Advisory report of the Superior Health Council no. 8751

Shorter deferral periods for blood donation following the implementation of pathogen reduction technology on platelet concentrates against the chikungunya and West-Nile viruses.

Inquiry on shortening the deferral periods for blood donation following the implementation of pathogen reduction technology on platelet concentrates: chikungunya and West Nile virus

This version was validated by the Board in September 2015[1]

INTRODUCTION AND ISSUE

On October 8, 2010, the Superior Health Council (SHC) received a request for an advisory report from the Chief Executive Officer of the Federal Agency for Medicines and Health Products[2] on the appropriateness of shortening the deferral periods for blood donation after applying pathogen reduction technology against the chikungunya and West-Nile viruses to platelet concentrates.

The SHC had already received a request for advice on the usefulness of pathogen reduction technology (PRT) as a means to inactivate various pathogens in fresh frozen plasma or in platelet concentrates. When carrying out these assessments, the SHC found that there are certain data (cf. Caridian, 2010; Cerus, 2010) with which it is possible to demonstrate the efficacy of these techniques in reducing the risk of plasma and platelets being contaminated with the West Nile (WNV) and chikungunya (CHIKV) viruses. In Belgium, platelet concentrates are subjected to pathogen reduction technology during their preparation. For these viruses, pathogen reduction technology could turn out to be efficacious enough for doing away with the need for a deferral period or for shortening the latter.

Belgium currently has a 28-day deferral period preventing anyone who has travelled outside the European Union as well as those returning from several neighbouring countries (including countries around the Mediterranean) from giving blood (SHC, 2007). Similarly, a several-week deferral period applies to anyone who has stayed in European countries or neighbouring countries in which there is a risk of WNV- or CHIKV-infection (ECDC, 2007; ECDC, 2010; Gould et al., 2010). In Belgium, the periods implemented were 21 days for the 2007 chikungunya-epidemic in northern Italy and 28 days for the West-Nile fever epidemic that has prevailed in Greece, Hungary, Romania and northern Italy since 2010 (SHC, 2007, FAMHP, 2010).

[1] The Council reserves the right to make minor typographical amendments to this document at any time. Amendments in substance are automatically included in an erratum. In this case, a new version of the advisory report is issued.
[2] Letter from Mr. X. De Cuyper, Chief Executive Officer of the Federal Agency for Medicines and Health Products (reference: FAGG/LM/112626) of 06/10/2010, addressed to Mr.J. Nève, SHC Chairman.

The number of symptomatic travellers identified in Belgium upon their return from an affected area, evolves in parallel with the chronology of the outbreaks described in the literature (Van den Bossche et al., 2015; Fig. 5).

The European Directive 2014/110/EU has recently provided that a temporary deferral for WNV is not necessary if a nucleic acid amplification test was carried out and its results were negative. This Directive has now been transposed into Belgian law (Royal Decree of 2 July 2015). At the moment, blood donations collected in Belgium are not subjected to any nucleic acid testing (NAT) for WNV and CHIKV. A thorough analysis of the financial impact has yet to be conducted. The EDQM (2013) takes the view that, when there is no test available, travellers with clinical WNV disease may be authorised to give blood 120 days after the symptoms have cleared. In Europe, the deferral periods for WNV do not apply when the blood is used exclusively for plasma intended for fractionation.

ADVICE

Pathogen reduction technology makes it possible to enhance the safety of platelet concentrates to varying degrees depending on the virus strain.

Based on an assessment of all the available data, the SHC advises that, in order to shorten the deferral periods for blood donation, the infectious load must be fully removed taking into account an additional 3 log safety margin. The viraemic phase that follows the infection with WNV and CHIKV is subdivided into two stages: thus, the viral load rises sharply shortly after the infection (viraemic phase) before dropping rapidly to levels that can no longer be detected in the plasma by means of nucleic acid testing. This drop is concomitant with the rise in neutralising antibodies.

