centrations. All sites were contacted and actively solicited to report any adverse reaction related to the use of Mirasol treated platelets.

Results: A total of 11 centers in Europe participate in the program. The evaluation program includes assessment of Mirasol treated platelets and Mirasol treated plasma. Currently 166 platelet products collected on Trima, Amicus, Haemonetics and Orbisac have been treated and evaluated; 110 treated products have been transfused. Overall evaluation of the ease of use of the process was rated as excellent. In vitro cell quality results from 7 centers and 111 products have been reported and are summarized in the table below. Swirl was rated positive in all products using either a numeric rating (3 to 0) or as presence/absence of swirl. No platelet loss following treatment and storage was reported. All products met European guidelines in terms of platelet content and for sample pH. No adverse reactions were reported after transfusion of the platelets to patients at any of the participating sites. Evaluation of the Mirasol system for plasma is ongoing.

Conclusions: The results presented here are consistent with previous data obtained from in vitro and in vivo studies, and demonstrate that Mirasol treated platelets are safe can be incorporated effectively in the routine blood bank and transfusion setting.

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SUCCESSFUL TREATMENT OF A POST-PARTUM BLEEDING WITH THE VIRUS INACTIVATED FRESH FROZEN PLASMA SAFE: A CASE REPORT

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Background: Disseminated Intravascular Coagulation (DIC) is a disorder characterized by both acute generalized, widespread activation of coagulation, which results in thrombotic complications due to the intravascular formation of fibrin, and diffuse hemorrhages, associated to deficit of platelets and factors of the coagulation (Factors V, VIII, X, XIII, fibrinogen, prothrombin, anti-trombin, C Protein). The DIC may occur in a variety of disorders among which: sepsis, severe infections, malignancies, obstetric or vascular disorders, and severe toxic or immunological reactions. Nevertheless, the obstetric pathology represents the main causes of DIC and between these the post-partum hemorrhage shock constitutes an obstetric and transfusion emergency. However, clinical and laboratory diagnosis of the syndrome remains difficult, as there is no single laboratory test, sensitive and specific enough, to allow a definitive diagnosis of DIC.

Aims: In the present work we present the case of an hemorrhagic shock developed in a nullipara at $39°$ week of gestation, complicated by a DIC, after an emergency caesarean section.

Methods: A 33-year-old nullipara was hospitalized by our Gynecology and Obstetrics Division in the 2008, in order to be submitted to caesarean section. The patient was treated in the last two months by acetil-salicylic acid because of an unknown proneness to thrombosis. The patient was submitted to an emergency caesarean and developed after 4 hours by the birth a vaginal laceration and uterine atony, accompanied by postpartum hemorrage. The clinical study showed that the patient appeared pale, with rapid decrease of the blood-pressure values (70/40 mmHg), heart rate of 110 bpm, diuresis < 500cc and high breath rate. The laboratory analyses show an Hb of 7.2 mg/dL and Hct of 22%. The patient was therefore submitted to the transfusion of 3 unities of red cell concentrates buffy coat removed (RCC). The laboratory analyses showed after 2 hours the following results: progressive reduction of the platelets (PLT) $< 80.000/\mu l$, fibrinogen < 200 mg/dL, very high values of D-Dimer, INR > 2.

Results: These results lead to a DIC post-partum diagnosis and the patient was treated by virus inactivated fresh frozen plasma safe. In particular, it was transfused 3 unity/die until the normalization of the coagulations parameters that it happened in turn of 3 days. The screening of the coagulation and the virology analyses performed after 1 month showed a complete remission and a general state of health of the patient.

Conclusions: Numerous studies have shown that after a DIC diagnosis the therapeutic results depend from the timeliness of the recognition of the disease symptoms and from the capacity to make an early diagnosis, in order to identify the cases at risk and to establish a therapy. The data of the our work show that the fresh-frozen plasma safe, remains still today, although the recent use of factors recombinant activated factor VII (FVIIa), a therapeutic treatment of high efficacy and safety in the control of the bleeding post-partum DIC correlated.

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THE INTRODUCTION OF A NOVEL PRION PROTEIN (PRPSC) REMOVAL TECHNOLOGY FOR THE PHARMACEUTICALLY LICENSED PLASMA (OCTAPLAS∩)

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Background: Four possible transmissions of variant Creutzfeldt-Jakob Disease (vCJD) through non-leukocyte depleted red blood cell concentrates in the UK have made prion diseases a matter of concern in today's blood therapy. All manufacturers of plasma-derived biopharmaceuticals are requested to perform appropriate prion safety evaluations of their product portfolio, and an improved safeguarding in terms of risk for prion transmission is promoted when possible from a technological point of view and feasible in terms of appropriateness and quality outcome.

Aims: The aim of our studies was to evaluate the incorporation of a novel prion protein removal technology into the manufacturing process of Octaplas®, a solvent/detergent (S/D) treated human plasma. The PrPSc removal was achieved by a chromatographic step, utilising an affinity ligand selected for prion protein binding that was developed by the company PRDT (Pathogen Removal and Diagnostic Technologies Inc., USA).

Methods: A validated downscale model of the adapted manufacturing process was used as basis for all investigational studies. Exogenous spike materials derived from brains of hamsters infected with hamster-adapted scrapie 263 K were used to investigate the PrPSc binding capacity of the resin. Standard Western blot assays were used for the detection and determination of PrPSc levels in the various samples.

Results: Our studies demonstrate that PrPSc binds rapidly and with a very high affinity to the novel prion protein-affinity-resin. Based on the amount of PrPSc captured, as determined by Western blotting of both the product fractions and resin, a very high and robust binding capacity in the order of 6 log10 ID50 bound per ml resin was demonstrated in this particular cell-free Octaplas® matrix.

Summary/Conclusions: The incorporation of this new chromatographic technology to remove pathogenic prions, potentially present in plasma, during Octaplas® manufacturing has been shown to be both technologically possible and feasible. The robust, reproducible PrPSc binding demonstrated by the PRDT affinity resin will further improve the safety margin of Octaplas® in terms of prion diseases such as variant Creutzfeldt-Jakob disease.

ORIGINAL ARTICLE

Emerging infectious disease agents and their potential threat to transfusion safety

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BACKGROUND: Emerging infections have been identified as a continuing threat to human health. Many such infections are known to be transmissible by blood transfusion, while others have properties indicating this potential. There has been no comprehensive review of such infectious agents and their threat to transfusion recipient safety to date.

STUDY DESIGN AND METHODS: The members of AABB's Transfusion Transmitted Diseases Committee reviewed a large number of information sources in order to identify infectious agents with actual or potential risk of transfusion transmission now or in the future in the US or Canada; with few exceptions, these agents do not have available interventions to reduce the risk of such transmission. Using a group discussion and writing process, key characteristics of each agent were identified, researched, recorded and documented in standardized format. A group process was used to prioritize each agent on the basis of scientific/epidemiologic data and a subjective assessment of public perception and/or concern expressed by regulatory agencies.

RESULTS: Sixty-eight infectious agents were identified and are described in detail in a single Supplement to **TRANSFUSION**. Key information will also be provided in web-based form and updated as necessary. The highest priorities were assigned to *Babesia* species, Dengue virus, and vCJD.

CONCLUSION: The information is expected to support the needs of clinicians and transfusion medicine experts in the recognition and management of emerging infections among blood donors and blood recipients.

INTRODUCTION

The concept of emerging infectious disease (EID) has developed over the last 2 decades, as it became apparent that full control of infectious disease had not been achieved. From 1997 data, the World Health Organization (WHO) estimated that infectious diseases were responsible for about 33% of all deaths worldwide primarily in the developing world, and these diseases remain one of the principal challenges to human survival (WHO The World Health Report 1998: Life in the 21st Century. A vision for all. World Health Organization, Geneva, 1998; http://www.who.int/whr/1998/en/index.html). Emerging infections are defined as those whose incidence in humans has increased within the past 2 decades or threatens to increase in the near future.[1] Emergence may be due to evolution of an existing organism, to the spread of a new agent, to the recognition of an infection that has been present in the population but has gone undetected, or to the realization that an established disease has an infectious origin. Emergence also may be used to describe the reappearance of a known infection after a decline in incidence.[1] The first emerging infection to have a major effect on blood safety was human immunodeficiency virus (HIV), the agent responsible for acquired immunodeficiency syndrome (AIDS), and the lessons learned from

that epidemic have sensitized us to the need to be alert to those emerging infections that can impact blood safety in the future.

Any infection with an asymptomatic blood-borne phase has the potential for transmission by transfusion, whether the infectious phase is prolonged, as is the case for hepatitis B virus (HBV) or HIV, or short, as in the case of West Nile virus (WNV) or dengue virus (DENV). Other characteristics that are necessary for transmission by transfusion are the survival/persistence of the infectious agent in collected blood or components, and its ability to cause infection by the intravenous route. Transfusion transmission will be of little relevance unless the agent also causes identifiable disease in the recipient. The frequency with which an infection is transmitted to blood recipients depends directly upon the length of the asymptomatic blood-borne period, how often blood is donated during this period, and the immune status of the recipient population. A number of factors relating both to the infectious agent and to the genetic and immunologic makeup of the recipient also will determine the frequency and severity of the disease resulting from the infection.

Concern about transfusion-transmitted infections seems to be driven by two sets of factors. First is the public health impact of the infection, characterized by its frequency and the severity of the outcomes, and by the risk of secondary transmissions that can be determined in more or less quantitative terms. Second is the public reaction to the disease, which appears to be driven, in some examples, more by emotional aspects and is not readily quantitated. The public response may be disproportionate to the severity of the infection. However, both aspects must be considered when responding to the threat of transfusion-transmitted infections. Priority setting in response to potential and emerging transfusion-transmitted infections must evaluate both the public health aspects, driven by scientific data, and the public response to, or perception of, the agents and their risk.

