. Busch, M.P., et al., *The Viral Activation Transfusion Study (VATS): rationale, objectives, and design overview.* Transfusion, 1996. **36**(10): p. 854-9.

62. Collier, A.C., et al., *Leukocyte-reduced red blood cell transfusions in patients with anemia and human immunodeficiency virus infection: the Viral Activation Transfusion Study: a randomized controlled trial.* Jama, 2001. **285**(12): p. 1592-601.
63. Fiebig, E.W., et al., *Lymphocyte subset analysis on frozen whole blood.* Cytometry, 1997. **29**(4): p. 340-50.
64. Lee, T.H., et al., *Quantitation of white cell subpopulations by polymerase chain reaction using frozen whole-blood samples. Viral Activation Transfusion Study.* Transfusion, 1998. **38**(3): p. 262-70.
65. Fiebig, E., et al., *Assessment of donor T-cell function in cellular blood components by the CD69 induction assay: effects of storage, gamma radiation, and photochemical treatment.* Transfusion, 2000. **40**(7): p. 761-70.
66. Lederman, M.M., et al., *'Modeling' relationships among HIV-1 replication, immune activation and CD4+ T-cell losses using adjusted correlative analyses.* Aids, 2000. **14**(8): p. 951-8.
67. Roback, J.D., et al., *Multicenter evaluation of PCR methods for detecting CMV DNA in blood donors.* Transfusion, 2001. **41**(10): p. 1249-57.
68. Drew, W.L., et al., *Cytomegalovirus glycoprotein B groups in human immunodeficiency virus-infected patients with incident retinitis.* J Infect Dis, 2002. **186**(1): p. 114-7.
69. Roback, J.D., et al., *CMV DNA is rarely detected in healthy blood donors using validated PCR assays.* Transfusion, 2003. **43**(3): p. 314-21.
70. Asmuth, D.M., et al., *Absence of HBV and HCV, HTLV-I and -II, and human herpes virus-8 activation after allogeneic RBC transfusion in patients with advanced HIV-1 infection.* Transfusion, 2003. **43**(4): p. 451-8.
71. Drew, W.L., et al., *Absence of activation of CMV by blood transfusion to HIV-infected, CMV-seropositive patients.* Transfusion, 2003. **43**(10): p. 1351-7.
72. Murphy, E.L., et al., *Increased human T-lymphotropic virus type II proviral load following highly active retroviral therapy in HIV-coinfected patients.* J Acquir Immune Defic Syndr, 2003. **33**(5): p. 655-6.
73. Yomtovian, R., et al., *WBC reduction in RBC concentrates by prestorage filtration: multicenter experience.* Transfusion, 2001. **41**(8): p. 1030-6.
74. Lee, T.H., et al., *Quantitation of residual WBCs in filtered blood components by high-throughput, real-time kinetic PCR.* Transfusion, 2002. **42**(1): p. 87-93.
75. Ariga, H., et al., *Residual WBC subsets in filtered prestorage RBCs.* Transfusion, 2003. **43**(1): p. 98-106.
76. Kruskall, M.S., et al., *Survival of transfused donor white blood cells in HIV-infected recipients.* Blood, 2001. **98**(2): p. 272-9.
77. Reed, W., et al., *Sample suitability for the detection of minor white cell populations (microchimerism) by polymerase chain reaction.* Transfusion, 1998. **38**(11-12): p. 1041-5.
78. Lee, T.H., et al., *Survival of donor leukocyte subpopulations in immunocompetent transfusion recipients: frequent long-term microchimerism in severe trauma patients.* Blood, 1999. **93**(9): p. 3127-39.
79. Wang-Rodriguez, J., et al., *Immune response to blood transfusion invery-low-birthweight infants.* Transfusion, 2000. **40**(1): p. 25-34.

80. Reed, W.F., et al., *Detection of microchimerism by PCR is a function of amplification strategy.* Transfusion, 2001. **41**(1): p. 39-44.
81. Lee, T.H., et al., *Quantitation of genomic DNA in plasma and serum samples: higher concentrations of genomic DNA found in serum than in plasma.* Transfusion, 2001. **41**(2): p. 276-82.
82. Chou, S.H., et al., *Increased engraftment and GVHD after in utero transplantation of MHC-mismatched bone marrow cells and CD80low, CD86(-) dendritic cells in a fetal mouse model.* Transplantation, 2001. **72**(11): p. 1768-76.
83. Reed, W., et al., *Non-invasive determination of the paternal HLA haplotype of a fetus using kinetic PCR to detect fetal microchimerism in maternal plasma.* Bone Marrow Transplant, 2002. **29**(6): p. 527-9.