If it has been shown that the platelets no longer contain any infectious viruses at the end of their shelf life, the deferral period for blood donation may be shortened depending on the efficacy of the methods used as well as the following restrictions:

a) The SHC warns that, in the event of other blood components being collected when preparing the platelet concentrates, the deferral periods must be shortened according to the most stringent reduction rate;

b) In addition, the PRT methods must effectively reduce any other pathogen for which there is a deferral period and which the donor could have been co-infected with;

c) Whenever it is possible to validate a reduction performance of at least 3.7 $\log_{10}$ of the infectious load$^{\Delta}$, the deferral period may be shortened and allowed to end after the viraemic phase, *i.e.* 11 days for the WNV-strains and 20 days for the CHIKV-strains.

d) A deferral period is no longer necessary when the efficacy reaches at least 6.5 $\log_{10}$ of the infectious load$^{\Delta}$ for WNV-strains and at least 10.7 $\log_{10}$ for CHIKV-strains.

$^{\Delta}$ expressed in $TCID_{50}$/mL plasma taking into account a safety margin of 3 log

Keywords and MeSH terms [3]

MeSH	Keywords	Sleutelwoorden	Keywords	Schlüsselwörter
West Nile virus	West Nile virus, WNV	West-Nijlvirus, WNV	Virus du Nil occidental, WNV	West Nile-Virus, WNV
Chikungunya virus	Chikungunya virus, CHIKV	Chikungunya virus, CHIKV	Virus Chikungunya, CHIKV	Chikungunya-Virus, CHIKV
safety	pathogen reduction, PRT	Pathogeenreductie, PRT	réduction des pathogènes, PRT	Pathogenreduktion, PRT
platelets	platelets	bloedplaatjes	plaquettes sanguines	Thrombozyten
blood donation	blood donation	bloedgeven	don de sang	Blutspende
–	deferral period	uitsluitingsperiode	période d'exclusion	Ausschlussperiode

MeSH (Medical Subject Headings) is the controlled vocabulary thesaurus used for indexing articles for PubMed http://www.ncbi.nlm.nih.gov/mesh

METHODOLOGY

After analysing the request, the Chair of the field "Blood and blood products" and the working group identified the necessary areas of expertise. On this basis, expert knowledge in virology was added to the fields of expertise "blood transfusion" and "cell biology". The experts of this working group provided a general and an *ad hoc* declaration of interests and the Committee on Deontology assessed the potential risk of conflicts of interest.

This advisory report is based on a review of the scientific literature and on reports from international organisations competent in this field, as well as on the views of the experts.

Once the advisory report had been approved by the working group, it was ultimately validated by the Board.

FURTHER DETAILS AND ARGUMENTATION

Abbreviations used: RNA = ribonucleic acid; CHIKV = Chikungunya virus; gEq = genome equivalents; IgG = immunoglobulin G; IgM = immunoglobulin M; $\log_{10}$ = logarithm to the base 10; NAT = nucleic acid testing; PCR = polymerase chain reaction; PFU = plaque forming units; PRT= pathogen reduction technology; RT-PCR = reverse transcription polymerase chain reaction; $TCID_{50}$ = 50 % median tissue culture infectivity dose; WNV = West Nile virus.

1. The appropriateness of pathogen reduction

In the argumentation below, the term *pathogen reduction technology* has been preferred over the term *pathogen inactivation technology,* as this does not necessarily imply that the contamination of the blood components has been entirely inactivated, but that it has been reduced.

[3] The Council wishes to clarify that the MeSH terms and keywords are used for referencing purposes as well as to provide an easy definition of the scope of the advisory report.

One of the conclusions drawn by the SHC in 2008 (SHC 2008) was the following: "the pathogen reduction methods that have been validated and are safe for platelet concentrates are efficient techniques that offer the advantage of being able to reduce the risk of infectious agent transmission, such as enveloped viruses, (gram-positive and gram-negative) bacteria and protozoa, but not prions" (*"les méthodes de réduction des pathogènes validées et sûres pour les concentrés plaquettaires constituent des techniques efficaces qui offrent le bénéfice de pouvoir réduire les risques de transmission des agents infectieux, tels que les virus enveloppés, les bactéries (Gram positives et Gram négatives) et les protozoaires, mais pas les prions* »

The usefulness of PRT depends on factors that affect the recipients, such as the severity of the post-transfusion clinical disease (e.g. Pealer et al., 2002 for WNV) and the minimum load considered to be infective (Goodrich et al., 2010; Petersen & Busch, 2010). In this context, it should not be overlooked that patients receiving platelets are typically prone to infections: with some 70 % immunocompromised, they are more greatly affected by this issue. In spite of this, there has been no confirmed case so far of (severe) post-transfusion clinical disease following CHIKV-transmission (Petersen & Epstein, 2014).