Many mechanisms lead to the emergence of infectious diseases.[2] Most dramatic is the appearance of a completely new human infection. This most often reflects a circumstance in which a zoonotic infection crosses over into the human population. A classic example is HIV/AIDS, which is thought to have occurred as a result of cross-species transmission of simian immunodeficiency viruses from monkeys to great apes and then to humans in Africa.[3] The original transmission perhaps occurred as a result of preparation of bushmeat derived from apes for human consumption. Such species crossings may be accompanied, or facilitated, by genetic changes in the infectious agent.[2,4] In the case of HIV, the subsequent transmission of the virus resulted from a variety of human behaviors involving sexual and injection drug use networks, travel, and blood-borne transmissions. A second example of such a species jump was severe acute respiratory syndrome (SARS), a disease caused by an animal coronavirus previously unrecognized in humans and again probably transmitted from exotic mammals used as a food source.[5] Another cause for emergence is the expansion of existing infections into a larger geographic region and/or a greater proportion of a susceptible population, often brought about by ecological and/or behavioral changes or by population movement. A striking example of this has been the emergence of WNV in the Americas.[6,7] It is unclear how the virus, which is primarily a bird-mosquito pathogen for which humans become an unintentional host, initially entered the United States (US), but its subsequent spread across the continent and into the Caribbean and Latin America has been extraordinarily rapid and complete. Other examples of potentially transfusion-transmissible agents that are expanding geographically include DENV and chikungunya viruses (CHIKV), *Plasmodium* species (malaria), *Babesia* species, and *Trypanosoma cruzi* (the agent of Chagas disease).[8-12] Another source of apparent emergence is the new recognition of existing human agents, often as a result of pathogen discovery techniques. Examples of this include human herpesvirus-8 (HHV-8), the nonpathogenic GB viruses (GBV-C, initially termed hepatitis G virus or HGV),[13] and Torque teno viruses (TTV/SEN-V).[14] Some infections emerge (or re-emerge) as a result of the breakdown of public health measures for previously controlled infections including the failure of initially effective vaccines, antibiotics, or vector control programs; examples are the re-emergence of malaria in areas of prior control and the geographical spread of DENV and CHIKV. Finally, otherwise benign infections may become serious pathogens in the face of modern medical treatments, particularly those involving immunosuppression. This group includes agents like cytomegalovirus (CMV) and other herpesviruses, human parvovirus B19 (B19V), and *Babesia* species.

Several factors that can contribute to the emergence of an infection frequently work together. Environmental change (often as a result of human interventions) is a major source, but changes in living conditions also may have a significant impact. Urbanization, particularly in the developing world, can lead to very crowded conditions with limited hygiene. Social disruption and conflict also have been associated with numerous outbreaks. Of particular relevance to transfusion medicine is travel, which has been a major factor in the spread of emerging infection (consider, for example, HIV, *Plasmodium* species, *T. cruzi*, DENV, and CHIKV).[3] Sometimes the spread of an emerging agent is a result of the importation of an animal host (for example, the introduction of monkeypox into the US), a vector such as a mosquito, or even a food. Mobile reservoirs (e.g., birds) can transport pathogens from one region to another over long distances (for example, WNV or the highly pathogenic H5N1 influenza A virus). In the

context of transfusion, while donor travel may not initiate or expand an epidemic, it can result in the transmission of an exotic, foreign agent to a recipient.

There have been a number of articles and reviews on the subject of emerging infections and their impact, or potential impact, on transfusion-associated illnesses,[9,10,15] but there does not appear to be any systematic review identifying a wide range of such agents and their key properties. Neither is there any comprehensive guidance to transfusion service and clinical staff on how best to recognize and manage emerging transfusion-transmitted infections. In this context, it is important to note that while decisions and recommendations about the overall management of blood safety are likely to be made at the institutional level (i.e., regulatory agency, blood system, or professional organization), individual practitioners also have specific responsibilities. A blood center or transfusion service physician may need to decide whether to accept or defer and how to counsel a donor with a history of disease, infection, or exposure, while a caregiver may be faced with the challenge of diagnosing, recognizing the link to blood, and reporting a suspected posttransfusion infection.

The intent of this Supplement is to provide a set of tools identifying, describing, and prioritizing those EID agents that have an actual or potential risk of transmission by transfusion and for which there is no currently implemented intervention. Of necessity, this list of emerging agents is not, and can never be, exhaustive due to the nature of EID agents, but it does reflect the consensus opinion of a group of experts. The major part of the Supplement consists of a set of 68 Fact Sheets, each of which provides referenced, systematic information about a single agent. (See Appendix 2.) Included is standard background information about each agent, along with an assessment of those characteristics specifically related to transfusion transmission. Although it is not intended that the Fact Sheets should provide specific recommendations about donor or patient management, consensus opinions about prudent approaches to a number of issues (such as donor deferral periods) are included wherever possible based on facts that are currently inferred or known. Additionally, an attempt has been made to rank the agents according to the consensus opinion about their anticipated impact upon blood safety. Such a ranking should not be regarded as definitive, and another group of experts may come to different conclusions. However, it may serve to focus attention on agents that merit more immediate attention in the development of plans for future interventions and might serve to focus the attention of the clinician on possible starting points for the diagnosis of an unfamiliar infection potentially associated with transfusion. It is critical to remember, however, that the very essence of emerging infections is that their evolution and manifestations are inherently unpredictable.

Ideally, there should be systems in place to deal with emerging infections, not only generally, but also specifically in the context of transfusion safety. Primarily, this is the responsibility of agencies that are charged with the maintenance of public health, the management of the blood supply, and its regulation. As it is unlikely that the first occurrence of an emerging infection will be seen in a transfused recipient, it is important that there be a system of assessing the threat and risk of emerging infections for their potential impact on blood safety and availability. This requires a process for evaluating each emerging infection for its transmissibility by this route and for estimating the severity and potential extent of the threat. The risk assessment should help to define the need for, and urgency of, development and implementation of interventions to reduce the risk of transmission of the agent. Such interventions, if implemented, must then be evaluated for efficacy and modified as appropriate.

There is no simple formula for recognizing that a transfusion-transmitted infection has occurred, particularly in the case of a rare or unusual disease agent. Nevertheless, many such events have been recognized by astute clinicians. Knowledge of the potential for transmission of an emerging infection can be valuable and very likely contributed to the relatively early recognition of transfusion transmission of WNV.[16,17] Unusual posttransfusion events with a suspected infectious origin should be brought to the attention of experts in infectious diseases and public health agencies for assistance in identification and follow-up. Investigation of illness occurring a few days or more after transfusion can diagnose infections using serologic or molecular evidence of infectious agents in posttransfusion samples. However, such detection is by no means definitive. A pretransfusion patient sample is extremely helpful if available, as this will reveal whether an infection predated the transfusion. Clinicians may not realize that type and crossmatch samples and diagnostic blood specimens may be available for up to 2 weeks following collection before they are discarded by the laboratory. Finally, recall and further testing of associated donors can tell us whether one or more of them was the likely source of the infection. Ideally, if the responsible agent can be isolated from both donor and recipient, molecular analyses, such as nucleic acid sequencing, can assist in identifying or excluding the same agent from the two sources. When a connection is made, testing of co-component recipients can further confirm transmission from a single donor via multiple blood components.

There are significant problems in recognizing that infections with very long incubation periods may have been transmitted by transfusion; this was illustrated by HIV/AIDS, which did not result in well-defined illness until long after the infection occurred. This delayed early recognition of transfusion-transmitted HIV and concealed

the magnitude of the infectious donor and infected recipient populations. Proactive approaches to transfusion transmission of EIDs, especially those with lengthy incubation periods, include the serologic or molecular evaluation of appropriate donor-recipient sample repositories, or engaging in active surveillance such as that used in the United Kingdom (UK) to identify the transmission of variant Creutzfeldt-Jakob disease (vCJD) by transfusion.[18,19] However, donor-recipient repositories require a large investment to create and maintain and have other limitations such as adequacy of sample size to detect rare events, the timing of collection of the retained samples (which may predate the agent's emergence), and the geography of collected samples which may be outside of the affected area.[20] Hemovigilance programs, while valuable for other reasons, are unlikely to contribute substantially to the identification of newly emerging post-transfusion infections since they are generally designed to identify well-defined acute outcomes.

An important component of preparedness is the establishment of close relationships between blood establishments, regulatory authorities, public health agencies, the medical community, and industry. This was exemplified by the rapid and effective response in the US to the emergence of WNV and the subsequent recognition of its transfusion transmissibility. Appropriate donor screening tests were rapidly developed and deployed within less than a year of the confirmation of the threat to blood safety.[7,21,22]

Although it is reasonable to consider plans for the management of an emerging transfusion-transmitted infection, it is not clear when, whether, or how a response to a potential threat should be triggered. Ideally, given the availability of suitable resources, studies to assess the actual extent and nature of the risk conveyed by high-priority agents would be undertaken. Assessment of the prevalence and incidence of the infection in the donor population and of the nature and dynamics of emergence would provide valuable information, as would investigation of the infectivity of the agent and of potential interventions. These activities may be time-consuming and inappropriate in the face of an explosive outbreak, although they may offer a foundation for decision making in the face of a less dramatic emergence, as was done for *T. cruzi* antibody blood donation screening in the US.[23,24]

The question of when to take specific actions to prevent or mitigate transfusion transmission of an EID agent is beyond the scope of this Supplement. However, the information provided should contribute materially to the factual background necessary to make such decisions and help establish some guiding principles for decision making. Interventions may be based upon questioning prospective donors about their medical risk or exposure histories (although neither the sensitivity nor the specific-

ity of this approach have proven adequate historically as a standalone strategy, with the exception of the dramatic reduction in HIV transfusion transmission in the early 1980s in San Francisco following targeted questioning[25]) and upon the use of laboratory tests, or a combination of these approaches. The availability of a specific donor screening test, however, should make it possible to eliminate or at least refine donor questions.[26] Finally, there is hope that pathogen-reduction methods will provide a generalized, proactive intervention to eliminate or reduce the risk of transmission of emerging infections by transfusion; however, such methods are not yet available for all blood components and their success and safety have not undergone extensive validation in the real world.