84. Utter, G.H., *Blood transfusion is associated with donor leukocyte microchimerism in trauma patients.* J Trauma, 2004.
85. Carrier, E., et al., *Induction of tolerance in nondefective mice after in utero transplantation of major histocompatibility complex-mismatched fetal hematopoietic stem cells.* Blood, 1995. **86**(12): p. 4681-90.
86. Carrier, E., et al., *Recruitment of engrafted donor cells postnatally into the blood with cytokines after in utero transplantation in mice.* Transplantation, 1997. **64**(4): p. 627-33.
87. Carrier, E., et al., *Microchimerism does not induce tolerance after in utero transplantation and may lead to the development of alloreactivity.* J Lab Clin Med, 2000. **136**(3): p. 224-35.
88. Lee, T.H., et al., *Donor WBCs can persist and transiently mediate immunologic function in a murine transfusion model: effects of irradiation, storage, and histocompatibility.* Transfusion, 2001. **41**(5): p. 637-42.
89. Donahue, J., et al., *Microchimerism does not induce tolerance and sustains immunity after in utero transplantation.* Transplantation, 2001. **71**(3): p. 359-68.
90. Lee, T.-H., *Immune mechanism responsible for clearance of donor WBCs in a murine transfusion model.* Blood, 2004.
91. Montalvo, L., *Clinical investigation of post-transfusion blood group typing using a rapid normalized quantitative PCR.* Tranfusion, 2004.
92. Murphy, E.L., et al., *Pulmonary function testing in HTLV-I and HTLV-II infected humans: a cohort study.* BMC Pulm Med, 2003. **3**(1): p. 1.
93. Orland, J.R., et al., *Prevalence and clinical features of HTLV neurologic disease in the HTLV outcomes study.* Neurology, 2003. **61**: p. 1588-94.
94. Murphy, E.L., *Respiratory and urinary tract infections, arthritis, and asthma associated with HTLV-I and HTLV-II infection.* Emerg Inf Dis, 2004. **10**: p. 109-116.
95. Murphy, E.L. and T.-H. Lee, *Higher HTLV proviral load is associated with HTLV-I vs. HTLV-II, HTLV-II subtype A versus. B, and with male sex and a history of blood transfusion.* J Infect Dis, 2004.
96. Sunzeri, F.J., et al., *Rapid simultaneous detection of multiple retroviral DNA sequences using the polymerase chain reaction and capillary DNA chromatography.* Blood, 1991. **77**(4): p. 879-86.
97. Lee, T.H., et al., *Quantitative assessment of HIV-1 DNA load by coamplification of HIV-1 gag and HLA-DQ-alpha genes.* Aids, 1991. **5**(6): p. 683-91.

98. Mayer, A., et al., *Separation and detection of DNA polynucleotides using capillary electrophoresis. Application to detection of polymerase chain reaction-amplified human immunodeficiency virus and HLA DNA.* Arch Pathol Lab Med, 1991. **115**(12): p. 1228-34.
99. Sheppard, H.W., et al., *HIV-1 PCR and isolation in seroconverting and seronegative homosexual men: absence of long-term immunosilent infection.* J Acquir Immune Defic Syndr, 1993. **6**(12): p. 1339-46.
100. Busch, M.P., et al., *Screening of blood donors for idiopathic CD4+ T-lymphocytopenia.* Transfusion, 1994. **34**(3): p. 192-7.
101. Lee, T.H., et al., *Circulating HIV-1-infected cell burden from seroconversion to AIDS: importance of postseroconversion viral load on disease course.* J Acquir Immune Defic Syndr, 1994. **7**(4): p. 381-8.
102. Sabino, E.C., et al., *Identification of low-level contamination of blood as basis for detection of human immunodeficiency virus (HIV) DNA in anti-HIV- negative specimens.* J Acquir Immune Defic Syndr, 1994. **7**(8): p. 853-9.
103. Johnson, D., D. Hirschkorn, and M.P. Busch, *Evaluation of four alternative methodologies for determination of absolute CD4+ lymphocyte counts. The National Heart, Lung, and Blood Institute Retrovirus Epidemiology Donor Study.* J Acquir Immune Defic Syndr Hum Retrovirol, 1995. **10**(5): p. 522-30.
104. Klitz, W., et al., *New HLA haplotype frequency reference standards: high-resolution and large sample typing of HLA DR-DQ haplotypes in a sample of European Americans.* Tissue Antigens, 2003. **62**(4): p. 296-307.
105. Grams, S.E., et al., *Three new DP alleles identified in a study of 800 unrelated bone marrow donor-recipient pairs.* Tissue Antigens, 2001. **58**(4): p. 272-5.
