

# Pathogen Inactivation

## A New Paradigm for Preventing Transfusion-Transmitted Infections

Jeffrey McCullough, MD

**Key Words:** Pathogen inactivation; Blood safety; Transfusion-transmitted infection

DOI: 10.1309/RAPQ3NXG3MV9AL94

### Abstract

*Remarkable improvements have been made in blood safety since the onset of the HIV epidemic. However, the current paradigm does not prevent all transfusion-transmitted infections and is reactive to new agents, thus accepting that some patients may be harmed before preventive measures are introduced. Several methods are now available that selectively damage DNA and RNA, thus inactivating pathogens contaminating blood components while not damaging the cells or plasma proteins of the blood component. Clinical trials have been completed and pathogen-inactivated platelets and plasma are widely used in Europe. A recent consensus conference recommended implementation of pathogen inactivation when a feasible and safe method is available that inactivates a broad spectrum of pathogens. The shortcomings of our present paradigm for preventing transfusion-transmitted diseases are described, along with a summary of the status of pathogen inactivation.*

It is time for a critical look at pathogen inactivation. Although this article is not intended to be a complete review, a summary of the status of the developmental work of pathogen inactivation is necessary to provide a perspective in which to consider this new paradigm.

### Pathogen Inactivation Methods

Solvent detergent treatment of plasma was developed years ago at the New York Blood Center and has become a mainstay for improving the safety of plasma derivatives. The method was also used to prepare units of solvent detergent-treated plasma made from pools of about 2,500 donors but otherwise similar to ordinary fresh frozen plasma (FFP). This product is no longer available in the United States, but a similar product is used in some other countries. The solvent detergent method is effective for viruses with a lipid envelop such as HIV, hepatitis B virus, and hepatitis C virus (HCV) but does not activate nonenveloped viruses such as hepatitis A virus (HAV) or parvovirus.

Methylene blue, when added to plasma and exposed to visible light, can inactivate most viruses and bacteria<sup>1-4</sup> by generation of reactive oxygen species, mostly singlet oxygen. Methylene blue is not effective against intracellular viruses, and so the blood component must undergo leukocyte reduction as part of pathogen inactivation by methylene blue. The treated plasma can then be frozen and used much the same as FFP. There is some loss of plasma coagulation factors in the methylene blue treatment process,<sup>3,5</sup> but methylene blue plasma is used routinely in Great Britain, Belgium, France, Portugal, Spain, and Denmark and is being considered for adoption by several other countries.

Three other methods are under development that target and damage DNA or RNA to prevent organisms from reproducing. Thus, any pathogens contaminating the blood components may be inactivated. Because none of the standard components (RBCs, platelets, and plasma) require nucleic acid replication for successful in vivo effect, this pathogen inactivation approach should not interfere with the effectiveness of the blood component. Navigant (Lakewood, CO) has used vitamin B<sub>2</sub>, or riboflavin, and UV light, and Cerus (Concord, CA) has used the psoralen compound amotosalen and UV light for platelets and plasma and a bifunctional alkylator for pathogen inactivation in RBCs.

Riboflavin damages DNA and RNA by intercalating with nucleic acids, and, when exposed to 265 to 370 nm of UV light, photolysis of the compound induces guanine oxidation, resulting in single-strand breaks.<sup>6-8</sup> It is thought that damage is so extensive to the guanine bases that DNA repair and replication are not possible.<sup>6,9</sup>

Amotosalen acts by selectively binding to nucleic acids and lipids but not proteins. Amotosalen intercalates into the helical regions of DNA or RNA and forms fixed cross-links on exposure to UV light of wavelengths of 320 to 400 nm. The cross-links are so extensive that they prevent separation of the strands of nucleic acids, which, in turn, prevents nucleic acid replication.<sup>10,11</sup> Amotosalen binds to ribosomal RNA and inhibits synthesis of proteins, such as cytokines, and, thus, may also reduce the likelihood of platelet transfusion reactions. The compound used by Cerus for pathogen inactivation of RBCs is a 3-part compound with an acridine portion that targets the nucleic acid, a reactive ester that links this to the bifunctional alkylator that damages the nucleic acids by cross-linking the DNA or RNA.<sup>12,13</sup>

### Toxicity of Compounds Used for Pathogen Inactivation

Amotosalen has a good safety profile in studies of general pharmacology, acute and repeated dose toxicity, genotoxicity, carcinogenicity, phototoxicity, reproductive and neonatal toxicity, and occupational safety.<sup>14-19</sup> The toxicity of riboflavin has been well studied as a vitamin, and, when it is subjected to UV light, 4 breakdown products result that are normal breakdown products in the body.<sup>9</sup> The toxic effects of these and the amounts infused as part of pathogen-inactivated components have been well studied and are within safety limits.<sup>20</sup> The alkylator compound used for RBC pathogen inactivation seems to have a satisfactory toxicity, genotoxicity, and mutagenicity profile.<sup>12,16,21,22</sup>

### Pathogens Inactivated

All 3 of these compounds inactivate pathogens very effectively. Amotosalen inactivates 10<sup>4</sup> to 10<sup>6</sup> of enveloped and nonenveloped viruses, gram-negative and gram-positive

bacteria, and protozoa<sup>13,23-33</sup> **Table 11.**<sup>34</sup> Although prions are not inactivated by amotosalen, the risk of transfusion-transmitted prion disease is not known. The nonenveloped parvovirus is inactivated at least at the 10<sup>5</sup> level.<sup>35</sup> Riboflavin inactivates 10<sup>4</sup> to 10<sup>6</sup> of intracellular and extracellular HIV, West Nile virus (WNV), porcine parvovirus, *Staphylococcus epidermidis*, *Escherichia coli*, vesicular stomatitis virus, *Staphylococcus aureus*, *Bacillus cereus*, and *Klebsiella pneumoniae*.<sup>36-43</sup> The alkylator used for pathogen inactivation of RBCs also has a very robust pathogen inactivation profile.<sup>13,44,45</sup>

Because most commercial assays detect full-length and incomplete and, thus, noninfectious particles, it is difficult to determine the true level of infectivity in apparently healthy blood donors. The extent to which these pathogen inactivation processes are in excess of the levels of these pathogens that would be expected in an apparently healthy blood donor who

**Table 11**  
Inactivation of Pathogens in Platelet Concentrates After Photochemical Treatment With Amotosalen and UV-A Light\*

Pathogen	Log Reduction in Organisms
Enveloped viruses	
HIV (cell-free)	>6.2
HIV (cell-associated)	>6.1
CMV	>5.9
Hepatitis B virus	>5.5
Hepatitis C virus	>4.5
Duck hepatitis B virus	>6.2
Bovine viral diarrhea virus	>6.0
Human T-cell leukemia virus type I/II	4.7/5.1
West Nile virus	>6.0
Nonenveloped viruses	
Blue tongue	6.1-6.4
Parvovirus B19	4.0-4.9
Gram-negative bacteria	
<i>Escherichia coli</i>	>6.4
<i>Serratia marcescens</i>	>6.7
<i>Klebsiella pneumoniae</i>	>5.6
<i>Pseudomonas aeruginosa</i>	4.5
<i>Salmonella choleraesuis</i>	>6.2
<i>Yersinia enterocolitica</i>	>5.9
<i>Enterobacter cloacae</i>	5.9
Gram-positive bacteria	
<i>Staphylococcus aureus</i>	6.6
<i>Staphylococcus epidermidis</i>	>6.6
<i>Streptococcus pyogenes</i>	>6.8
<i>Listeria monocytogenes</i>	>6.3
<i>Corynebacterium minutissimum</i>	>6.3
<i>Bacillus cereus</i>	>6.0
Gram-positive anaerobic bacteria	
<i>Lactobacillus</i> species	>6.9
<i>Propionibacterium acnes</i>	>6.7
<i>Clostridium perfringens</i>	>7.0
<i>Bifidobacterium adolescentis</i>	>6.5
Protozoa	
<i>Trypanosoma cruzi</i>	>5.3
<i>Plasmodium falciparum</i>	>7.0
<i>Leishmania mexicana</i>	>5.2

CMV, cytomegalovirus.