Usually interventions are not implemented until it is clear that transfusion transmission has occurred, although in some cases the potential outcome of transmission is considered to be so severe that regulators require action even in the absence of documented transmission as was the case for vCJD (a decision that was based on, and cited in retrospect to justify, a rather stringent form of the precautionary principle[27,28]). Regulators have considered, and some have even implemented, precautions to reduce the risk of transmission of the non-pathogenic simian foamy virus (SFV) out of fear that infection occurring in a human might be accompanied by a mutation conferring disease-causing capability to the virus.[29]

The precautionary principle is often cited when decisions about interventions to reduce the risk of transfusion-transmitted infections are discussed. It is suggested that in the absence of any specific information about the efficacy of an intervention, implementation is appropriate as long as it does no harm. Commentary on the precautionary principle suggests that it should not be invoked without some evaluation to assure that the measure is not extreme and does not exceed other measures taken in similar circumstances.[30] The hope is that information in this Supplement will contribute to informed decision making and to appropriate and well-reasoned application of "precautionism."

FRAMEWORK FOR ASSESSMENT

The Supplement is a project undertaken by the AABB Transfusion-Transmitted Diseases (TTD) Committee. The TTD provides consultation on all facets of transfusion and transplantation-related diseases and associated donor management and laboratory testing. It consists of volunteer members with expertise in various areas of infectious disease and blood center operations, and includes liaisons from the Food and Drug Administration (FDA), Centers for Disease Control and Prevention (CDC), the US Department of Defense (DOD), the

American Society of Hematology, and the Association of Public Health Laboratories.

The TTD committee undertook this project to collate, in a series of Fact Sheets, information concerning EID agents posing demonstrated or potential risk to the safety of transfusion or transplant recipients (Appendix 2). Specifically, the task was to review and prioritize the status of current and EID agents that could be transfusion transmitted with potential adverse outcomes for recipients. Creation of such a thorough review document was judged to be useful for overall policy development and strategic planning. In this document, most of the emphasis is placed on transfusion-transmitted disease agents; however, it should be noted that many issues relevant to agents transmitted by transfusion will overlap with agents that are transplant transmitted. Due to the potential for blood-borne transmission of agents that are transplant transmitted, several agents for which organ transmission has been documented, even in the absence of transfusion transmission, have been included (e.g., rabies virus and lymphocytic choriomeningitis virus).[31,32]

The AABB TTD committee was aware of previous efforts to develop tools for communicating the threat of EID agents entering the blood supply and was able to obtain current versions of Héma-Québec internal EID charts (G. Delage, vice president, Medical Affairs) and information from the US DOD; materials being developed by the US Public Health Service were requested but not made available. Findings of meetings such as the CDC conference on tick-borne diseases[33] and FDA conferences on parvovirus B19, malaria, and donor behavioral risks were scrutinized:

- www.fda.gov/downloads/BiologicsBloodVaccines NewsEvents/WorkshopsMeetingsConferences/ TranscriptsMinutes/UCM055339.pdf
- www.fda.gov/downloads/BiologicsBloodVaccines NewsEvents/WorkshopsMeetingsConferences/ TranscriptsMinutes/UCM054429.pdf
- www.fda.gov/downloads/BiologicsBloodVaccines NewsEvents/WorkshopsMeetingsConferences/ TranscriptsMinutes/UCM054430.pdf

Although the original intent was to prepare a series of consolidated matrices, it was apparent that the amount of information collected exceeded the format of a simple matrix and instead the document evolved into multipage Fact Sheets for each specific agent.

In determining which specific EID agents should be included in this review, the following factors were considered (agents for which the FDA requires donor testing have been excluded from this publication):

- The agent must infect or pose a potential risk to humans.

- With good documentation, the agent must be transmissible by transfusion or by organ/tissue transplantation or such transmission must be biologically plausible (i.e., the agent is present in plasma or associated with blood cells or donor tissue during a time when the donor is asymptomatic or has a biological basis to suggest the possibility to replicate in blood, tissue, or organs).
- The agent must have the possibility of being introduced into the blood supply during an epidemic, following an act of bioterrorism, or inadvertently during its emergence.
- The agent must lack a current intervention strategy that is widespread and known to be effective.

Many of these agents are considered pandemic threats or to have epidemic potential outside the US and Canada, but the primary focus for this Supplement was the presence, or threat, of the agent emerging in the US and Canada.

Development of the Fact Sheets was an iterative process. First a list of potential agents that met the proposed definitions for inclusion was developed and the format for presenting the information was devised. Both naturally occurring agents and agents that could be considered bioterrorism threats were included. The preliminary draft of each Fact Sheet was prepared by an individual TTD member. Each Fact Sheet was then subjected to broad discussion and underwent several additional review cycles by various groups within the TTD. Articles in conventional and esoteric publications were scrutinized to document blood-borne capability. This review included intensive fact checks and standardization of format, culminating in a final review by the entire TTD committee.

Each Fact Sheet contains data published primarily in peer-reviewed journals or texts that are relevant to the agent and its potential to be transfusion transmitted, and when available also includes data relevant to transplant-transmitted agents. They include general background and epidemiologic information, as well as information specific to blood donation. In lieu of a comprehensive bibliography, a suggested reading list is provided for each agent that includes the most relevant citations on the agent's capacity for transfusion transmission, and the source articles documenting transfusion-transmission events. The list of suggested reading includes one or more recent review articles or book chapters that will familiarize the reader with the agent's general biologic and epidemiologic characteristics.

Pertinent consensus categories that were selected by the TTD core leadership for the Fact Sheets were designed to provide the necessary background for each agent as well as other data that were deemed relevant for a discussion of their threat to recipient safety. These include:

Disease Agent:
Disease Agent Characteristics:
Disease Name:
Priority Level:
- Scientific/Epidemiologic evidence regarding blood safety:
- Public perception and/or regulatory concern regarding blood safety:
- Public concern regarding disease agent:

Background:
Common Human Exposure Routes:
Likelihood of Secondary Transmission:
At-Risk Populations:
Vector and Reservoir Involved:
Blood Phase:
Survival/Persistence in Blood Products:
Transmission by Blood Transfusion:
Cases/Frequency in Population:
Incubation Period:
Likelihood of Clinical Disease:
Primary Disease Symptoms:
Severity of Clinical Disease:
Mortality:
Chronic Carriage:
Treatment Available/Efficacious:
Agent-Specific Screening Question(s):
Laboratory Test(s) Available:
Currently Recommended Donor Deferral Period:
Impact on Blood Availability:
- Agent-specific screening question(s):
- Laboratory test(s) available:

Impact on Blood Safety:
- Agent-specific screening question(s):
- Laboratory test(s) available:

Leukoreduction Efficacy:
Pathogen Reduction Efficacy for Plasma Derivatives:
Other Prevention Measures:
Suggested Reading:

Documentation of transfusion transmission and its clinical outcomes was a key component of this project. When documentation was lacking, agents were evaluated for their *potential* for transfusion transmission. For the former, the task was relatively straightforward. For the latter, it was much more difficult because of a paucity of data and difficulties in proving the negative proposition that transfusion is not implicated in transmission. The occurrence of an asymptomatic blood phase and its duration (whether a long-term chronic carrier state or shorter-term acute infection) were the critical elements used to estimate this "risk" of transmission. Available evidence about the persistence or survival of an infectious agent during blood component manufacture and storage was examined where possible. Treatment availability and its effectiveness were reviewed.

Many sources were used to judge the plausibility of blood transmission. Obviously, peer-reviewed publications and textbooks are of great importance and have been cited in the suggested reading, but for several of the judgments, the necessary data have not appeared in those venues. Other reputable and easily accessible public data sources such as the ProMed electronic mailing list server and websites like those of the US CDC and the WHO were used as appropriate:

- http://www.promedmail.org/pls/otn/f?p=2400:1000
- http://www.cdc.gov/
- http://www.who.int/en

An estimate of the level of concern about the health threat from each agent was undertaken to suggest thresholds for intervention(s) to reduce or prevent transmission by transfusion. This was an unavoidably subjective process that included a review of the biology and epidemiology of the agents, an estimate of public and regulatory concerns about the agents and an assessment of the availability of sensitive and specific donor screening approaches. The priority assessment also was driven by questions such as an agent's geographic location/presence, projections of changes in geographic distribution (where it was going), and where and how frequently prospective donors would be at potential risk of exposure. In addition, the existence of surveillance adequate to allow recognition and reporting of putative transfusion transmissions, whether from physicians treating patients or reports to state or local health departments, federal or national public health agencies or blood collection facilities was considered. The deliberation also took into account the incubation period for each agent and a rough estimation of the period of time that would be required to realize that there is a potential public health threat. In so doing, the TTD committee applied the experience and interventions used for prior emergent transfusion-transmitted agents to evaluate the selected EID agents. These included the potential impact of behavioral and testing interventions in addition to pathogen reduction strategies. Pathogen reduction strategies that were analyzed included the impact of leukoreduction, physicochemical methods used in the manufacture of clotting factors, and methods used outside the US or in development in the US for cellular components and transfusable plasma. Possible interventions were evaluated relative to their potential to be effective and their estimated impact on the donor base.

If there is a shortcoming to what is known about the many agents for which blood is a plausible vector, it is our passive surveillance system for transfusion transmission. For example, how often do clinicians seeing DENV in endemic areas and during epidemics obtain the critical history related to transfusion? Are significant rates of transfusion-transmitted DENV obscured during an epidemic of vector-borne infection? The judgments made in

these Fact Sheets should lead to discussions of where and how to enhance surveillance by clinicians, the transfusion medicine community, and public health agencies if we think the threat from an agent rises to the level of gathering more evidence.