106. Hickman, H.D., et al., *Non-conservative substitutions distinguish previously uncharacterized HLA-A molecules.* Tissue Antigens, 2001. **57**(2): p. 95-102.
107. Steiner, L.L., et al., *Four new DP alleles identified in a study of 500 unrelated bone marrow donor-recipient pairs.* Tissue Antigens, 1999. **53**(2): p. 201-6.
108. Stranford, S.A., et al., *Reduction in CD8+ cell noncytotoxic anti-HIV activity in individuals receiving highly active antiretroviral therapy during primary infection.* Proc Natl Acad Sci U S A, 2001. **98**(2): p. 597-602.
109. Delwart, E., et al., *Homogeneous quasispecies in 16 out of 17 individuals during very early HIV-1 primary infection.* Aids, 2002. **16**(2): p. 189-95.
110. Hecht, F.M., et al., *Use of laboratory tests and clinical symptoms for identification of primary HIV infection.* Aids, 2002. **16**(8): p. 1119-29.
111. Barbour, J.D., *Higher CD4+ T cell counts associated with low viral pol replication capacity among treatment naive adults in early HIV-1 infection.* J Infect Dis, 2004.
112. Elkins, M.K., *Longitudinal Analysis of B Cell Repertoire and Antibody Gene Rearrangements during Early HIV Infection.* AIDS, 2003.
113. Barbour, J.D., *Persistence of primary drug resistance among recently HIV-1 infected adults.* AIDS, 2004.
114. Romeo, J.M., et al., *Analysis of hepatitis C virus RNA prevalence and surrogate markers of infection among seropositive voluntary blood donors.* Hepatology, 1993. **17**(2): p. 188-95.

115. Heneine, W., et al., *Molecular subtyping of human T-cell lymphotropic virus type 2 by single-strand conformation polymorphism analysis. Retrovirus Epidemiology Donor Study Group.* J Clin Microbiol, 1995. **33**(12): p. 3260-3.
116. Busch, M.P., et al., *Frequency of human immunodeficiency virus (HIV) infection among contemporary anti-HIV-1 and anti-HIV-1/2 supplemental test-indeterminate blood donors. The Retrovirus Epidemiology Donor Study.* Transfusion, 1996. **36**(1): p. 37-44.
117. Schreiber, G.B., et al., *The risk of transfusion-transmitted viral infections. The Retrovirus Epidemiology Donor Study.* N Engl J Med, 1996. **334**(26): p. 1685-90.
118. Korelitz, J.J., et al., *A method for estimating hepatitis B virus incidence rates in volunteer blood donors. National Heart, Lung, and Blood Institute Retrovirus Epidemiology Donor Study.* Transfusion, 1997. **37**(6): p. 634-40.
119. Busch, M.P., et al., *False-negative testing errors in routine viral marker screening of blood donors. For the Retrovirus Epidemiology Donor Study.* Transfusion, 2000. **40**(5): p. 585-9.
120. Kleinman, S.H., et al., *Frequency of HBV DNA detection in US blood donors testing positive for the presence of anti-HBc: implications for transfusion transmission and donor screening.* Transfusion, 2003. **43**(6): p. 696-704.
121. Busch, M.P., *A new strategy for estimating risks of transfusion-transmitted viral infections based on rates of detection of recently infected donors.* JAMA, 2004.
122. Kuhns, M.C., *Lack of correlation between HBsAg and HBV DNA levels in blood donors who test positive for HBsAg and anti HBc: implications for future HBV screening policy.* Tranfusion, 2004.
123. Stramer, S.L., *Detection of HIV-1 and HCV infections among antibody-negative U.S. blood donors by nucleic acid amplification testing.* N Engl J Med, 2004.
124. Busch, M.P., et al., *Absence of evidence of infection with divergent primate T-lymphotropic viruses in United States blood donors who have seroindeterminate HTLV test results.* Transfusion, 2000. **40**(4): p. 443-9.
125. Pellett, P.E., et al., *Multicenter comparison of serologic assays and estimation of human herpesvirus 8 seroprevalence among US blood donors.* Transfusion, 2003. **43**(9): p. 1260-8.
126. Kaplan, J.E., et al., *Male-to-female transmission of human T-cell lymphotropic virus types I and II: association with viral load. The Retrovirus Epidemiology Donor Study Group.* J Acquir Immune Defic Syndr Hum Retrovirol, 1996. **12**(2): p. 193-201.
127. Mariani, R., et al., *CCR2-64I polymorphism is not associated with altered CCR5 expression or coreceptor function.* J Virol, 1999. **73**(3): p. 2450-9.