\* Reproduced with permission from McCullough J et al.<sup>34</sup> Data are summarized from references in the original article.

would have passed the standard medical or health evaluation is difficult to conclude. However, it seems that these 3 compounds are very effective inactivating transfusion-transmitted pathogens, including those for which no prevention strategy is currently in place.

## Graft-vs-Host Disease

An additional benefit from the pathogen inactivation technology is the potential to eliminate the risk of transfusion-transmitted graft-vs-host disease (GVHD). Although the damage to or prevention of replication of nucleic acids is not damaging to the cells or proteins of interest for transfusion therapy, the process also prevents replication of lymphocytes in the blood components.<sup>46-52</sup> Leukocyte depletion is not necessary to achieve this effect, which means that transfused pathogen-inactivated blood components should not cause transfusion-related GVHD. This has been established in animal studies, although a classic randomized, controlled clinical trial may be difficult to carry out in humans. Some centers in Europe have discontinued irradiating pathogen-inactivated platelets produced with the amotosalen method and have not observed transfusion-related GVHD due to the use of these unirradiated products.<sup>53,54</sup>

## In Vitro and Animal Studies of Cell Function and Pathogen Inactivation

Platelets treated with amotosalen have normal results of in vitro platelet function studies, including biochemical studies, aggregation, morphologic/hypotonic shock response, and surface markers.<sup>55-58</sup> Amotosalen-treated platelets were as hemostatically effective as normal control platelets in a thrombocytopenic rabbit model.<sup>59</sup> A recent abstract<sup>60</sup> reported some platelet damage in vitro that was dependent on the UV light dose. From the data presented, it is difficult to tell how the dose and specific wavelength of light used relate to the light used in the amotosalen and riboflavin methods. Coagulation factor levels and routine laboratory tests of hemostasis in amotosalen pathogen-inactivated plasma are not different from the levels in untreated plasma.<sup>61-63</sup> Amotosalen is an effective inactivator of hepatitis B virus and HCV in a chimpanzee model.<sup>64</sup>

Riboflavin-treated platelets also have essentially normal in vitro functional properties.<sup>37,41,42,65</sup> Plasma treated with riboflavin for pathogen inactivation also has essentially normal levels of plasma coagulation factors.<sup>66,67</sup>

RBCs treated with the alkylator have normal in vivo test results, including morphologic features, osmotic fragility, potassium efflux, hemolysis during storage, adenosine

triphosphate levels, 2,3-diphosphoglycerate levels, oxygen affinity, and RBC antigen strength.<sup>12,45</sup> The posttransfusion survival of alkylator-treated RBCs was normal in mice<sup>12</sup> and dogs,<sup>68</sup> but there seemed to be a slight but not statistically significant decreased recovery.

## In Vivo Phase 1 Studies

Amotosalen-treated platelets given to healthy research subjects had slightly decreased recovery and survival.<sup>69</sup> In 13 thrombocytopenic patients, amotosalen-treated platelets had a slightly decreased posttransfusion recovery but were equally as effective as control platelets in correcting the bleeding time and time to the next transfusion.<sup>70-72</sup>

Riboflavin-treated platelets have shown normal in vivo recovery and survival in studies of radiolabeled cells in healthy research subjects.<sup>65,73</sup>

Autologous amotosalen pathogen-inactivated FFP was equally effective as autologous untreated FFP in correcting the prothrombin time and partial thromboplastin time and elevating the levels of factors II, VII, IX, and X in healthy subjects treated with warfarin and then transfused with their own untreated or pathogen-inactivated FFP.<sup>61,74</sup>

RBCs subjected to pathogen inactivation by the alkylator have slightly decreased autologous in vivo recovery and normal survival.<sup>75-77</sup>

## Clinical Trials of Pathogen-Inactivated Components

A prospectively randomized, controlled trial of amotosalen pathogen-inactivated buffy coat vs control platelets in 103 patients showed that both platelet products were effective in increasing the posttransfusion platelet count, and there were no differences in transfusion reactions or adverse events.<sup>78</sup> A prospectively randomized, controlled trial of amotosalen pathogen-inactivated apheresis platelets<sup>79</sup> vs untreated apheresis platelets in 645 patients showed equivalence of both products in control and prevention of bleeding and fewer transfusion reactions in patients receiving pathogen-inactivated platelets.<sup>34</sup> This is the largest clinical trial of platelets ever reported and was unique in that the primary end point was hemostasis rather than platelet count. Patients in this study<sup>34</sup> who received the pathogen-inactivated platelets had significantly lower posttransfusion corrected count increments and received more transfusions. However, these patients also received lower doses of platelets owing to processing losses. When the data for patients who received doses of pathogen-inactivated platelets similar to doses received by control subjects were analyzed, the posttransfusion count increments

were similar to those for control subjects,<sup>80</sup> indicating that much of the observed difference was due to a difference in dose. This observation was confirmed on a large scale in Europe where use of pathogen inactivation has not led to an increased number of platelet transfusions.<sup>81</sup> Although no overall difference in adverse reactions was seen in the SPRINT study, there were small but significant increases in several types of adverse reactions in patients receiving pathogen-inactivated platelets.<sup>34</sup> In a more detailed report of these adverse events, it does not seem that these pathogen-inactivated platelets represented a higher-than-usual risk for patients.<sup>82</sup>

A phase 3 clinical trial of pathogen-inactivated FFP showed that improvements in hemostasis and laboratory test results were equal and not significantly different in 121 patients with acquired coagulopathies primarily due to liver disease.<sup>83,84</sup> There were no differences in the use of blood components or in bleeding complications, indicating that pathogen-inactivated FFP was as effective as untreated FFP for the treatment of acquired coagulopathy. Pathogen-inactivated FFP has also been equally as effective as untreated FFP in patients with congenital coagulopathies<sup>85,86</sup> and for replacement during plasma exchange for thrombotic thrombocytopenic purpura.<sup>87</sup>

A phase 3 trial of alkylator pathogen-inactivated RBCs has been reported.<sup>88</sup> This trial was halted prematurely owing to the development of RBC antibodies in patients who were receiving multiple transfusions of similar pathogen-inactivated RBCs but in a different trial. The reported trial<sup>88</sup> involved 223 patients undergoing cardiovascular surgery, and even though the trial was halted prematurely, statistical analysis revealed that the end point had been met. There were no significant differences in a combined end point involving cardiac and renal function. Thus, the trial can be considered successful, although the specific pathogen inactivation method will not be used in the future owing to antibody formation in multiply transfused patients. The manufacturer has modified the pathogen inactivation method<sup>44,89</sup> in a way that it believes will no longer lead to antibody formation and will soon propose to reopen the RBC trial using the new modified method.