A primary use of the Fact Sheets is to present methods to decrease the risk of a given agent in the event of a transfusion-transmitted threat. However, each Fact Sheet can also serve as a medical and technical resource to blood providers including medical directors or other clinicians and blood center or transfusion service staff in the event that donors or transfused patients present with a history of or evidence for infection with one of these agents. Importantly, readers must understand that these Fact Sheets do not represent regulatory guidance, but instead serve as an indicator of what is known, and as such can be used as a starting point to develop policy.

The agents included in this Supplement have been chosen based on what we know now and the current judgment of the members of the TTD committee. During the time it has taken to complete this work, additional Fact Sheets already have been incorporated into the document and multiple revisions have been made to the original Fact Sheets. As new information is accumulated, the TTD will edit the Fact Sheets. Thus, additions and deletions to the Supplement are to be expected and encouraged; a 3-year cycle is planned. Readers are encouraged to challenge the assessments, provide data, and suggest edits. Much of the value of the Supplement will be realized in the future if its audience sees it as a living document. Mechanisms to receive public review/comments will be communicated through AABB publication channels; those comments may result in revisions of Fact Sheets prior to regularly scheduled revisions, depending on the nature of the comment. The Fact Sheets published in this Supplement will be posted on the AABB website. Modification of the Fact Sheets or new additions to the Supplement will be posted on the AABB website.

PRIORITIZATION OF SPECIFIC AGENTS

The prioritization effort is intended to suggest where intellect and resources should be spent in planning for the future. This is an especially important message for developers of blood donation screening tests or pathogen reduction methods since the lead time for research and development and clinical trials to bring products to the market place is generally many years.

Each agent was assigned a priority risk level under three different categories: scientific/epidemiologic evidence regarding blood safety, public perception and/or regulatory concern regarding blood safety, and public concern regarding the disease agent.

Scientific/epidemiologic risk assessment was based on a review of available data. Factors taken into consider-

ation included proof of transfusion transmission; prevalence of the agent in an asymptomatic population that might donate blood, blood components, or in some cases organs and tissues; the possibility of an outbreak resulting in increased incidence over a specific time frame; the attack rate and severity of disease caused by the agent; and the availability of effective prevention strategies or treatment. If there was no published or other credible documentation that an agent had been transmitted by transfusion to humans, it could be assigned a risk of theoretical or absent. Theoretical was used if transmission was biologically plausible because the agent had a blood phase during asymptomatic infection. Absent was used if this possibility was judged to be remote or if the agent was not associated with any known human disease.

Classification of the public perception and/or regulatory concern for each agent's risk was an inherently more subjective process. Regulatory concern was judged primarily on whether such agents had been discussed in public forums such as the FDA's Blood Products Advisory Committee (BPAC) or as part of other regulatory deliberations (e.g., simian foamy virus, monkeypox, and variants of HIV, HBV or HTLV). The category of public concern about an agent was assessed by reviewing whether the agent had been discussed in the popular press in relationship to transfusion and whether queries to the AABB or major blood providers had occurred.

Each category received an assessment of risk of high, moderate, low, very low, or absent. As previously discussed, the scientific/epidemiologic risk category also included a classification of theoretical. When risk could not be precisely classified, an agent was assigned an intermediate categorization or a range (e.g., from low to very low) depending on where in the US/Canada, or outside the US/Canada, concern exists.

The overall prioritization of risk was a synthesis of the ratings in each category based on consensus of the group with greater emphasis given to ratings in the first two categories: scientific/epidemiologic risk and public and/or regulatory concern regarding blood safety. Categories of agents deemed to be of high or moderate priority are indicated by color: red, orange, and yellow as indicated below. The agents that have not been given a priority status at this time were placed in a white category for the purpose of the Tables in this Supplement; most of these agents remain on the watch list of potential threats, while some have been assessed to have very little possibility of causing disease in transfusion recipients.

Red. Agents with low to high scientific/epidemiologic evidence of risk regarding blood safety with the potential for severe clinical outcomes. This priority also may be influenced by the committee's estimate of the risk of emergence of these agents in the US and Canada as well as public and/or regulatory concern.

Orange. Agents with sufficient scientific/epidemiologic evidence of risk in regard to blood safety that might support their elevation to a higher priority in the future.

Yellow. Agents with absent to low scientific/epidemiologic evidence of risk regarding blood safety for which there is public and/or regulatory concern.

White. Agents that were evaluated but no higher priority appears warranted at this time. This category represents a watch list, subject to modification as circumstances change.

Appendix 1, Table A1 provides a complete listing of all agents by group (i.e., prions, viruses, rickettsiae, bacteria, protozoa, and nematodes); Tables A2 through A11 provide the priority scores for each agent by group. A complete assignment of the prioritized agents is provided in Tables A12 through A14. Those agents associated with documented cases of transfusion transmissions are listed in Table A15. Agents that are vector-borne are listed in Table A16. The Fact Sheets are included as Appendix 2.

Agents classified in the red, orange, and yellow categories are as follows:

- Red category agents (highest priority): human variant Creutzfeldt-Jakob disease, dengue viruses, and *Babesia* species (Table A12).
- Orange category agents: Chikungunya virus, St Louis encephalitis virus, *Leishmania* species, *Plasmodium* species, and *T. cruzi* (Table A13).
- Yellow category agents: chronic wasting disease prions, human herpesvirus 8, HIV variants, human parvovirus B19, influenza A virus subtype H5N1, simian foamy virus, *Borrelia burgdorferi*, and hepatitis A virus (Table A14).

Red category agents

Human variant Creutzfeldt-Jakob disease (vCJD)

The assignment of the risk of transfusion transmission of vCJD in the US is based on scientific/epidemiologic evidence of transfusion transmissibility and was influenced by several opposing factors. In favor of a higher risk were: 1) data from the UK indicating that if a donor is incubating vCJD, there appears to be a risk of transfusion transmission[18] and potentially a risk to hemophiliacs who received UK-derived plasma products prior to the implementation of interventions to decrease BSE that were put into effect in 1996 (vCJD abnormal prion protein found in a patient with hemophilia at postmortem, Health Protection Agency, CJD Section, London, UK, http://www.hpa.org.uk/webw/HPAweb&HPAwebStandard/HPAweb_C/1195733818681?p=1225960597236) and 2) the rapid mortality associated with clinical disease and the lack of effective treatment.[34] In favor of a lower risk were: 1) the presumed very low to absent rate of carriers of the agent in

the general US population and 2) the possibility of an even lower carrier rate in the US blood donor population due to travel deferrals based on time spent in the UK and Europe. These resulted in a priority rating of low for the scientific/epidemiologic risk category. However, based on high public concern about this agent in the UK and other areas of the world that has influenced public perception in the US about "mad cow disease," considerable FDA attention to the issue of vCJD transfusion transmission, and the very difficult donor counseling issues and potential for large numbers of donor deferrals attendant on implementation of a test for vCJD, this agent was assigned a high rating with regard to public concern about blood safety. With all factors taken into account, this led to the assignment of the red priority category for vCJD.

Dengue viruses (DENV)

The assignment of the risk of transfusion transmission of DENV in the US is based on scientific/epidemiologic evidence of transfusion transmissibility,[35,36] and was influenced by several opposing factors. In favor of a higher risk were: 1) the common occurrence of asymptomatic infections and occurrence of viremia during the asymptomatic period; 2) the demonstration of relatively high rates of virus-specific RNA detection in studies of blood donors from endemic areas;[37,38] 3) the occurrence of epidemics that affect a relatively high percentage of the population at any one time; 4) the presence of competent mosquito vectors in large parts of the US; 5) the demonstration of high seroprevalence rates in US populations on the Texas-Mexico border;[39] and 6) the occurrence of localized epidemics in Hawaii and Texas and in Puerto Rico from where collected blood may be imported into the continental US. In favor of a lower risk were: 1) the low incidence of autochthonous transmission in the US and 2) travel deferral for visits to malarial endemic locations that extensively overlap with DENV-endemic areas that should defer returning donors with asymptomatic DENV infection. A consideration of all of these factors led the committee to assign a value of low for scientific/epidemiologic risk in regard to blood safety. However, in non-US DENV-endemic areas, this assignment would be moderate to high based on the prevalence of the agent. Public concern for blood safety was judged to be very low to absent in the US but moderate to high in some dengue-endemic areas. Overall, these considerations led to the classification of DENV as red priority category agents.

Babesia species

Based on the large number of transfusion-transmitted cases reported and the perception there is gross underreporting,[40,41] *Babesia* was assigned a risk rating of moderate to high on scientific/epidemiologic grounds regarding blood safety. Pertaining to public concern, the

agent was assigned a very low rating nationally but a moderate rating in areas known to be endemic for the agent (i.e., states in the Northeast and upper Midwest). Regulatory concern is evidenced by sponsorship of a workshop on transfusion-associated babesiosis in September 2008 by the FDA (http://www.fda. gov/downloads/BiologicsBloodVaccines/NewsEvents WorkshopsConferences/TranscriptsMinutes/ UCM051501.pdf). Overall, the increasing recognition of transfusion-transmitted cases, the severe outcomes that can occur in immunocompromised and asplenic transfusion recipients, and the lack of effective interventions to prevent transfusion transmission led to the classification of this agent in the red priority category.

Orange category agents

Chikungunya virus (CHIKV)
Due to the lack of any proven transfusion-transmitted cases, CHIKV was assigned a scientific/epidemiologic risk rating of theoretical in regard to blood safety. However, several scientific and epidemiologic factors were judged to contribute to the potential for an increased risk from this agent in the future. These include the rapid re-emergence of the infection in the Indian Ocean and parts of Africa and Asia since 2005,[42] the geographical spread of the virus by travelers returning from endemic areas to nonendemic regions, the presence of appropriate mosquito vectors in various geographic locations including the US, the increased vector efficiency of newly emerged strains, and the presence of asymptomatic viremia in infected individuals.[43,44]

St Louis encephalitis virus (SLEV)
Due to the lack of any proven transfusion-transmitted cases, SLEV was assigned a scientific/epidemiologic risk rating of theoretical regarding blood safety. However, due to its close phylogenetic relationship and similar epidemiology to WNV, and the possibility for large outbreaks of St Louis encephalitis in the US, this agent was judged to have the potential to be a blood safety concern in the future.