128. Busch, M.P., et al., *Very low level viremia in HCV infectious unit missed by NAT.* Transfusion, 2003. **43**(8): p. 1173-4.
129. Operskalski, E.A., et al., *HCV viral load in anti-HCV-reactive donors and infectivity for their recipients.* Transfusion, 2003. **43**(10): p. 1433-41.
130. Delwart, E.L. and N.D. Kalmin, *First case of human immunodeficiency virus transmission via an RNA-screened blood donation.* Vox Sang, 2004. **86**: p. 171-177.
131. Drew, W.L., et al., *Frequency and duration of plasma CMV viremia in seroconverting blood donors and recipients.* Transfusion, 2003. **43**(3): p. 309-13.

132. Biswas, R., et al., *Comparative sensitivity of HBV NATs and HBsAg assays for detection of acute HBV infection.* Transfusion, 2003. **43**(6): p. 788-98.
133. Fiebig, E.W., *Intermittent low-level viremia during eclipse phase of primary HIV-1 infection.* Tranfusion, 2004.
134. Glynn, S., *Intermittent low-level viremia in early HCV infection.* Tranfusion, 2004.
135. Mosley, J.W., *Viral and host factors in early hepatitis C virus infection.* Hepatology, 2004.
136. Kleinman, S.H., et al., *Evaluation of a p21e-spiked western blot (immunoblot) in confirming human T-cell lymphotropic virus type I or II infection in volunteer blood donors. The Retrovirus Epidemiology Donor Study Group.* J Clin Microbiol, 1994. **32**(3): p. 603-7.
137. Busch, M.P., et al., *Accuracy of supplementary serologic testing for human T-lymphotropic virus types I and II in US blood donors. Retrovirus Epidemiology Donor Study.* Blood, 1994. **83**(4): p. 1143-8.
138. Busch, M.P., et al., *Declining value of alanine aminotransferase in screening of blood donors to prevent posttransfusion hepatitis B and C virus infection. The Retrovirus Epidemiology Donor Study.* Transfusion, 1995. **35**(11): p. 903-10.
139. Korelitz, J.J., et al., *Relationship between antibody to hepatitis B core antigen and retroviral infections in blood from volunteer donors.* Transfusion, 1996. **36**(3): p. 232-7.
140. Ownby, H.E., et al., *Loss of volunteer blood donors because of unconfirmed enzyme immunoassay screening results. Retrovirus Epidemiology Donor Study.* Transfusion, 1997. **37**(2): p. 199-205.
141. Kleinman, S., et al., *False-positive HIV-1 test results in a low-risk screening setting of voluntary blood donation. Retrovirus Epidemiology Donor Study.* Jama, 1998. **280**(12): p. 1080-5.
142. Busch, M.P., *The Efficiency of HIV p24 Antigen Screening of U S Blood Donors: Projections versus Reality.* Infusionther Transfusionmed, 1998. **25**: p. 5-8.
143. Busch, M.P., et al., *Use of third-generation hepatitis C virus (HCV) enzyme immunoassay (EIA) to resolve second-generation HCV EIA-reactive and second-generation recombinant immunoblot assay-indeterminate blood samples: data to support current Food and Drug Administration guidance on HCV lookback.* Transfusion, 2000. **40**(1): p. 10-4.
144. Tobler, L.H., et al., *Lookback on donors who are repeatedly reactive on first-generation hepatitis C virus assays:justification and rational implementation.* Transfusion, 2000. **40**(1): p. 15-24.
145. Tobler, L.H., et al., *Performance of second- and third-generation RIBAs for confirmation of third-generation HCV EIA-reactive blood donations. Retrovirus Epidemiology Donor Study.* Transfusion, 2000. **40**(8): p. 917-23.
146. Tobler, L.H., et al., *Impact of HCV 3.0 EIA relative to HCV 2.0 EIA on blood-donor screening.* Transfusion, 2003. **43**(10): p. 1452-9.
147. Jackson, B.R., et al., *The cost-effectiveness of NAT for HIV, HCV, and HBV in whole-blood donations.* Transfusion, 2003. **43**(6): p. 721-9.
148. *Provisional surveillance summary of the West Nile virus epidemic--United States, January-November 2002.* MMWR Morb Mortal Wkly Rep, 2002. **51**(50): p. 1129-33.

149. Pealer, L.N., et al., *Transmission of West Nile virus through blood transfusion in the United States in 2002.* N Engl J Med, 2003. **349**(13): p. 1236-45.
150. Dodd, R.Y., *Emerging infections, transfusion safety, and epidemiology.* N Engl J Med, 2003. **349**(13): p. 1205-6.