A phase 3 clinical trial of a different pathogen inactivation method for RBCs was also begun and then halted prematurely owing to RBC antibody formation in recipients. That company elected not to attempt to resolve the antibody formation problem and has since gone out of business.

To summarize the current situation, pathogen inactivation is effective for buffy coat and apheresis platelets, and traditional methods of evaluating pharmacology, toxicity, and mutagenicity have given satisfactory results. A buffy coat platelet product (amotosalen) is being used in France, Germany, Belgium, Spain, Italy, Norway, Sweden, and Russia, and work to gain experience using the technology is underway in Switzerland, Austria, Luxembourg, and the

Czech Republic. A trial of a second buffy coat platelet product (riboflavin) is under way in Europe and will be completed in late 2007. A phase 3 trial of apheresis pathogen-inactivated platelets was completed in the United States several years ago and results were submitted to the US Food and Drug Administration (FDA), but no decision about licensure has been forthcoming, despite more than 80,000 transfusions having been given in Europe. The phase 3 trials of FFP are completed. Recently, this product was approved in Europe and is being produced there. It seems that the reasons for development of alloantibodies in the RBC phase 3 trial are understood, the method has been revised, and preparations are being made to propose a new trial.

## Discussion

### Shortcomings of the Present Paradigm

The shortcomings of our present paradigm for preventing transfusion-transmitted infections are as follows: (1) It applies only to known pathogens and transfusion-transmitted infections. (2) It does not address or prevent all currently known transfusion-transmitted infections. (3) It is reactive to the occurrence of new infectious agents and, thus, accepts that some patients will be harmed before steps can be taken to minimize transmission of the agent. (4) Current methods to detect and/or prevent transfusion of bacterially contaminated products are inadequate. (5) Many donors who do not pose a risk to patients are temporarily or permanently deferred because of the impreciseness of the present screening tests or deferral criteria.

There are several examples of these shortcomings. WNV is the first example. The blood banking–transfusion medicine community, industry, and regulators collaborated in a Herculean task of recognizing the problem, exploring donor deferral options, developing new tests, implementing them strategically, and creating a unique regulatory framework to enable this to occur in a time previously unimaginable.<sup>90</sup> These groups are all understandably proud of the accomplishment. However, during the summer of 2002, the mean risk of transfusion-transmitted WNV was 2.12 to 4.76/10,000 donations.<sup>91</sup> This implies that approximately 2,500 to 5,500 patients might have been infected (based on 12 million RBC transfusions). Of 23 patients described separately,<sup>92</sup> 7 died. The cost-effectiveness of screening for WNV has been estimated as \$897,000 per quality-adjusted life year for individual unit testing.<sup>93</sup>

Impressive as this response is, none of this would have been necessary had our transfusion-transmitted infection paradigm been based on pathogen inactivation. No additional money would have spent on this problem, countless hours of

meetings would have been unnecessary, blood bank operations would not have been disrupted and altered, suitable donors would not have been lost, and most important, patients would not have been infected or died as a result of transfusion-transmitted WNV infection.

A more recent example of a situation in which pathogen inactivation was used to intercede with a new infectious agent entering the blood supply occurred on the island of Le Reunion, a remote island in the Indian Ocean that is a department of France. A new epidemic of the RNA Chikungunya virus developed on the island, and more than 34% of the population became infected.<sup>94</sup> Although Chikungunya virus has not been reported to be transfusion-transmitted, its natural history is such that health officials believed that transfusion transmission was likely. Parenteral transmission by needle stick has occurred. Health and blood authorities believed it was not feasible to continue to collect blood owing to the widespread epidemic, so blood collection was halted on the island. An alternative was needed to maintain a blood supply. RBCs and frozen plasma were shipped from France, but owing to the distance and shipping time, it was not possible to provide platelets from France. Laboratory studies quickly established that amotosalen pathogen inactivation is effective against the Chikungunya virus,<sup>95</sup> so platelet pathogen inactivation procedures were put in place locally within a very short time. Platelets were then collected locally to meet the needs of the island.<sup>96</sup> Subsequent studies established that the outbreak was due to a new variant that may have enabled the virus to adapt to a new mosquito vector,<sup>94</sup> and there may have been as many as 2 million cases of Chikungunya virus infection worldwide in 2006.<sup>94</sup>

The outbreak involved an African virus in an Asian mosquito, and the possibility that this virus, like WNV, could be carried to the Americas<sup>94</sup> has already come true. At least 37 cases are known in the United States,<sup>97</sup> and although these occurred in returning travelers, this could become an epidemic similar to that resulting from WNV.

A third example of the shortcomings of our present paradigm is bacterial detection. If pathogen inactivation were in place, there would have been no need for the American Association of Blood Banks standard requiring methods to reduce bacterial contamination of platelets. Even with current testing methods, 20 septic reactions have been reported in about 1 million units of platelets.<sup>98</sup> Thus, the complex, expensive, and only partially effective testing that has resulted would not be necessary. As with the WNV example, the cost of test development, regulatory activities, countless hours of meetings, operational changes, and complexity of resulting logistics have increased the cost of blood products. All would have been unnecessary with a pathogen inactivation paradigm, and the transfusion of bacterially contaminated products with resulting harm to patients that continues at present, despite testing, would be avoided.

A fourth example of the shortcomings of our current paradigm is cytomegalovirus (CMV). Although leukocyte depletion greatly reduces transfusion-transmitted CMV, it still occurs in about 1% of patients,<sup>99</sup> even after CMV antibody screening of donated blood. The growing use of hematopoietic cell transplantation, complex chemotherapy regimens, and organ transplantation increases the number of patients who should receive CMV-safe blood. This, in turn, places greater demands on the supply of CMV-safe blood. Pathogen inactivation could eliminate the need for continued testing for CMV antibody and, thus, avoid problems due to lack of CMV-antibody-negative blood components.

A fifth example of the difference between a proactive pathogen inactivation paradigm and our present reactive paradigm is testing for *Trypanosoma cruzi*. A test is now available, and testing of donors has been initiated by some blood organizations, although universal testing is not required by the FDA. Pathogen inactivation is effective against *T cruzi*<sup>26,27</sup> and would make all of the test-related activity and costs unnecessary. More important, pathogen inactivation would have prevented the harm that has been done to some patients because our present transfusion-transmitted disease paradigm does not prevent transmission of *T cruzi* infection. Bacterial testing or adding a test for WNV or *T cruzi*, like the other infectious disease strategies in our current paradigm, is reactionary and accepts that patients are injured before preventive steps are implemented. Conversely, pathogen inactivation is a proactive approach to transfusion safety.