Leishmania species
These agents were assigned a scientific/epidemiologic risk rating of low in regard to blood safety. This risk rating was influenced by several opposing factors. In favor of a higher risk were: 1) transfusion transmission documented in at least three cases (and perhaps as many as 10) in which the transfused recipients were either infants or immunocompromised patients;[45] 2) the propensity for chronic carriage of the agent; and 3) the presence of asymptomatic parasitemia. In favor of a lower risk were: 1) lack of any documented transfusion transmission in

the US or Canada and 2) potentially effective prevention methods (specific geographical deferral for time spent in Iraq or travel to other areas where leishmaniasis and malaria are both endemic, and the widespread use of leukoreduction in North America). Public concern regarding blood safety was judged to be low based on discussions at BPAC meetings. Because these agents have been demonstrated to be transmissible by transfusion and because they can potentially be introduced into the US by returning military personnel (as well as by other travelers), it was judged that *Leishmania* species could be of increasing significance to blood safety in the future.

Plasmodium species
Malaria is a major infectious disease associated with transfusion in many emerging and developing countries. In contrast, this risk is much lower in developed countries due to lack of endemicity of the agent. The *Plasmodium* species were assigned a scientific/epidemiologic risk rating of low in the US and in most nonendemic countries in regard to blood safety due to their low prevalence coupled with the effective use of donor deferrals due to travel, residence, or having had malaria. However, the risk may be moderate to high in some nonendemic countries based on the demographics and travel patterns of their donor population. Similarly, public concern regarding both blood safety and transmissibility by other routes is likely to vary between endemic and nonendemic regions. In the US, the level of public concern was judged to be moderate. Several factors contribute to the possibility that malaria may increase as a transfusion risk. These include the re-emergence of the disease in nonendemic geographic regions due to immigration and travel, an increase in sporadic cases of "airport malaria," the occurrence of autochthonous transmission in nonendemic countries when plasmodia are introduced by immigrants (or rarely travelers), the possibility that global climate change could result in an expanded range of vectors, and the lack of a screening assay to interdict donors for whom risk is not recognized.

Trypanosoma cruzi
T. cruzi is included even though an FDA-licensed test for blood donor screening has been available since December 2006. The assignment of a blood safety scientific/ epidemiologic risk rating for this agent was influenced by the implementation of blood donor screening in 2007 by the majority of US blood centers. Based on evidence of transfusion transmission in Central and South America, documentation of transfusion-transmitted cases in North America prior to donor screening, and the risk mitigation achieved by donor screening, this agent was assigned a scientific/epidemiologic risk rating of low regarding blood safety. When assessing public concern for blood safety, *T. cruzi* was assigned a rating of moderate based

on public and regulatory attention given to the introduction of blood donor screening. Although the risk of transmission of this agent should be decreasing in the US, uncertainties persist regarding autochthonous transmission and the sensitivity of the donor screening assay. After 2 years of universal donor screening by most blood collection facilities in the US, testing strategies will likely be modified to a selective strategy, based upon at least one-time testing of every donor. The committee felt that this agent should be assigned an overall priority of orange, at least for the interval required for data relating to the efficacy of donor screening and the number of previous transfusion transmissions (as assessed by lookback investigations) to be collected and analyzed.

Yellow category agents

Chronic wasting disease (CWD)
This prion agent has never been detected in humans and no transfusion transmission has occurred, leading to a blood safety scientific/epidemiologic risk rating of theoretical. However, because of public awareness of another prion agent, vCJD, which is associated with mad cow disease and lethal human infection, it was judged that there was low to moderate public concern about the possibility that the CWD prion agent also might cross the species barrier.[46-48] Public concern specific to blood safety, however, was judged to be very low. Because of new scientific and public focus on this agent and its associated disease in deer and elk in the US and Canada, the limited amount of research that has been done to date, the potential for this prion agent to behave in a manner similar to vCJD, and the extensive opportunities for donor exposure attendant to the popularity of hunting, public health agencies (i.e., CDC) and regulatory agencies responsible for blood safety have expressed some concern even in the absence of any substantiating data. As a result, this agent was assigned an overall priority rating of yellow.

Human herpesvirus 8 (HHV-8)
Although HHV-8 transfusion transmission has been proven in Africa,[49] a material risk of such transmission in the US has not been convincingly established.[50] The US risk should be considerably lower than that in Africa based on several factors: the frequency of HHV-8 in the blood donor population is lower in the US, the storage age of blood components is longer, and leukoreduction is commonly, though not universally, employed. Despite transfusion transmission, clinical disease has not been documented to have resulted from such transmission. Taken together, these factors resulted in a scientific/epidemiologic risk rating of low in regard to blood safety. Public concern for blood safety also was judged to be low.

This agent was assigned an overall priority rating of yellow based on FDA concerns regarding potential transfusion transmission. These concerns would increase if donor deferral criteria were to be modified for males who have sex with males, due to the relatively high prevalence of infection in this population.

HIV variants
Due to the lack of any proven transfusion-transmitted cases, these agents were assigned a scientific/epidemiologic risk rating of theoretical regarding blood safety. Although the wild-type agent (HIV-1, Group M) is transfusion transmitted, transmission of HIV variants has not been documented. Assuming HIV variants are transmissible by transfusion, the risk should be minimal in the US due to low local prevalence, cross-reactivity of HIV screening tests, and use of questions for malaria exposure that would temporarily exclude donors who traveled to areas in Africa where HIV variation occurs at a high rate. Since HIV variants may result in AIDS, the public concern in regard to blood safety was judged to be low to moderate. In addition, the FDA has, appropriately, continued to be concerned about the ability of blood screening tests to detect all HIV variants and the ability of donor history questions to screen out all potentially at-risk donors. For these reasons, HIV variants have been assigned a priority rating of yellow.

Human parvovirus B19 (B19V)
Transfusion transmission of B19V from blood components has been proven, with at least four cases documented in the literature. However, the frequency of transmission has not been determined or estimated through established mathematical models. These factors resulted in the assignment of a scientific/epidemiologic risk rating of very low to low regarding blood safety. Public concern regarding blood safety risk was judged to be very low in the US with the exception of some concern among specific patient groups (i.e., patients with hemophilia, those with chronic anemia such as sickle cell disease or thalassemia, bone marrow transplant recipients, and other immunocompromised individuals). Based on historical transmissions of B19V to recipients of Factor VIII, there has been ongoing scientific and regulatory concern about the safety of plasma derivatives, leading many manufacturers and regulatory authorities to require B19V DNA qualification testing of incoming plasma and release testing of manufactured lots. When such B19V DNA testing is applied to recovered plasma, the issue of how to manage associated remaining in-date components from B19V DNA-positive donors has been a subject of much debate. For these reasons, this agent has been assigned a priority rating of yellow.

Influenza A virus, subtype H5N1

Due to the lack of any proven transfusion-transmitted cases, this highly pathogenic avian influenza agent was assigned a blood safety scientific/epidemiologic risk rating of theoretical. Furthermore, based on the biology of known influenza viruses and of H5N1, it was concluded that transfusion transmission was unlikely to occur.[51] However, because H5N1 has been discussed as an agent that could lead to a worldwide influenza pandemic, public concern about community transmission of this agent was judged to be high, whereas concern related specifically to blood safety was judged to be very low. The high profile of this agent in the public health sector, especially with regard to pandemic planning, led to assigning a priority rating of yellow.

Simian foamy virus (SFV)

Although SFV transfusion transmission has been documented in experiments in nonhuman primates, transmission by transfusion has not been demonstrated in humans. In addition, this agent has not been shown to cause any human disease. For these reasons, this agent was assigned a scientific/epidemiologic risk rating of theoretical with regard to blood safety. Public concern about this agent was judged to be absent. In contrast, some level of concern has been demonstrated by regulatory agencies. This concern relates to the theoretical possibility that clinical disease does occur but has not yet been recognized and/or that mutated strains of this agent may eventually show increased pathogenicity in humans.[2,4] In the US, SFV has been discussed at meetings of the BPAC without concern expressed by stakeholder groups or other members of the public (http://www.fda.gov/ohrms/dockets/ac/04/transcripts/2004-4074t1.htm). In contrast, Health Canada, the Regulatory agency in Canada (http://www.hc-sc.gc.ca/dhp-mps/brgtherap/activit/fs-fi/fact_simian_foamy_virus_spumeux_simien_feuillet-eng.php), requires a permanent deferral for potential blood donors whose employment involves contact with monkeys or their body fluids.[29] For these reasons, this agent has been assigned a priority rating of yellow.

Borrelia burgdorferi

Due to the lack of any proven transfusion-transmitted cases, this agent was assigned a scientific/epidemiologic risk rating of theoretical regarding blood safety. If transfusion-transmission does occur, it is likely to be rare. Conversely, given that this agent has been recognized and studied for many years, public concern about the general transmissibility of this agent and its resulting disease manifestations (i.e., Lyme disease) was judged to be moderate, whereas public concern regarding blood safety was judged to be very low. Based primarily on the public apprehension of Lyme disease in areas of the country with a higher prevalence of this disease, the agent has been assigned a priority rating of yellow.

Hepatitis A virus (HAV)

HAV transmission through blood is uncommon, but well documented, and can lead to secondary cases especially when transmission occurs among infants in neonatal intensive care units. Viremia often precedes the development of symptoms by 7-14 days. The presumed rarity of transfusion-associated cases is due to several factors that include a short viremic phase, low concentration of virus in the blood, absence of a carrier state, neutralization of the virus by specific antibody in other components concurrently administered, routine immunization in populations with high HAV incidence, and increasing prevalence of immunity in recipients with age. HAV remains in the yellow category based on the fact that the scientific/epidemiologic evidence regarding blood safety is low but public concern is low to moderate, especially during a community outbreak of the disease. In the US, risk of transmission during a common source epidemic is mitigated by the addition of a specific question to the donor questionnaire regarding exposure that leads to a 120-day deferral postexposure.