It is not entirely clear what the lowest infective load is after transfusion of a blood component. However, a recent study on the WNV-strain that circulates in North America (Kelly et al., 2013) shows that it could lie below the detection limit achieved with individual nucleic acid testing (NAT). Under these circumstances, proper pathogen reduction will require such residual concentrations in blood components to be inactivated.

To shorten, or even lift, any deferral periods imposed in the event of WNV- or CHIKV-epidemics, it is also important to take into account the (maximum) viral load that a given PRT treatment could reduce. When there is no pathogen-specific screening available, the appropriateness of PRT actually increases as the proportion of asymptomatic infected donors goes up and the phase of (maximum) viraemia in these donors extends for longer periods of time (see figure 1).

Conversely, PRT to shorten the deferral periods for a particular virus is clearly of limited use if it is not possible to achieve an equivalent reduction rate for one of the other components collected from the same donor. Note that there is no validated PRT-method available to date for red blood cell concentrates.

PRT can also be of limited use when this method cannot be used efficiently to reduce another pathogen present in the donor through co-infection (e.g. Myers & Carey, 1967; Gould et al., 2008; Caron et al., 2012; Baba et al., 2013; Reusken et al., 2013).

The appropriateness of shortening the deferral periods for blood donation after applying pathogen reduction technology against WNV and CHIKV in platelet concentrates depends both on parameters that are intrinsic to a given virus as well as on the pathogen reduction performance of these methods. The assessment will need to consider the viral load that could be reduced by applying PRT, but also the duration of (maximum) viraemia in these donors, the proportion of asymptomatic infected donors, the lowest infective load, the severity of post-transfusion clinical disease, the possibility of achieving an equivalent reduction rate for any of the other components collected from the same donor or for another pathogen present in the same donor as a result of co-infection.

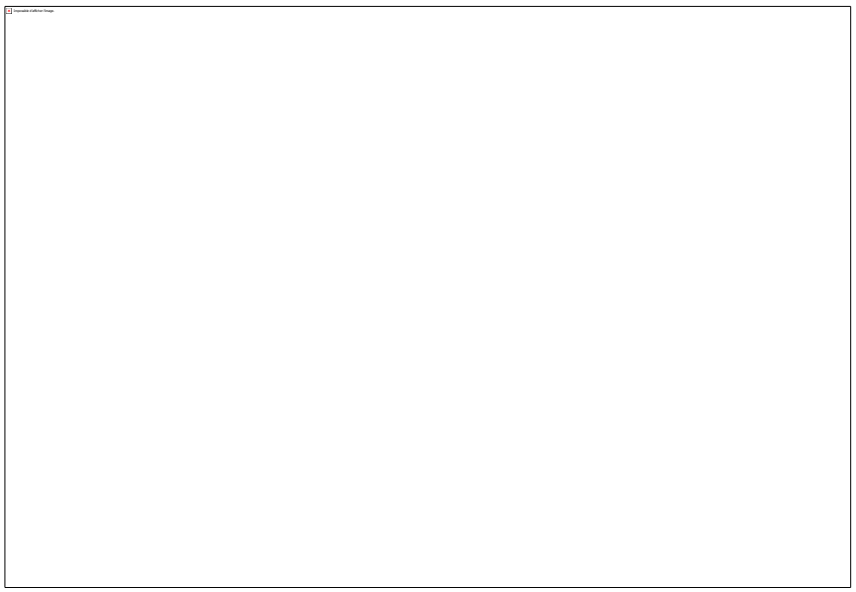
2. Pathogen reduction performance and the viral load

It may not be possible to provide evidence in support of the efficacy of PRT in preventing disease transmission in a clinical setting due to the substantial amount of data required for this type of study. Therefore, the efficacy of PRT was measured in terms of the logarithmic reduction in blood component contamination (Epstein & Vostal, 2003). This is considered appropriate for viruses found in plasma because the viral particles that have not been inactivated lack the ability to proliferate after treatment.

The pathogen reduction performance depends on the type of virus (enveloped or not) as well as on the specific method that was used (see figure 1).