The benefits of pathogen inactivation must be considered in relation to these new steps that could have been avoided, existing known transfusion-transmitted diseases, and expected new threats to the safety of the blood supply. We cannot continue to add test after test and new donor criterion after criterion.

### The Stakeholders

This body of work represents substantial progress, and pathogen inactivation has arrived at a realistic point. We all have a role in the evolution of this technology: industry, academia, the blood banking–transfusion medicine community, and regulators, but most of all, patients.

Industry has been able to generate substantial sums of money to develop pathogen inactivation to its present state. Developers have been exceptionally willing to share their results as indicated by the large number of resulting publications. This is in interesting contrast with the disappointing lack of publications from industry on hemoglobin-based oxygen carriers. Industry has the responsibility to continue thorough, careful development of pathogen inactivation technology, pursuing appropriate safety and efficacy issues. It has a responsibility to develop a product that can be implemented operationally in a realistic manner and at a cost that



can be incorporated into transfusion therapy and the blood supply system.

Academia has a responsibility to provide knowledge, expertise, advice, and research collaboration in the development of this technology as appropriate. These are small companies that can never have the breadth and depth of knowledge that exists in our universities. The companies have a responsibility to seek this knowledge, and we in academia should participate as appropriate in the development of these products.

The blood banking–transfusion medicine community has a responsibility to consider the potential of this paradigm-changing technology in a truly open-minded and, hopefully, imaginative way. Pathogen inactivation may be technically complex. However, the blood banking community has demonstrated the ability to implement complex processes. There were times when conducting radioimmunoassay or enzyme-linked immunosorbent assay in the blood bank setting was considered impossible, but this occurred, and, in the process, it improved patient safety but increased costs. The most recent example, of course, is nucleic acid amplification testing. Initially, the concept of applying nucleic acid amplification and detection technology in a relatively standard way to test tens of millions of specimens was almost laughable. At the FDA-sponsored conference on this topic,<sup>100</sup> the concept of routine widespread nucleic acid amplification testing was greeted with considerable skepticism. Now, of course, complex nucleic acid amplification testing is done routinely although at a substantial increase in cost to eliminate but a few hundred cases of transfusion-transmitted infection. Thus, the transfusion medicine community has demonstrated its ability to implement impressive technologically sophisticated advances as a part of the existing paradigm.

As we blood banking–transfusion medicine professionals evaluate pathogen inactivation and its potential role in transfusion therapy and product preparation, it must be in the broad perspective of a paradigm shift. It may increase some production costs and alter our current operations; it might be inconvenient, at least for a while. This has been true of every major development in the past 50 years. For example, it is more expensive to produce components than whole blood, and apheresis platelets are more expensive and difficult to produce than whole blood–derived platelets.

A paradigm shift occurred with the introduction of apheresis rather than separation of units of whole blood for the production of blood components. Equipment and an entirely new way of evaluating donors for suitability had to be developed, new donor risks identified, and steps put in place to minimize these risks. This increased costs and created new risks to donors with apheresis instead of whole blood donation. Examples of risks of apheresis donation that are different from whole blood donation include citrate toxicity, cell depletion, air embolus, mechanical hemolysis, and others.<sup>101</sup> However,

we adapted this new technology and moved to the paradigm of producing components at the donation site rather than in the traditional blood component laboratory. It seems likely that this trend will extend to more extensive bedside component production as instrument technology continues to evolve.

Regulators also have a responsibility in the evolution of pathogen inactivation. First, their expectations and requirements must be defined clearly and in advance so that the developers of this technology can see the course they must take to succeed. Regulators must be clear and consistent in adhering to these expectations and not change them frequently during product development. All pertinent regulators should be involved in establishing the expectations initially so that during product development, the regulators can speak with one voice and from a single point of view. The regulators' expectations should be scientifically sound and based on available data. However, some of the major benefits of pathogen inactivation are the elimination of existing transfusion-transmitted infections, thus improving patient safety while also eliminating some current activities such as bacterial detection, irradiation, CMV testing, and the likely prevention of new infectious threats to the blood supply. These must be incorporated into regulatory and risk-benefit decisions. Regulators must look beyond HIV, HCV, and human T-cell leukemia virus.

One aspect of pathogen inactivation may seem unique but is not different from other regulatory decisions regarding a new drug or biologic. The issue is the possibility of a very small risk or one that is not manifest until far into the future. Data from studies of the magnitude necessary for licensure will never be as extensive as what experience with a new agent or technology will provide after licensure. Therefore, regulators may be concerned that although data seem to be satisfactory, unexpected or unknown adverse events may occur when the agent or technology is applied on a large scale. The lack of effective postmarketing surveillance systems and the lack of “a systematic approach to identifying premarketing drug safety problems and turning them into high quality postmarketing studies”<sup>102</sup> further complicates difficult regulatory decisions. However, regulators must cope with this issue in all potential licensing decisions, and pathogen inactivation is not different fundamentally.

The issue then becomes the potential value vs unknown long-term risks. Two other approaches for postmarketing surveillance that might be useful in pathogen inactivation are the RADAR (Research on Adverse Drug Events and Reports) project to identify previously unrecognized adverse drug and device reactions<sup>103</sup> and the growing interest in hemovigilance. The benefit of pathogen inactivation is far beyond eliminating the small number of remaining traditional transfusion-transmitted diseases such as hepatitis and HIV. The benefits include the long list of the other transfusion-transmitted infections that are not prevented by present technology ■ **Table 2**, the remaining

bacterial contamination problem, and the nearly certain expectation that more WNV- or Chikungunya virus-type situations will occur with emerging agents or changes in known agents. In addition, irradiation of blood components could be eliminated, as could testing for CMV and WNV, and the incidence of platelet transfusion reactions reduced. We are at the end of the usefulness of the present paradigm and must move to a new one. It is incumbent on all of us to consider pathogen inactivation in this broad context.

Many practical issues would need to be addressed for pathogen inactivation to be implemented in the United States. The only product that has been submitted to the FDA for licensure is platelets collected by apheresis. In the United States, many platelets are produced from whole blood, so physicians would need to decide whether to adopt a technology that would apply to only some platelets and possibly result in a dual inventory of platelets—some pathogen-inactivated and others not. This could present difficult issues in managing the dual inventory and raise the question of whether the pathogen-inactivated platelets should be used for certain patients and not for others. Implementation of pathogen inactivation might be an easier decision in centers that produce only apheresis platelets. The method for pathogen inactivation of whole blood-derived platelets that is approved in Europe is for platelets produced from buffy coats. It is not clear whether the United States would convert to buffy coat platelets to adopt

pathogen inactivation. Canada is converting its platelet production to the buffy coat method, although for reasons other than pathogen inactivation. In the United States, there would be pressure to speed the conversion to apheresis platelets or for manufacturers to develop a method for pathogen inactivation of platelets produced with the platelet-rich plasma method unless the United States also converted to the buffy coat method.

This dual inventory would not be an issue for plasma, and, if pathogen-inactivated plasma were approved in the United States, use of the same technology for plasma and platelets would make adoption for both components more convenient. Amotosalen is applicable to plasma and platelets, and riboflavin should also be effective for both components. Thus, if both processes were approved, two suppliers would be available, which might alleviate some concerns about product availability. Many more units of RBCs are transfused than platelets or plasma, and the availability of pathogen-inactivated RBCs would have a huge impact on interest in implementing this technology.