Selected specific white category agents

In addition to the previously categorized agents, several agents on the watch list (i.e., white category agents) merit further discussion, either because of recently changing information (e.g., hepatitis E virus [HEV] and *Anaplasma phagocytophilum*) or because of concerns about the potential use of the agent in a bioterrorist attack. Some of these latter agents are discussed in more detail in the section on bioterrorism.

Hepatitis E virus (HEV)

A small number of cases of HEV transmission by blood transfusion has been documented[52] both in areas classically viewed as endemic for human infection as well as in developed, industrialized countries.[53] The sporadic (nontransfusion) cases in humans observed in the US have been mostly imported from endemic areas.[53] This has led to assignment of a scientific/epidemiologic risk rating of very low for this agent. The impact of this disease currently remains very low in the US and public concern is absent for blood safety and clinical disease. Nevertheless, the potential for human infection in the US remains a possibility given that an HEV reservoir exists in pigs. The existence of this reservoir, combined with several cases of transfusion transmission in other developed countries, indicates that HEV may increase in priority.

Anaplasma phagocytophilum

There have been two published case reports of transfusion-transmitted infection in the US (one as an abstract[54] and one by the CDC in Morbidity and Mortality Weekly Report[55]). Due to this low number of reported transfusion-transmitted cases, the scientific/epidemiologic risk assigned was very low. However, it is likely that more cases have occurred and have not been recognized or reported. Given the high seroprevalence rates in donors in some geographic locations, the demonstrated survival of the organism in refrigerated red cells, documented transfusion transmission in animal models, and an unknown period of asymptomatic bacteremia, it is possible that an increasing number of transfusion-transmitted cases will be recognized if surveillance is adequate.

CATEGORIZATION INTERSECTION: SCIENCE VERSUS THE PUBLIC'S PERCEPTION

As noted, categorization of agents on the scientific/epidemiologic scale was based on data that appeared in the scientific literature. In contrast, the public perception categorizations were developed by a more subjective process. Agents may have low scientific priorities but may be high on the public's "radar" screen if the agents are perceived as being those that might lead to significant human disease. As a means of showing the various conflicts or agreements in priorities, agents having red, orange, or yellow priority levels were plotted by factor-analytic representation[56] (Fig. 1) where the x-axis is the scientific/epidemiologic scale that ranges from a theoretical transfusion-transmission risk to higher levels of proof of transmission and/or disease severity and incidence. In contrast, the y-axis is public concern (i.e., *perception* of risk) ranging from absent to high that occurs when there is the perceived potential for dread, catastrophic or fatal consequences often accompanied by social stigma (e.g., HIV/AIDS) with lack of control over the outcome. Risk communication and risk management science have shown that the higher the perceived risk, the more people want to see the risk reduced, and the more they want to see strict regulation employed to achieve the desired reduction in risk. Risk management efforts are destined to fail unless they are structured using such a two-way process (expert data

synthesis, opinion, and public perception).[56] The lesson that we have learned since HIV emerged as a transfusion-transmitted agent is that what we do to protect the safety of the blood supply is dependent on both science and the expectations of the communities that we serve.

CATEGORY A AGENTS OF BIOTERRORISM: BLOOD SAFETY IMPLICATIONS AND ACTIONS IN THE EVENT OF AN ATTACK

Blood establishments need to be appropriately prepared in the event of a bioterrorist event. Key concerns are the management of the existing and future blood supply once an attack has been identified. This requires knowledge of the potential impact of agents of bioterrorism on blood safety and availability. Different actions may need to be taken depending upon the magnitude of the attack and the agent(s) involved.

The CDC has classified several agents that might be used for bioterrorism. Those considered to be of the gravest concern are classified as Category A (http://www.bt.cdc.gov/agent/agentlist-category.asp#a), and include the agents of anthrax, botulism, plague, small-pox, tularemia, and viral hemorrhagic fevers. These agents, exclusive of botulism, are listed in Appendix 1, Table A17. The characteristics and potential actions relative to these agents are summarized in Appendix 1,
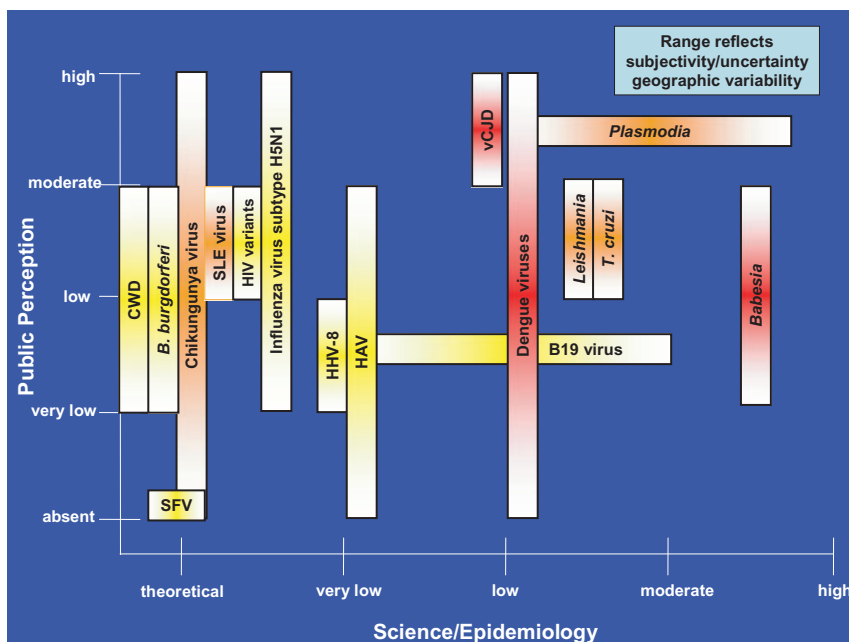

Fig. 1. EID agent priority matrix, where the y-axis represents the priority based on public perception ranging from absent to high and the x-axis represents the priority based on science and epidemiology ranging from theoretical to high (see text).

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Table A18. These high-priority agents include organisms that pose a risk to national security because they:

- Can be easily disseminated.
- Can be transmitted from person to person.
- Result in high morbidity or mortality rates and have the potential for major public health impact.
- Might cause public panic and social disruption.
- Require special action for public health preparedness.

The items that require specific consideration for blood organizations in relation to a bioterrorist attack are:

- The risk of infectivity of blood components if an exposed individual gives blood prior to the appearance of symptoms.
- The risk of contamination of collected blood as a result of direct or indirect deposition of the agent on blood containers (also putting staff at risk).
- The impact of a bioterrorist attack on the availability of donors and facility personnel.
- The impact of the attack upon the need for blood components.
- The impact of regulatory actions taken in response to an attack.

A major attack may compromise the safety of the blood supply to such an extent that blood collection operations would have to be shut down in the area impacted by the attack. In addition, it may be necessary to quarantine in-date products, at least until the nature, extent, and likely date of the attack is known. However, an attack of lesser magnitude (for example, the anthrax attacks in 2001) will probably require management of each donor and product according to individual circumstances.

The CDC bioterrorism page (http://www.bt.cdc.gov/agent/agentlist.asp) is a useful resource providing descriptive information on all agents and ways to manage suspected attacks or exposures. There are also links to other sites covering issues including reporting and clean-up. A series of articles in the *Journal of American Medical Association* provides very useful information and the specific reference is noted in each subtitle below. The key characteristics of the agents listed in Table A18 are outlined.

Anthrax[57]

Anthrax is caused by the gram-positive, spore-forming bacterium, *Bacillus anthracis*. Three forms of disease are recognized: inhalation anthrax resulting from respiratory exposure (most likely to occur in the event of a deliberate attack); gastrointestinal anthrax resulting from the consumption of contaminated food; and cutaneous anthrax manifesting as skin lesions, most often on the hand. It has

also become apparent that individuals may be colonized but exhibit no symptoms. Inhalation and gastrointestinal anthrax have incubation periods of 1-7 days, and if untreated, fatality rates of 97% and 25-60%, respectively. Cutaneous anthrax has an incubation period of 1-12 days and an untreated fatality rate of 20%. Anthrax is not transmitted from person to person. While *B. anthracis* is frequently present in the blood of ill patients, the FDA reports that bacteremia is thought to be extremely unlikely in asymptomatic individuals. Consequently, anthrax transmission by blood transfusion is not believed to occur provided blood is collected only from healthy donors with normal temperatures.

A bioterrorism attack would most likely occur through distribution of anthrax spores by the aerosol route and/or distribution of the spores in powdered form. Spores are resistant to sterilization by chemicals or heat and restoring a contaminated area to a safe condition is difficult and time-consuming. In the event of known contamination of a blood establishment, it would be necessary to evacuate staff and donors and to close the establishment pending remediation.

In 2001, following the anthrax attacks, the FDA issued Guidance for Industry entitled: "Recommendations for assessment of donor suitability and blood and blood product safety in cases of possible exposure to anthrax" (http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Blood/ucm076711.htm). In this document, the FDA did not recommend any changes to standard donor screening and blood collection practices to identify or otherwise query donors who may have been exposed to anthrax. (Note that the document was prepared after a rather limited exposure and that regulatory advice might change in the face of a massive attack.) The FDA did recommend deferral for donors with diagnosed anthrax, suspected skin lesions, or known colonization until they have completed a full course of antibiotic treatment and have been shown to be free of the bacteria. Donors with skin lesions suspected to be anthrax should be deferred until the lesion is later shown not to be anthrax or until the lesion is shown to be anthrax and the individual completes a full course of treatment and the infection is considered to be resolved.