Many PRT methods developed for treating platelets (e.g. Irsch & Lin, 2011; Marschner & Goodrich, 2011; Seltsam & Müller, 2011), usually display higher reduction rates for enveloped viruses than for various non-enveloped viruses (SHC, 2008). Given the fact that WNV and CHIKV are enveloped viruses, their responsiveness to treatment is fairly good. Resistance to physico-chemical treatment is also low to medium for enveloped viruses with single-stranded RNA such as WNV and CHIKV (Farshid, 2002).

Figure 1. Comparison of the efficacy of two PRT methods against a hypothetical virus, one with a reduction rate of 3 log/mL, the other with a rate of 5 log/mL (adapted according to Goodrich et al., 2010). The graph shows how viraemia progresses in an infected person, as well as the efficiency limits and periods of ineffectiveness depending on the viral load.



The efficacy of PRT methods also depends on the viral species, strain[4] or genotype (Farshid, 2002; Shimasaki et al., 2009; Farcet et al., 2012) as well as the viral load found in the component to process (SHC, 2008).

[4] For a given viral strain, the viral particles may also behave differently depending on the cells that replicate the virions (Rey, 2013).

However, there is an inherent difficulty in determining an absolute number of viral genome copies, especially in the context of detection methods covering different technological dosing platforms that may display different analytical sensitivities (Añez et al., 2015). It follows that the viral loads measured delineate average values for detectable units.

When the viral load is expressed in terms of the number of particles per volume, this does not, however, reflect the actual amount of infectious viruses, as there are viral particles present that are unable to replicate (Odelola & Oduye, 1977). The relation between the number of detectable genome equivalents (gEq) and the infectious virus concentration ($TCID_{50}$ or PFU[5]) can be estimated by means of *real time* RT-PCR analysis, but whether or not the results are consistent depends on the specific RNA sequences used in these real-time amplification experiments (Yap et al. 2010). For CHIKV, a 92 % correlation has been found to exist between the result obtained with such real time RT-PCR analysis on the one hand, and quantification based on viral-plaque formation on the other (Ho et al., 2010). It should be noted that the virus level obtained by means of the $TCID_{50}$ method is not equivalent to that obtained with the PFU method, even for a single viral strain and an identical cell line. In actual fact, these two laboratory tests are set up differently and the infectivity of the virus is highly sensitive to factors such as cell age, overlay media, etc.

Since the WNV envelope proteins are less strongly anchored to the nucleocapsid than is the case for the CHIKV, the WNV is more fragile than the CHIKV and, as a result, displays a higher $gEq/TCID_{50}$ ratio.

The sensitivity to PRT treatment varies depending on the viral species, strain or genotype. Its efficacy in reducing the viral load may vary according to changes in the latter in an infected individual.

3. The evolution of West Nile virus viraemia

The WNV belongs to the *Flaviviridae* family, which also includes other viruses such as the dengue, yellow fever, Japanese encephalitis viruses. It is transmitted to humans through mosquito bites. The WNV was initially considered harmless to humans, but an increasing number of pathogenic virus lineages have been described over time (see section 6). About 80 % of those infected never show any visible symptoms, but West Nile fever can occur in about 20 % of cases (Campbell et al., 2002; Petersen et al., 2010).

In a small proportion of those infected — about 1 in 150 — the disease may progress to severe symptoms involving the central nervous system (encephalitis) and even result in death (Petersen et al., 2013). The frequency with which the severe forms of the disease occur and the prognosis is poor depends on the patient's age (> 65 years) and immune system status (Weiss et al., 2001; Jean et al., 2005). Patients with high blood pressure or diabetes also appear to develop more severe symptoms (Samuel & Diamond, 2006). According to a multivariate analysis (Lindsey et al., 2012), there is also a link between chronic kidney disease, a history of cancer and a history of alcohol abuse on the one hand and severe disease on the other, whereas only immunosuppression is associated with fatal cases.