The cost of pathogen inactivation will certainly be an issue. It is not the scope of this report to deal with cost analysis. However, pathogen inactivation might not be as large an increased cost as might be expected. In addition to elimination of the patient care costs of the diseases transmitted, transmission of agents not now tested should be prevented and patients spared new infections. The countless hours spent in developing strategies to deal with new agents would be avoided, and the huge costs of testing and loss of donors owing to false-positive screening test results would be eliminated. In addition, irradiation of blood products; testing for bacterial contamination of platelets; testing for CMV and, possibly, for WNV could probably be eliminated; and 7-day storage of platelets could be reconsidered. Because plasma is replaced with a platelet additive solution during the pathogen inactivation process, more plasma is available for fractionation, thus providing some revenue. Because plasma is removed and because pathogen inactivation stops cytokine synthesis, transfusion reactions to platelets are decreased,<sup>34</sup> thus improving patient care and reducing the costs of managing these reactions. It seems unlikely that most currently required transmissible disease testing would be eliminated, but potential future costs of screening for *T. cruzi*, malaria, and *Leishmania* species could probably be avoided, and we would be prepared if an epidemic such as severe acute respiratory syndrome or avian flu should occur.

The group with the most concern about pathogen inactivation, however, is patients. They must be the primary focus of all of us in blood banking and transfusion medicine. It is our responsibility to provide adequate and safe transfusion therapy. We must pursue and embrace the developments that can contribute to this. We must recognize and accept that adoption of these improvements may be complex, disrupt our present routine operations, and pose operational or physical

**Table 2**  
Transfusion-Transmitted Infections

Pathogen	Disease	Routinely Found in the United States	
		Yes	No
Hepatitis B and C viruses	Hepatitis	X	
HIV	AIDS	X	
Hepatitis E and G viruses	Hepatitis		X
Human T-lymphotropic virus I/II	Malignant lymphoma	X	
Cytomegalovirus	Retinitis, hepatitis, pneumonia	X	
Epstein-Barr virus	Viral syndrome, ? malignancy		X
Human herpesvirus-8	Kaposi sarcoma		X
Parvovirus B19	Aplastic anemia		X
Gram-negative or gram-positive bacteria	Sepsis	X	
<i>Treponema pallidum</i>	Syphilis	X	
<i>Borrelia burgdorferi</i>	Lyme disease		X
<i>Rickettsia rickettsii</i>	Rocky Mountain spotted fever		X
<i>Ehrlichia chaffeensis</i>	Ehrlichiosis		X
<i>Trypanosoma cruzi</i>	Chagas disease		X
<i>Babesia microti</i>	Babesiosis		X
<i>Leishmania donovani</i>	Leishmaniasis		X
<i>Plasmodium</i> species	Malaria		X
West Nile virus	Meningitis; encephalitis	X	
Dengue virus	Hemorrhagic fever		X
Prion	Creutzfeldt-Jakob disease		X

challenges. Patients have a right to expect no less of us—that we use our expertise and creativity to continue to improve transfusion therapy

## Conclusions

Pathogen inactivation with solvent detergent has been used for plasma derivatives for years, and a pathogen-inactivated frozen plasma product is used in Europe presently. Substantial progress has been made in the development of pathogen-inactivated platelet, plasma, and RBC products. Safety profiles for the additives in these pathogen-inactivated methods are good. A platelet product is available in Europe, and more than 80,000 units have been administered to thousands of patients. A phase 3 trial of pathogen-inactivated platelets was completed in the United States several years ago, and a request for licensure has been submitted to the FDA. A clinical trial of a different pathogen-inactivated platelet product is under way in Europe, and this product has recently been licensed there. Pathogen-inactivated plasma (using methylene blue) is used in some European countries, and phase 3 trials of a different FFP product have also been completed. This product has been approved in Europe, and is beginning to be implemented. Three phase 3 trials of pathogen-inactivated RBC products were halted prematurely owing to antibody formation in some patients. However, before one of these trials was closed, an adequate number of patients had been entered to demonstrate clinical effectiveness of the pathogen-inactivated RBC product. The manufacturer believes the cause of the antibody formation has been eliminated and expects to reopen the RBC trial soon.

In vitro and in vivo data show that most pathogen-inactivated blood products are slightly compromised compared with untreated products. Most of our traditional methods of evaluating blood products have involved in vitro analyses and studies of in vivo survival in a small number of healthy research subjects and patients in relatively stable condition. In contrast, studies of pathogen-inactivated blood products have looked at the most important issue, which is clinical effectiveness. Platelet studies evaluated prevention or control of bleeding, not just platelet counts. RBC studies looked at clinical outcome, not just the change in hemoglobin level. Thus, at least to some extent, the information available about pathogen-inactivated products is better than that on which we have based existing transfusion therapy. Although to some extent these new pathogen-inactivated products are not the same as those we have used for years, they do not have to be the same. What they have to be is effective for patients. If pathogen inactivation represents a true advance, the blood banking–transfusion medicine community is creative enough to adopt the technology successfully. We blood banking–transfusion

medicine professionals must anticipate and embrace change. Whether pathogen inactivation will prove to be safe and effective is still being determined, but experience with thousands of transfusions in Europe is encouraging. We must consider pathogen inactivation in the broad context of history, the shortcomings of our present paradigm, and the future of transfusion safety for patients.

---

*From the Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis.*

*Address reprint requests to Dr McCullough: Dept of Laboratory Medicine and Pathology, University of Minnesota, 420 Delaware St SE, Minneapolis, MN 55455.*

*Disclosure: The author has a financial relationship with Cerus and Navigant through service on advisory boards or committees and through receipt of research funds in the past.*

## References

1. Muller-Breitkreutz K, Mohr H. Hepatitis C and human immunodeficiency virus RNA degradation by methylene blue/light treatment of human plasma. *J Med Virol.* 1998;56:239-245.
2. Wagner SJ. Virus inactivation in blood components by photoactive phenothiazine dyes. *Transfus Med Rev.* 2002;16:61-66.
3. Zeiler T, Riess H, Wittmann G, et al. The effect of methylene blue phototreatment of plasma proteins and in vitro coagulation capability of single-donor fresh-frozen plasma. *Transfusion.* 1994;34:685-689.
4. Lambrecht B, Mohr H, Knuver-hopf J, et al. Photoinactivation of viruses in human fresh plasma by phenothiazine dyes in combination with visible light. *Vox Sang.* 1991;60:207-213.
5. Morrado M, Villar A, Jimenez-Yuste VM, et al. Effects of methylene blue-treated plasma on coagulation factors [abstract]. *Blood.* 1999;94:247a.
6. Kumar VK, Lockerbi O, Keil SD, et al. Riboflavin and UV-light based pathogen reduction: extent and consequence of DNA damage at the molecular level. *Photochem Photobiol.* 2004;80:15-21.
7. Tsugita A, Okada Y, Uehara K. Photosensitized inactivation of ribonucleic acids in the presence of riboflavin. *Biochim Biophys Acta.* 1965;103:360-363.
8. Korycka-Dahl M, Richardson T. Photodegradation of DNA with fluorescent light in the presence of riboflavin, and photoprotection by flavin triplet-state quenchers. *Biochim Biophys Acta.* 1980;610:229-234.
9. Hardwick CC, Herivel TR, Hernandez SC, et al. Separation, identification and quantification of riboflavin and its photoproducts in blood products using high-performance liquid chromatography with fluorescence detection: a method to support pathogen reduction technology. *Photochem Photobiol.* 2004;80:609-615.
10. Wollowitz S. Fundamentals of the psoralen-based Helinx technology for inactivation of infectious pathogens and leukocytes in platelets and plasma. *Semin Hematol.* 2001;38:4-11.
11. Wollowitz S. Targeting DNA and RNA in pathogens: mode of action of amotosalen HCl. *Transfus Med Hemother.* 2004;31:11-16.