In the event that a donor reports postdonation information of anthrax, in-date blood components from prior donations should be quarantined and retrieved promptly; this should date back to the time of exposure or 60 days prior to onset of illness, whichever is shortest. The FDA also recommends recipient notification and that medical directors should determine an appropriate course of action in the event of postdonation illness among donors suspected of having been exposed. Should direct contamination of components with anthrax spores occur, blood products would have to be destroyed.

Botulism[58]

Botulism results from exposure to a toxin produced by the bacterium *Clostridium botulinum*. The toxin is extraordinarily potent and a lethal human dose is thought to be 0.09-0.15 µg intravenously, 0.70-0.90 µg by inhalation and 70 µg orally. Natural forms of botulism are foodborne, wound, and intestinal, but all have similar clinical outcomes, namely an acute, afebrile descending flaccid paralysis that may lead to death from paralysis of the muscles of respiration. The incubation period depends on the quantity of preformed toxin to which an individual is exposed and, in the case of foodborne exposure, may be from 2 hours to 10 days, but is usually 18-36 hours. A bioterrorist attack is likely to occur as a result of distribution of the toxin by the aerosol route or perhaps by deliberate contamination of food. An attack via the water supply is technically not feasible.

There is no possibility of person-to-person transmission of *C. botulinum*. It would seem very unlikely, if not impossible, that a lethal dose could be transmitted by blood transfusion, as such transmission would require that the donor would have to have a lethal dose of preformed toxin in the blood. Thus, no Fact Sheet was developed for *C. botulinum*. Donor deferral and quarantine and retrieval of components will not be needed unless an attack targeted a blood establishment for contamination of products.

Plague[59]

Plague is caused by the gram-negative bacterium, *Yersinia pestis*. There are two major forms of disease: bubonic, usually acquired by the bites of fleas that have fed on bacteremic rodents; and pneumonic, resulting from aerosol (respiratory) exposure. Primary or secondary septicemic plague also may occur. Pneumonic plague is the form most likely to occur as a result of a bioterrorist attack, although intentional transmission using infected fleas has occurred. The overall mortality of bubonic plague is about 15%, but 50-60% of cases may die in the absence of treatment. Untreated pneumonic disease is usually fatal. Pneumonic disease may be transmitted from person to person through respiratory droplets. The incubation period is 1-7 days or 1-4 days for primary pneumonic plague. A bioterrorist attack would manifest itself as an outbreak of rapidly fatal respiratory illness 1-6 days after the attack; secondary cases would occur. Affected individuals may have been bacteremic briefly at some time prior to the appearance of illness; therefore, their donated blood must be presumed to be infectious. Quarantine and retrieval of blood collected up to 10 days prior to the recognition of the outbreak would probably be prudent in the event of a large outbreak. Presenting donors exposed to known cases should be deferred (probably for 2 weeks or until completion of a prophylactic course of antibiotics). Staff or donors with respiratory symptoms should be referred for medical evaluation and treatment. In the event of a major regional outbreak, it may be necessary to suspend blood collection in that region until the outbreak is terminated. Significant residual contamination of facilities is unlikely, as the bacterium is very sensitive to environmental inactivation.

Smallpox[60]

Smallpox is caused by a large DNA virus known as the variola virus. Unlike other potential bioweapons, smallpox has been eradicated in nature. The disease spreads rapidly from person to person by droplet and aerosol routes and by direct contact: contaminated surfaces such as bedding and clothing also may spread the infection. Individuals are most infectious from the time that the rash appears until 7-10 days thereafter. The usual incubation period is 12-14 days (with a 7-17 day range), and there is asymptomatic viremia starting about 3-4 days after exposure. The mortality is up to 30% and there is no known effective treatment. A biological attack would likely involve aerosol release that would be recognized as a cluster of cases. Each case may infect 10-20 others and isolation of cases would be required. Although there is no reported case of transmission of smallpox by transfusion, this remains a possibility. Therefore, robust interventions would be required for blood establishments, including deferral of cases and case-contacts and quarantine and recovery of blood components collected up to 21 days prior to the first case. Second wave cases would complicate the situation. There also would be obvious concern about staff exposure and it may be necessary to cease all operations until the extent of the outbreak is determined. Environmental contamination from the attack itself will not be a serious issue as the virus is not expected to persist in the environment for more than 2 days. It is likely that an attack would result in the implementation of widespread vaccination programs. The FDA has provided guidance on donor management in the context of a smallpox vaccine program (http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Blood/ucm075115.htm). It should be noted that this guidance requires donor deferral and such a deferral may have a significant impact upon product availability (particularly for platelets). An attack with smallpox is likely to be extremely challenging to the blood system.

Tularemia[61]

Tularemia is caused by a small, gram-negative bacterium, *Francisella tularensis*. This organism infects a number of wild animals naturally and may be found as an environmental contaminant. Humans may be infected via the aerosol or droplet route, accidental inoculation, the bite of

fleas or other arthropods, or even via food or water. The agent is highly infectious, with as few as 10 organisms causing disease. However, person-to-person transmission does not occur. It is likely that a bioterrorism attack would be accomplished via an aerosol release. This would result in an outbreak of acute, febrile illness with respiratory symptoms. The incubation period is 3-5 days, with a range of 1-14 days. Although subjects may be incapacitated in a matter of days, the untreated disease may be prolonged and can last weeks to months. Untreated inhalation tularemia may have a mortality rate of 30-60%. Bacteremia does occur and deferral of cases and individuals thought to be at risk of primary exposure would be necessary, pending successful completion of therapy or of antibiotic prophylaxis. Additionally, quarantine and recovery of products collected from the time of the presumed attack until its recognition would be advisable. Although the organism is relatively hardy, simple surface decontamination with 10% bleach would be adequate and residual contamination from the attack is not anticipated to be a problem.

Viral hemorrhagic fevers[62]

This is a complex area as about eight different viruses belonging to four different groups are thought to be potential threats for a bioterrorist attack. However, there are some general shared characteristics. All are enveloped RNA viruses and all tend to cause a similar illness. Person-to-person spread by direct contact with infected blood and body fluids is a primary route of transmission, especially among those providing direct care for infected persons. Viruses that might be used in this context include Ebola, Marburg, Lassa, New World arenaviruses, Rift Valley fever, yellow fever, Omsk hemorrhagic fever, Crimean-Congo hemorrhagic fever, and Kyasanur Forest disease viruses. A deliberate attack would most likely involve an aerosol release of virus. The result would be an outbreak of undifferentiated febrile illness 2-21 days later. Clinical manifestations could include rash, hemorrhagic manifestations, and shock. Case fatality rates would depend upon the agent used, but vary from a low of 0.5% (Omsk hemorrhagic fever) to a high of 90% (Ebola). Diagnosis would not be easy and it might take time before the cause of the outbreak was established. Depending on the agent used, considerable caution may have to be used in patient care. Some, but not all, of the viruses are somewhat responsive to selected antiviral drugs. These viruses are present in the blood, but little is known about viremia in the presymptomatic phases. Quarantine and recovery of products from infected, and possibly exposed, donors collected during the incubation period would have to be undertaken. If the involved virus were not identified, the presumed maximum incubation period would have to be used; this could be 4 weeks or more. Secondary cases would be most likely to occur among caregivers with direct contact with symptomatic cases; consequently, it may be necessary to defer such individuals from donation. The viruses are not expected to survive very long in the environment, so there should be no significant facility clean-up issues for blood establishments. Finally, it should be noted that the clinical effects of these agents may result in a need for blood components, and especially platelets, to correct the bleeding.

PATHOGEN REDUCTION AS A SAFETY STRATEGY FOR EID

Introduction

This Supplement presents detailed information on selected EID agents, including their biology, potential threat to recipient safety, and existing interventions. Historically, the transfusion medicine community's response to EID agents has been to add new donor deferral criteria and/or new screening tests. EIDs will continue to appear, and continuous addition of deferrals and screening tests may not be sustainable. Furthermore, this approach involves waiting for disease emergence, identification of the agent, understanding of epidemiological risk factors of infection to craft donor deferrals, and then development and implementation of an assay. During this interval before introduction of the assay, morbidity and mortality may accumulate. This reactive strategy of donor qualification via questions and tests will continue unless more broad-reaching interventions are developed and implemented. Pathogen reduction (PR) offers a proactive strategy to address these threats; if a PR technology offers a broad spectrum of inactivation, there is a high likelihood that it will inactivate the new agent, thereby preventing infections and perhaps obviating the need for the introduction of new donor deferral criteria and new screening tests. PR should also be of benefit in those situations where an assay fails to detect an infectious agent due to a low level of antigen/antibody/nucleic acid in the test sample during the "window period" when the analyte has not reached its detection threshold. Finally, PR may interdict agents with very long incubation periods or unrecognized pathogenicity for which the association with transfusion may be obscure.

The intent of the following section is to provide an overview of what is known regarding PR systems that have progressed to clinical trials or have been implemented in other countries. It provides a brief review of each PR method by blood component including: the company name and the name of the technology, the company's website (as a source of additional unpublished data), a description of how the process would be used, the agent's mechanism of action, a composite table of published inactivation results, and a brief summary of the clinical performance and regulatory status. There is also brief commentary on limitations of PR, including

the long and complex regulatory cycle (especially in the US and Canada), reductions in product efficacy or toxicology associated with PR technologies, and lastly, agents that might be resistant either inherently or due to high concentrations of the agent in the product. This review does not address costs associated with the use of these technologies.

PLATELET PATHOGEN REDUCTION SYSTEMS

There are three technologies for PR in platelets, none of which is licensed for clinical use in the US or Canada:

- Cerus Corporation INTERCEPT Blood System™ (http://www.cerus.com) Concord, CA, USA;
- CaridianBCT Biotechnologies Mirasol® PRT (http://www.caridianbct.com/) Lakewood, CO, USA; and
- MacoPharma's Theraflex© UV. (http://www.macopharma.com/) Tourcoing, France.