151. Food and Drug Administration, C.f.B.E.a.R., *Workshop on development of donor screening assays for West Nile Virus.* 2002.
152. Hollinger, F.B. and S. Kleinman, *Transfusion transmission of West Nile virus: a merging of historical and contemporary perspectives.* Transfusion, 2003. **43**(8): p. 992-7.
153. Giachetti, C., et al., *Highly sensitive multiplex assay for detection of human immunodeficiency virus type 1 and hepatitis C virus RNA.* J Clin Microbiol, 2002. **40**(7): p. 2408-19.
154. *Update: Detection of West Nile virus in blood donations--United States, 2003.* MMWR Morb Mortal Wkly Rep, 2003. **52**(38): p. 916-9.
155. Diamond, M.S., et al., *A critical role for induced IgM in the protection against West Nile virus infection.* J Exp Med, 2003. **198**(12): p. 1853-62.
156. Wang, Y., et al., *CD8+ T cells mediate recovery and immunopathology in West Nile virus encephalitis.* J Virol, 2003. **77**(24): p. 13323-34.
157. Kelley, T.W., et al., *The neuropathology of West Nile virus meningoencephalitis. A report of two cases and review of the literature.* Am J Clin Pathol, 2003. **119**(5): p. 749-53.
158. Prince, H.E. and W.R. Hogrefe, *Detection of West Nile virus (WNV)-specific immunoglobulin M in a reference laboratory setting during the 2002 WNV season in the United States.* Clin Diagn Lab Immunol, 2003. **10**(5): p. 764-8.
159. Krugman, S., et al., *Infectious hepatitis: detection of virus during the incubation period and in clinically inapparent infection.* N Engl J Med, 1959. **261**: p. 729-34.
160. Busch, M., et al., *Oversight and monitoring of blood safety in the United States.* Vox Sang, 1999. **77**(2): p. 67-76.
161. Busch, M.P., S.H. Kleinman, and G.J. Nemo, *Current and emerging infectious risks of blood transfusions.* Jama, 2003. **289**(8): p. 959-62.
162. Chamberland, M.E., et al., *Emerging infectious disease issues in blood safety.* Emerg Infect Dis, 2001. **7**(3 Suppl): p. 552-3.
163. Leiby, D.A., *Parasites and other emergent infectious agents*, in *Blood Safety in the New Millennium*, S.L. Stramer, Editor. 2001, American Association of Blood Banks: Bethesda, MD. p. 55-78.
164. Switzer, W.M., et al., *Frequent simian foamy virus infection in persons occupationally exposed to nonhuman primates.* J Virol, 2004. **78**(6): p. 2780-9.
165. Heneine, W., et al., *Human infection with foamy viruses.* Curr Top Microbiol Immunol, 2003. **277**: p. 181-96.
166. Hussain, A.I., et al., *Screening for simian foamy virus infection by using a combined antigen Western blot assay: evidence for a wide distribution among Old World primates and identification of four new divergent viruses.* Virology, 2003. **309**(2): p. 248-57.
167. Boneva, R.S., et al., *Simian foamy virus infection in a blood donor.* Transfusion, 2002. **42**(7): p. 886-91.

168. Busch, M.P. and R.Y. Dodd, *NAT and blood safety: what is the paradigm?* Transfusion, 2000. **40**(10): p. 1157-60.
169. Grant, P.R. and M.P. Busch, *Nucleic acid amplification technology methods used in blood donor screening.* Transfus Med, 2002. **12**(4): p. 229-42.
170. Miley, W.J., et al., *Real-time polymerase chain reaction assay for cell-associated HTLV type I DNA viral load.* AIDS Res Hum Retroviruses, 2000. **16**(7): p. 665-75.
171. Jones, M.S., *Two new DNA viruses found in patients with acute viral infection syndrome.* Nat Med, 2004.
172. Britto, C., et al., *Polymerase chain reaction detection: new insights into the diagnosis of chronic Chagas disease.* Mem Inst Oswaldo Cruz, 1999. **94 Suppl 1**: p. 305-6.
173. Cummings, K.L., *Rapid quantitation of Trypanosoma cruzi in host tissue by real-time PCR.* Mol Biochem Parasitol, 2004. **129**: p. 53-59.
174. Lee, T.H., et al., *Quantitation of residual white cells in filtered blood components by polymerase chain reaction amplification of HLA DQ-A DNA.* Transfusion, 1994. **34**(11): p. 986-94.
175. Tobler, L.H., et al., *Use of the Procleix HIV-1 and HCV discriminatory assays to detect HIV and HCV RNA in whole blood.* Transfusion, 2002. **42**(11): p. 1525-7.