12. Cook D, Stassinopoulos A, Wallowitz S, et al. In vivo analysis of packed red blood cells treated with S-303 to inactivate pathogens. *Blood*. 1998;92(suppl 1):503a.
13. Dupuis K, Alfonsol A. Helinx technology used in the INTERCEPT blood system, inactivates high titers of emerging insect-borne pathogens in platelets and red cells [abstract]. *Blood Banking Transfusion Med*. 2003;1(suppl 1):S292.
14. Ciaravino V. Preclinical safety of a nucleic acid-targeted Helinx component: a clinical perspective. *Semin Hematol*. 2001;38:12-19.
15. Ciaravino V, McCullough T, Dayan AF. Pharmacokinetic and toxicology assessment of INTERCEPT (S-59 and UVA treated) platelets. *Hum Exp Toxicol*. 2001;20:533-550.
16. Ciaravino V, McCullough T, Cimino G. The role of toxicology assessment in transfusion medicine. *Transfusion*. 2003;34:1481-1492.
17. Ciaravino V, McCullough T, Cimino G, et al. Preclinical safety profile of plasma prepared using the INTERCEPT Blood System. *Vox Sang*. 2003;85:171-182.
18. Lin L, Conlan MG, Tessman J, et al. Amotosalen interactions with platelet and plasma components: absence of neoantigen formation after photochemical treatment. *Transfusion*. 2005;45:1610-1620.
19. Conlan MG, Corash L, Flament J, et al, for the euroSPRITE SPRINT, and STEP Study Groups. Intercept platelets and plasma do not result in neoantigenicity: result of seven phase 3 clinical trials [abstract]. *Vox Sang*. 2004;87:6.
20. Reddy H, Dayan AD, Cavagnaro J, et al. Toxicity testing of a novel riboflavin-based technology for pathogen reduction and white cell inactivation (Mirasol PRT™ System). *Transfus Med Rev*. In press.
21. Ciaravino V, Stassinopoulos A, Singh Y, et al. INTERCEPT red blood cells are not genotoxic or carcinogenic in a series of in vitro and in vivo tests. *Blood*. 2002;100(suppl 1):143b.
22. Ciaravino V, McCullough T, Woods N, et al. Absence of carcinogenicity in a 26-week intravenous study with S-303-treated mouse red blood cells in C57BL/6TAC-TRP53TML heterozygote mice [abstract]. *Blood Banking Transfusion Med*. 2003;1(suppl 1):S365.
23. Lin L, Cook DN, Wiesehahn GP, et al. Photochemical inactivation of viruses and bacteria in platelet concentrates by use of a novel psoralen and long-wavelength ultraviolet light. *Transfusion*. 1997;37:423-435.
24. Lin L, Alfonso R, Behrman B, et al. Photochemical treatment of platelet concentrates with a novel psoralen and UVA to enhance the safety of platelet transfusion. *Infusion Ther Transfusion Med*. 1998;25:39-48.
25. Lin L, Corten L, Murthy KK, et al. Photochemical inactivation of hepatitis B (HBV) and hepatitis C (HCV) virus in human platelet concentrates as assessed by a chimpanzee infectivity model [abstract]. *Blood*. 1998;92:502a.
26. Van Voorhis WC, Barrett LK, Eastman T, et al. *Trypanosoma cruzi* inactivation in human platelet concentrates and plasma by a psoralen (amotosalen HCl) and long-wavelength UV. *Antimicrob Agents Chemother*. 2003;47:475-479.
27. Van Voorhis W, Barrett L, Eastman T, et al. Helinx inactivates the malaria, Chagas' disease and leishmaniasis protozoa in human platelet concentrates [abstract]. *Blood*. 2002;100:708a.
28. Sawyer L, Dupuis K, Alfonso R, et al. The INTERCEPT Blood System for platelets inactivates high titers of a variety of blood-borne pathogens in platelets [abstract]. *Hematol J*. 2003;4:228.
29. Lin L, Hanson CV, Alter HJ, et al. Inactivation of viruses in platelet concentrates by photochemical treatment with amotosalen and long-wavelength ultraviolet light. *Transfusion*. 2005;45:580-590.
30. Eastman RT, Barrett LK, Dupuis K, et al. *Leishmania* inactivation in human pheresis platelets by a psoralen (amotosalen HCl) and long-wavelength ultraviolet irradiation. *Transfusion*. 2005;45:1459-1463.
31. Lin L, Dikeman R, Molini B, et al. Photochemical treatment of platelet concentrates with amotosalen and long-wavelength ultraviolet light inactivates a broad spectrum of pathogenic bacteria. *Transfusion*. 2004;44:1496-1504.
32. Pinna D, Sampson-Johannes A, Clementi M, et al. Amotosalen photochemical inactivation of severe acute respiratory syndrome coronavirus in human platelet concentrates. *Transfus Med*. 2005;15:269-276.
33. Roback JD, Conlan M, Drew WL, et al. The role of photochemical treatment with amotosalen and UVA light in the prevention of transfusion-transmitted cytomegalovirus infections. *Transfus Med Rev*. 2006;20:45-56.
34. McCullough J, Vesole DH, Benjamin RJ, et al. Therapeutic efficacy and safety of platelets treated with a photochemical process for pathogen inactivation: the SPRINT Trial. *Blood*. 2004;104:1534-1541.
35. Sawyer L, Hanson D, Castro G, et al. Inactivation of parvovirus B19 in human platelet concentrates by treatment with amotosalen and ultraviolet A illumination. *Transfusion*. 2007;47:1062-1070.
36. Ruane PH, Edrich R, Gampp D, et al. Photochemical inactivation of selected viruses and bacteria in platelet concentrates using riboflavin and light. *Transfusion*. 2004;44:877-885.
37. Goodrich L, Ghielli M, Hansen E, et al. Riboflavin and UV light inactivate enveloped and non-enveloped viruses in platelet concentrates under conditions which maintain cell quality [abstract]. *Vox Sang*. 2002;83:111. Abstract 333.
38. Li J, de Korte D, Woolum MD, et al. Pathogen reduction of buffy coat platelet concentrates using riboflavin and light: comparisons with pathogen-reduction technology-treated apheresis platelet products. *Vox Sang*. 2004;87:82-90.
39. Goodrich L, Douglas I, Urioste M. Riboflavin photoinactivation procedure inactivates significant levels of bacteria and produced a culture negative product [abstract]. *Transfusion*. 2002;42:16S.
40. Goodrich RP. The use of riboflavin for the inactivation of pathogens in blood products. *Vox Sang*. 2000;78:211-215.
41. Goodrich RP, Li J, Edrich R, et al. The Mirasol PRT system for pathogen reduction of platelets and plasma: an overview of current status and future trends. *Transfus Apheresis Sci*. 2006;35:5-17.
42. Goodrich RP, Edrich RA, Goodrich LL, et al. The antiviral and antibacterial properties of riboflavin and light: applications to blood safety and transfusion medicine. In: Silva E, Edwards AM, eds. *Flavins: Photochemistry and Photobiology*. Cambridge, England: Royal Society of Chemistry; 2006. *Comprehensive Series in Photochemistry and Photobiology*; Vol 6.
43. Cardo LJ, Rentas FJ, Ketchum L, et al. Pathogen inactivation of *Leishmania donovani infantum* in plasma and platelet concentrates using riboflavin and light. *Vox Sang*. 2006;90:85-91.
44. Sawyer LR, Mababangloob R, Patel N, et al. Inactivation of pathogens in red blood cells by a modified treatment process utilizing S-303 and glutathione [abstract]. *Vox Sang*. 2006;91(suppl 3):31-32.
45. Cook D, Stassinopoulos A, Merritt J, et al. Inactivation of pathogens in packed red blood cell (PRBC) concentrates using S-303 [abstract]. *Blood*. 1997;90:409a.