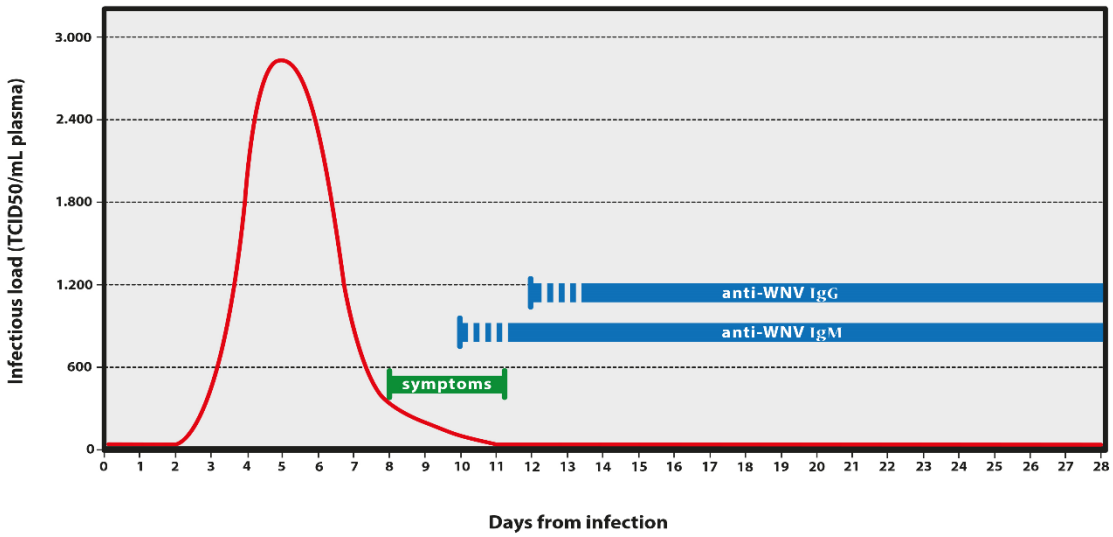
[5] $TCID_{50}$ quantification identifies the cytopathic effect in 50 % of inoculated cells by performing serial dilutions of the virus sample, whereas the PFU assay quantifies the number of viral plaques formed in a cell culture.

After an incubation period of about 2 days after infection, WNV viraemia remains high for an average of 8 days (Fig. 2). With this virus, the symptoms typically appear *after* the peak of infection (Gea-Banaloche et al., 2004; Custer et al., 2009). Sometimes, the symptoms may persist for up to 14 days (Campbell, 2002) and the viraemia may last for up to 11 days after infection (Mostashari et al., 2001; Biggerstaff & Petersen, 2002).

When WNV viraemia has reached its peak, the viral particle rate reaches 10 to 10^6 viral particles/mL plasma (Stramer et al., 2005; Tobler et al., 2008; Goodrich et al., 2010; Petersen & Busch, 2010; Zou et al., 2010).

Upon experimental infection of advanced cancer patients (Southam & Moore, 1954), the rise in viral load was proportional to the initial charge inoculated. However, 89 % of patients showed no symptoms apart from mild fever. It follows that a high viral load usually makes it possible to predict how future symptoms will develop (Southam & Moore, 1954). Still, the WNV viraemia does not always progress in a manner that is consistent with the severity of the symptoms, as there are reports that asymptomatic blood donors could carry 10^5 viral particles/mL plasma (Tobler et al., 2008). Zou et al. (2010) have even shown that the maximum viral load in some asymptomatic donors may exceed that of donors who displayed symptoms at the time of donation. The incubation period after transfusion- or transplantation-borne transmission is 4x longer than after having sustained a mosquito bite (Rudolph et al., 2014).

Figure 2. Kinetics of post-infection viraemia for a person acutely infected with the WNV (maximum infectious load). The neutralising antibodies appear gradually (hatched in blue).



Very recently, Dodd et al. (2015) highlighted the stratification of viral loads detected in 1,477 blood donors who tested positive for WNV-NAT in the US between 2003 and 2012. The profile — graphically depicted as a "boxplot" — shows a continuity of the upper adjacent values to the extreme value, ranging from 28,750 to 720,000 gEq/mL plasma, *i.e.*$10^{5,86}$ viral particles/mL. These viral loads amount to 25 % of the distribution of RNA levels detected in these asymptomatic donors.

As regards WNV, Pfleiderer et al. (2008) estimated that an infectious dose of 1 $TCID_{50}$ amounts to 340 gEq/mL.

It has been shown that a low viral load is infective in the event of transfusion-borne transmission (Lanciotti et al., 2000) and contamination has been found to occur when handling small amounts of infected blood in the laboratory (CDC, 2002). However, it remains unclear what the lowest infective load is for debilitated or immunocompromised patients (Busch et al., 2008; Petersen & Busch, 2010). Moreover, the transfusion risk is considered low after the viraemic phase and this has been linked to the concomitant presence of IgM and IgG (Busch et al., 2008) or regulatory T cells (Lanteri et al., 2009). However, Macedo et al. (2004) and Rios et al. (2008) take the view that blood components remain infectious in the presence of low levels of viral RNA and anti-WNV antibodies. Indeed, Kelly et al. (2013) have recently described a fatal case linked to the transfusion of platelets collected from an asymptomatic infected blood donor, in spite of the fact that WNV-specific IgM and neutralising antibodies were found. The blood donation did not react to individual NAT screening.