Cerus Corporation INTERCEPT Blood System for platelets

Platelets suspended in approximately 65% additive solution (InterSol, Fenwal, Inc., Lake Zurich, IL) and 35% plasma are treated by adding amotosalen, a psoralen compound, to the platelets and delivering 3 Joules (J)/cm^2 ultraviolet A light (320-400 nm) in approximately 5 minutes. This results in irreversible cross-linking of nucleic acids. Unreacted amotosalen and photoproducts are adsorbed during incubation of illuminated platelets in a separate container containing the Compound Adsorption Device (CAD) for at least 4 hours and up to 16 hours; the platelets are transferred to a final container for storage and transfusion.[63] The process is shown in Fig. 2.

CaridianBCT Biotechnologies Mirasol for platelets

Platelets suspended in 100% plasma are treated by adding riboflavin (the normal nutrient, vitamin B2) to the platelets, then delivering 6.2 J/cm^2 ultraviolet (UV) light (265-370 nm) in approximately 10 minutes. This results in irreversible photo-oxidative damage to nucleic acid. After illumination, the product is ready for transfusion without further processing.[64,66] The process is shown in Fig. 3A. Alternatively, CaridianBCT Biotechnologies is developing a process in which a hyperconcentrated

platelet with low plasma content can be collected and photochemically treated; after treatment, platelet additive solution (PAS) is then added to yield a platelet stored in 65% PAS and 35% plasma (Fig. 3B) (Raymond Goodrich, pers. comm., 2009).

MacoPharma Theraflex UV for platelets

Platelets suspended in approximately 65% Storage Solution for Platelets (SSP, including magnesium and potassium, MacoPharma, Tourcoing, France) and 35% plasma are loosely placed on a quartz plate in bags to produce a thin platelet layer (approximately 4-5 mm), then treated
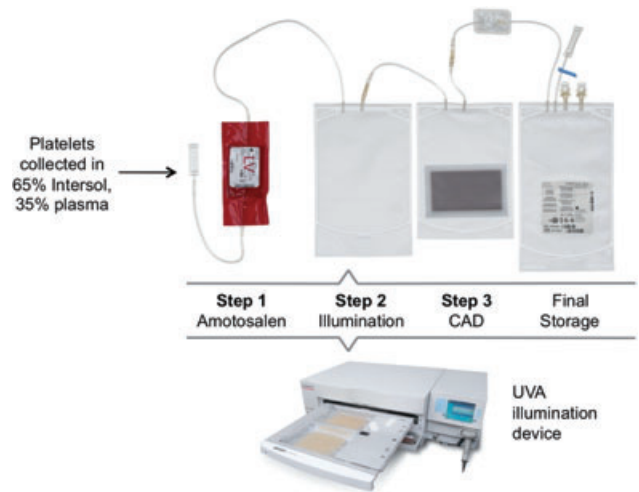

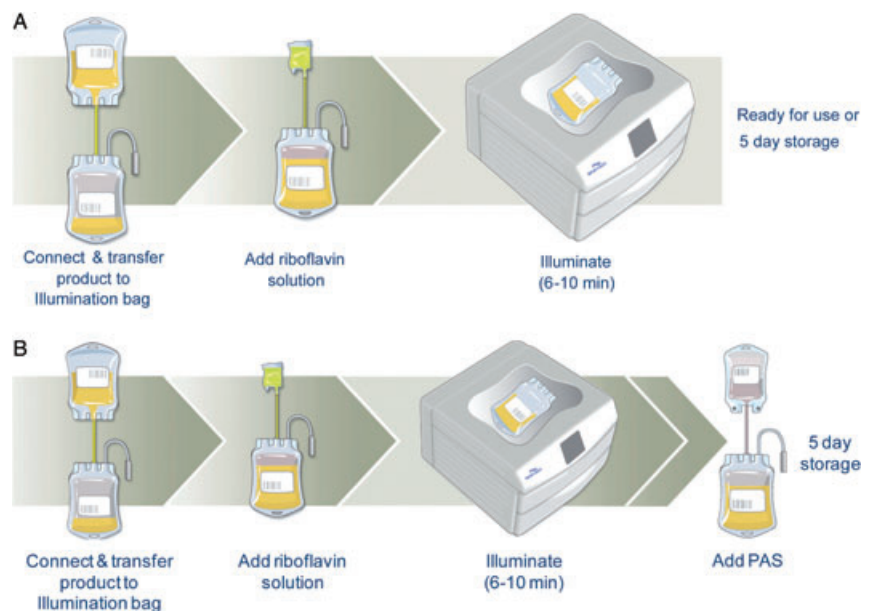Fig. 2. Cerus Corporation INTERCEPT Blood System for platelets.

Fig. 3. CaridianBCT Biotechnologies Mirasol for platelets. (A) Platelets stored in 100% plasma. (B) Platelets stored in PAS.

by exposure to 0.3 J/cm^2 monochromatic wavelength UV light (254 nm) with intense agitation (approximately 100 cycles/min) for about 1 minute.[65] No photoactive compound is required; nucleic acid damage presumably occurs due to cyclobutyl ring formation. After illumination, the platelets are transferred into a storage container and are ready for transfusion with no further processing. The process is shown in Fig. 4.
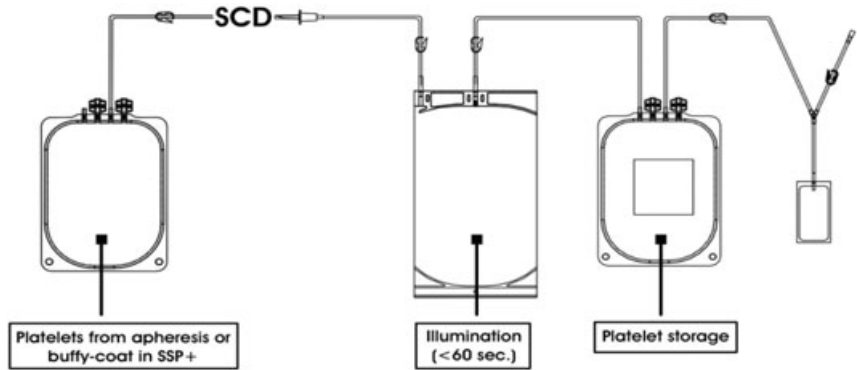

Fig. 4. MacoPharma Theraflex UV for platelets.

Pathogen reduction in platelets

Data that have been published in peer-reviewed journals are provided in Tables 1 through 4. Data from abstracts or unpublished data available from studies conducted by the manufacturers are included as personal communications from the companies' representatives. Cerus (INTERCEPT) has demonstrated >6.4 log activity for CHIKV in platelets (Lily Lin, pers. comm., 2009). CaridianBCT Biotechnologies (Mirasol) has demonstrated inactivation of the following agents: **Viruses**: Sindbis virus (3.2 logs); influenza A virus (>5.3 logs); infectious bovine rhinotracheitis virus (2.1 logs); HAV (2 logs); HBV (>4 logs by PCR); bovine enterovirus (3.0 logs); pseudorabies virus (2.5 logs); encephalomyocarditis virus (3.2 logs); CHIKV (2-3 logs); **Bacteria/Yeast**: *Acinetobacter baumannii* (1.8 logs); *Klebsiella oxytoca* (1 log); *K. pneumoniae* (2.8 logs); *Streptococcus mitis* (3.7 logs); *S. pyogenes* (2.3 logs); *Yersinia enterocolitica* (3.3 logs); *Candida albicans* (1.8 logs); and **Protozoa**: *Plasmodium falciparum* (>3.0 logs); and *Babesia microti* (>5.0 logs) (Raymond Goodrich, pers. comm., 2009). MacoPharma Theraflex UV process for platelets using only UVC and agitation has been presented at meetings held by the AABB, ISBT, and German Society for Transfusion Medicine and Immunohaematology and has shown inactivation against the following viruses: WNV (>5 logs); Sindbis virus (5.6 logs); encephalomyocarditis virus and porcine parvovirus (5-6 logs); vesicular stomatitis virus (VSV) (>6 logs); Suid herpesvirus 1 (3.7 logs); HIV-1 (1.4 logs) (Frank Tolksdorf and Stefan Reichenberg, pers. comm., 2009).

TABLE 1. Log$_{10}$ reduction of known transfusion-transmitted viruses in platelets

Virus	INTERCEPT[63]	Mirasol[64,66]
Enveloped		
HIV-1		
• Cell-free	>6.2	5.9 (includes cell-associated)
• Cell-associated	>6.1	See above
• Proviral	To limit of detection	4.5
HBV	>5.5 CID$_{50}$*	
HCV	>4.5 CID$_{50}$	
HTLV-I, cell-associated	4.7	
HTLV-II, cell-associated	5.1	
CMV, cell-associated	>5.9	
WNV	>6.0[67]	5.2
Nonenveloped		
B19V	4-5.5[68]	
HAV	0†	

* Chimp Infectious Dose where 50% of the animals become infected.
† No reduction was observed (Lily Lin, pers comm., 2009).

TABLE 2. Log$_{10}$ reduction of model viruses in platelets

Virus	Model for	INTERCEPT[63]	Mirasol[64,66]
Enveloped			
Vesicular stomatitis	Enveloped viruses	>5.8	>6.3
Influenza A (H5N1)	Influenza viruses	≥5.9[69]	
Duck HBV	HBV	>6.2 ID$_{50}$*	
Bovine viral diarrhea	HCV	>6.0	
SARS		>6.2[70]	
Vaccinia		>5.2	
Nonenveloped			
Bluetongue	Nonenveloped viruses	6.1-6.4	
Calicivirus	Nonenveloped viruses	1.7-2.4	
Simian adenovirus 15	Nonenveloped viruses	0.7-2.3	
Human adenovirus 5	Nonenveloped viruses	>5.7	
Porcine parvovirus	Nonenveloped viruses	0†	≥5.0

* Infectious dose where 50% of the ducks are infected.
† No reduction was observed.

PLASMA PATHOGEN REDUCTION SYSTEMS

The same three companies have processes for PR in plasma. Cerus and CaridianBCT Biotechnologies use the same photoactive substance and process as employed in