46. Grass JA, Hei DJ, Metchette K, et al. Inactivation of leukocytes in platelet concentrates by psoralen plus UVA. *Blood*. 1998;91:2180-2188.
47. Corash L, Lin L. Novel processes for inactivation of leukocytes to prevent transfusion-associated graft-versus-host disease. *Bone Marrow Transplant*. 2004;33:1-7.
48. Fiebig E, Hirschhorn DF, Maino VC, 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;40:761-770.
49. Grass JA, Wafa T, Reames A, et al. Prevention of transfusion-associated graft-versus-host disease by photochemical treatment. *Blood*. 1999;93:3140-3147.
50. Fast LD, DiLeone G, Cardarelli G, et al. Mirasol PRT treatment of donor white blood cells prevents the development of xenogeneic graft-versus-host disease in Rag 2<sup>-/-</sup>γc<sup>-/-</sup> double knockout mice. *Transfusion*. 2006;46:1553-1560.
51. Fast LD, DiLeone G, Li J, et al. Functional inactivation of white blood cells by Mirasol treatment. *Transfusion*. 2006;46:642-648.
52. Conlani M, Lin J-S, Flament J, et al. INTERCEPT platelets treated with Helinx technology to inactivate T-cells prevented transfusion-associated graft vs host disease (TAGVHD): results of 3 clinical trials. *Blood Banking Transfusion Med*. 2003;1(suppl 1):S292.
53. Osselaer JC, Doyen C, Messe N, et al. Routine use of platelet components prepared with photochemical treatment (INTERCEPT platelets): impact on clinical outcomes and resources [abstract]. *Blood*. 2005;106(11):129a.
54. Van Haute I, Benoit M, Bardon V. Therapeutic efficacy and safety of transfusion of pathogen inactivated platelets to pediatric patients [abstract]. *Vox Sang*. 2006;91(suppl 3):177.
55. Janetzko K, Klinger M, Mayaudon V, et al. Storage characteristics of split double-dose platelet concentrates derived from apheresis and treated with amotosalen hydrochloride and UVA light for pathogen inactivation. *Transfus Med Hemother*. 2002;29:193-198.
56. Knutson F, Alfonso R, Dupuis K, et al. Photochemical inactivation of bacteria and HIV in buffy-coat-derived platelet concentrates under conditions that preserve in vitro platelet function. *Vox Sang*. 2000;78:209-216.
57. Picker SM, Speer R, Gathof BS. Functional characteristics of buffy-coat PLTs photochemically treated with amotosalen-HCl for pathogen inactivation. *Transfusion*. 2004;44:320-329.
58. van Rhenen DJ, Vermeij J, Mayaudon V, et al. Functional characteristics of S-59 photochemically treated platelet concentrates derived from buffy coats. *Vox Sang*. 2000;79:206-214.
59. Lin C, Bardossy L, Drerup J, et al. Demonstration of in vivo hemostatic efficacy of human PCT platelets using a thrombocytopenic rabbit bleeding time model [abstract]. *Vox Sang*. 2000;78(suppl 1). Abstract 0098.
60. Pyke AD, Blajchman MA. The pathogen inactivation of platelets: studies of the possible mechanisms of the platelet dysfunction seen following photochemical treatment [abstract]. *Blood*. 2006;108:175a.
61. Hambleton J, Wages D, Radu-Radulescu L, et al. Pharmacokinetic study of FFP photochemically treated with amotosalen (S-59) and UV light compared to FFP in healthy volunteers anticoagulated with warfarin. *Transfusion*. 2002;42:1302-1307.
62. Cazenave JP, Isola H, Wiesel ML, et al. Stability of coagulation factors in plasma prepared with photochemical treatment (INTERCEPT™) stored for one year [abstract]. *Vox Sang*. 2006;91(suppl 3):185.
63. Singh Y, Sawyer LS, Pinkoski LS, et al. Photochemical treatment of plasma with amotosalen and long-wavelength ultraviolet light inactivates pathogens while retaining coagulation function. *Transfusion*. 2006;46:1168-1177.
64. Corten L, Wieseahn GP, Smyers JM, et al. Photochemical inactivation of hepatitis B and hepatitis C viruses in human plasma as assessed in a chimpanzee infectivity model [abstract]. *Blood*. 2000;96:60a.
65. Goodrich RP, Janssen M, Ghielli M, et al. Correlation of in vitro parameters and in vivo recovery and survival values for PRT treated platelets in normal human donors. *Transfusion*. 2003;43:79A.
66. Buytaert-Hoefen KA, Hendrix B, Gampp D, et al. Protein quality in Mirasol treated fresh frozen plasma. *Transfusion*. 2006;46(suppl):114A.
67. Buytaert-Hoefen KA, Gampp D, Edrich R, et al. Mirasol pathogen reduction treated fresh frozen plasma maintains protein quality. *Vox Sang*. 2006;91(suppl 3):183.
68. Corash L. Inactivation of viruses, bacteria, protozoa and leukocytes in platelet and red cell concentrates. *Vox Sang*. 2000;78:205-210.
69. Snyder E, Raife T, Lin L, et al. Recovery and life span of <sup>111</sup>indium-radiolabeled platelets treated with pathogen inactivation with amotosalen HCl (S-59) and ultraviolet A light. *Transfusion*. 2004;44:1732-1740.
70. Corash L, Behrman B, Rheinschmidt M, et al. Post-transfusion viability and tolerability of photochemically treated platelet concentrates [abstract]. *Blood*. 1997;90:267a.
71. Slichter SJ, Corash L, Grabowski M, et al. Viability and hemostatic function of photochemically-treated platelets in thrombocytopenic patients [abstract]. *Blood*. 1999;94:376a.
72. Slichter SJ, Raife TJ, Davis K, et al. Platelets photochemically treated with amotosalen HCl and ultraviolet A light correct prolonged bleeding times in patients with thrombocytopenia. *Transfusion*. 2006;46:731-740.
73. AuBuchon JP, Herschel L, Roger J, et al. Efficacy of apheresis platelets treated with riboflavin and ultraviolet light for pathogen reduction. *Transfusion*. 2005;45:1335-1341.
74. Wages D, Smith D, Walsh J, et al. Transfusion of therapeutic doses of virally inactivated fresh frozen plasma in healthy subjects [abstract]. *Blood*. 1997;90:409a.
75. Greenwalt TJ, Hambleton J, Wages D, et al. Viability of red blood cells treated with a novel pathogen inactivation system [abstract]. *Transfusion*. 1999;39:109s.
76. Hambleton J, Greenwalt T, Viele M, et al. Post transfusion recovery after multiple exposures to red blood cell concentrates (RBCs) treated with a novel pathogen inactivation process [abstract]. *Blood*. 1999;94:376a.
77. Rios JA, Hambleton J, et al. Viability of red cells prepared with S-303 pathogen inactivation treatment. *Transfusion*. 2006;46:1778-1786.
78. van Rhenen D, Gulliksson H, Cazenave JP, et al. Transfusion of pooled buffy coat platelet components prepared with photochemical pathogen inactivation treatment: the euroSPRITE trial. *Blood*. 2003;101:2426-2433.
79. Pineda A, McCullough J, Benjamin RJ, et al. Pathogen inactivation of platelets using a photochemical treatment with amotosalen HCl and ultraviolet light: process used in the SPRINT Trial. *Transfusion*. 2006;46:562-571.
80. Murphy S, Snyder E, Cable R, et al. Platelet dose consistency and its effect on the number of platelet transfusions for support of thrombocytopenia: an analysis of the SPRINT Trial of platelets photochemically treated with amotosalen HCl and ultraviolet A light. *Transfusion*. 2006;46:24-33.