Studies on the partitioning of WNV in blood (Rios et al., 2007; Lai et al., 2012; Lanteri et al., 2014) have revealed that this virus adheres to the red blood cells and that the viral load could be 10 to 25 times higher in whole blood than in plasma. Moreover, viral RNA remains in whole blood for 3 months (Lanteri et al., 2014). That does not necessarily mean that this virus is infectious. However, the WNV also has been transmitted through organs from a donor with high titres of anti-WNV antibodies and no detectable virus in the plasma (Nett et al., 2012).

In nature, the WNV maintains a bird-mosquito-bird infection cycle. Even though it is able to replicate at high temperatures, as in birds with a fever (Andrade et al., 2011), the WNV does not maintain a man-mosquito-man infection cycle. The results of Bai et al. (2010) also show that polymorphonuclear neutrophils serve as a reservoir for WNV at the beginning of the infection, which prevents them from exercising their putative protective role properly and can lead to transfusion-borne transmission.

For the WNV, the kinetics of post-infection viraemia go through an acute phase that can last for up to 11 days after infection. The symptoms typically appear after the viraemic phase. About 80 % of those infected never display any visible symptoms.

The **infectious load reaches its peak** at around 2,940 $TCID_{50}$/mL plasma (*i.e.* **3.47 log_{10}**).

The lowest viral load considered infective when transfusing platelets potentially lies below the detection limit achieved with individual nucleic acid testing (NAT).

4. The evolution of chikungunya virus viraemia

The CHIKV belongs to the *Togaviridae* family, which also includes other viruses such as the Semliki Forest, Sindbis and Ross River viruses. It is typically transmitted to humans through bites from tropical mosquitoes. Three pathogenic virus lineages have been described in humans (see section 6). There is general agreement over the fact that infection causes high fever accompanied by severe and debilitating symmetrical joint pain as well as muscle pain in about 80 % of those infected (Sergon et al., 2007). After a short-term improvement following the acute phase, the symptoms can last for weeks or months and up to 66.5 % of patients report muscular stiffness and/or muscle pain more than a year after the onset of infection (Borgherini et al., 2008; Moro et al., 2012). Simon et al. (2015) estimated that 5 % of these patients suffer from chronic inflammatory rheumatism.

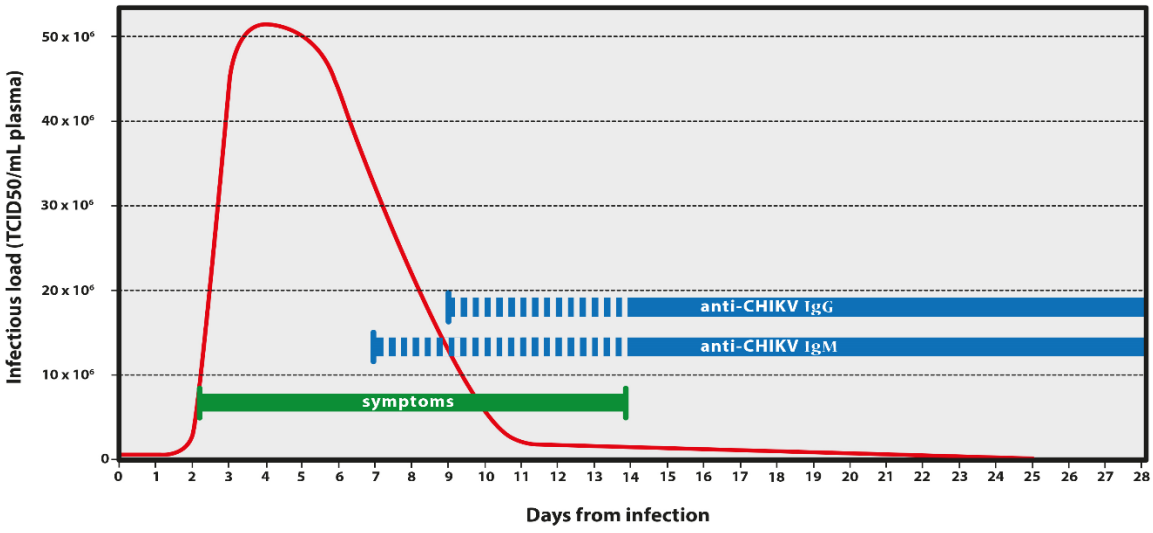
According to Staikowski et al. (2012), the course of disease is benign in those aged under 65: indeed, none of the under-65-year-olds infected during the outbreak in Reunion Island needed to be hospitalised. Conversely, Yoon et al. (2015) report that the number of asymptomatic infected individuals increases with age from around 30 % among children to 90 % among infected donors aged over 50. Their study probably reflects the degree of immunity acquired as a result of previous exposures in the Philippines. Severe complications, which, for a long time, were considered rare, include myocarditis, meningitis, encephalitis and acute flaccid paralysis. This rarely results in death, with fatal cases primarily involving elderly people with underlying diseases or other co-infections. Mother-to-child transmission is common only in late, near-term pregnancy, and has serious consequences for the foetus (Ramful et al., 2007; Gérardin et al., 2008).