81. Cazenave JP. Clinical experience with pathogen inactivated platelets. Presented at: Pathogen Inactivation: Making Decisions About New Technologies Consensus Conference; March 29-30, 2007; Toronto, Canada.
82. Snyder E, McCullough J, Slichter SJ, et al. Clinical safety of platelets photochemically treated with amotosalen HCl and ultraviolet A light for pathogen inactivation: the SPRINT Trial. *Transfusion*. 2005;45:1864-1875.
83. Mintz PD, Bass NM, Petz LD, et al. Photochemically treated fresh frozen plasma for transfusion of patients with acquired coagulopathy of liver disease. *Blood*. 2006;107:3753-3760.
84. Wages DC, Bass N, Keefe E, et al. Treatment of acquired coagulopathy by transfusion of fresh frozen plasma (FFP) prepared using a novel, single unit photochemical pathogen inactivation process [abstract]. *Blood*. 1999;94:247a.
85. deAlarcon P, Benjamin R, Shopnick R, et al. An open-label trial of fresh frozen plasma treated by the Helinx™ single-unit photochemical pathogen inactivation system in patients with congenital coagulation factor deficiencies [abstract]. *Blood*. 2000;96:61a.
86. de Alarcon P, Benjamin R, Dugdale M, et al. Fresh frozen plasma prepared with amotosalen HCl (S-59) photochemical pathogen inactivation (PCT-FFP): transfusion of patients with congenital coagulation factor deficiencies. *Transfusion*. 2005;45:1362-1372.
87. Mintz PD, Neff A, Mackenzie M, et al. A randomized, controlled phase III trial of therapeutic plasma exchange with fresh-frozen plasma (FFP) prepared with amotosalen and ultraviolet A light compared to untreated FFP in thrombotic thrombocytopenic purpura. *Transfusion*. 2006;46:1693-1704.
88. Benjamin RJ, McCullough J, Mintz PD, et al. Therapeutic efficacy and safety of red blood cells treated with a chemical process (S-303) for pathogen inactivation: a phase III clinical trial in cardiac surgery patients. *Transfusion*. 2005;45:1730-1749.
89. North A, Garratty G, Schott MA, et al. A modified treatment process for preparation of S-303 RBC for pathogen inactivation substantially reduces the potential for cross-reactivity [abstract]. *Vox Sang*. 2006;91(suppl 3):126.
90. Peterson LR, Epstein JS. Problem solved? West Nile virus and transfusion safety [editorial]. *N Engl J Med*. 2005;353:516-517.
91. Biggerstaff BJ, Petersen LR. Estimated risk of transmission of the West Nile virus through blood transfusion in the US, 2002. *Transfusion*. 2003;43:1007-1017.
92. Pealer LN, Marfin AA, Petersen LR, et al. Transmission of West Nile virus through blood transfusion in the United States in 2002. *N Engl J Med*. 2003;349:1236-1245.
93. Custer B, Busch MP, Marfin AA, et al. The cost-effectiveness of screening the US blood supply for West Nile virus. *Ann Intern Med*. 2005;143:486-492.
94. Charrel RN, de Lamballerie X, Raoult D. Chikungunya outbreaks: the globalization of vectorborne diseases. *N Engl J Med*. 2007;356:769-771.
95. Sawyer L, Dupuis K, Sampson-Johannes A, et al. Inactivation of Chikungunya virus in plasma and platelets using Helinx technology, as utilized in the INTERCEPT Blood System [abstract]. *Vox Sang*. 2006;91(suppl 3):74.
96. Rasongles P, Isola H, Kientz D, et al. Rapid implementation of photochemical pathogen inactivation (INTERCEPT) for preparation of platelet components during an epidemic of Chikungunya virus [abstract]. *Vox Sang*. 2006;91(suppl 3):32.
97. Farnon E. Update: Chikungunya fever diagnosed among international travelers: United States, 2006. *MMWR Morb Mortal Wkly Rep*. 2007;56:276-277.
98. Eder AF, Weiss JW, Notari EP, et al. Residual risk of septic transfusion reactions from bacterially-screened platelets, pheresis linked to double-needle collection procedures [abstract]. *Transfusion*. 2006;46:1A.
99. Bowden RA, Slichter SJ, Sayers M, et al. A comparison of filtered leukocyte-reduced and cytomegalovirus (CMV) seronegative blood products for the prevention of transfusion-associated CMV infection after marrow transplant. *Blood*. 1995;86:3598-3603.
100. Hewlett IK, Epstein JS. Food and drug administration conference on the feasibility of genetic technology to close the HIV window in donor screening. *Transfusion*. 1997;37:346-351.
101. McCullough J. Blood donor medical assessment and blood collection. In: *Transfusion Medicine*. 2nd ed. Philadelphia, PA: Elsevier Churchill Livingstone; 2005:53-75.
102. Psaty BM, Burke S. Institute of Medicine on drug safety. *N Engl J Med*. 2006;355:1753-1759.
103. Bennett CL, Nebeker JR, Lyons E, et al. The Research on Adverse Drug Events and Reports (RADAR) project. *JAMA*. 2005;293:2131-2140.