After an incubation period of about two days, CHIKV viraemia rises dramatically and usually drops suddenly around day 6, but it can remain high for 8 days (Appassakij et al., 2013; Fig. 3). Appassakij et al. (2013) and Chusri et al. (2014) confirmed that the infectious load decreases rapidly as soon as anti-CHIKV IgM antibodies appear, though viral RNA could remain detectable until up to 17 days after the onset of infection. However, the incubation period may sometimes last for up to 2 weeks (Robinson, 1955; Beltrame et al., 2007) and in this case, viraemia may remain high until up to 20 days after infection.

For this virus, the duration of presymptomatic viraemia is still poorly understood. Usually, the symptoms appear soon after infection — sometimes one day later (Simon et al., 2007; Gallian et al., 2014) and according to estimates, some 4 – 25 % of those infected show no obvious symptoms during the initial stages of the infection (Josseran et al., 2006; Ng et al., 2009).

When viraemia peaks, the CHIKV viral load may be particularly high and exceed 10^{10} viral particles/mL plasma (Panning et al., 2008; Hoarau et al., 2010; Petersen et al., 2010; Win et al., 2010). Based on calibration to synthetic reference RNA, Lanciotti et al. (2007), Santhosh et al. (2007) and Appassakij et al. (2013) assessed that the maximum infectious load reached $10^{6,8}$, 10^{7} or $10^{8,5}$ PFU/mL plasma, respectively.

Figure 3. Kinetics of post-infection viraemia for a person acutely infected with the CHIKV (maximum infectious load). The neutralising antibodies appear gradually (hatched in blue).



There are few data available to date on the viral load in asymptomatic infected blood donors. During the great epidemic that raged in Reunion Island, PRT was systematically implemented in addition to screening for the virus as well as to very comprehensive donor exclusion (Angelini et al., 2006; Cazenave et al., 2006). Cazenave et al. (2006) report that, out of 521 tested apheresis platelet concentrates, a single donation turned out to be slightly positive. These concentrates are part of a larger study (Rasonglès et al., 2009) involving 1,950 platelet donations. However, the results of nucleic acid testing were not disclosed[6]. Appassakij et al. (2013) and Gallian et al. (2014) report that in some asymptomatic blood donors, the infectious load reached values above 10^8 gEq/mL of plasma at the time of donation. The first results on the CHIKV epidemic in Puerto Rico have just been made public (Busch, 2015): they indicate for the first time that only 4 % of infection cases are actually reported, with antibodies appearing in 23.4 % of blood donors without there being any visible symptoms. In about 10 % of these donors, the viral loads exceeded 10^5 gEq/mL. Chiu et al. (2015) found that, among three donors with viral RNA, one asymptomatic blood donor displayed a load of $10^{7,96}$ gEq/mL.

As regards the CHIKV, Carletti et al. (2007) as well as Vanlandingham et al. (2013) estimated that an infectious dose of 1 $TCID_{50}$ amounts to 200 gEq/mL.

It is unclear what the lowest dose that will be infective in the event of a potential transfusion-borne transmission of the virus to debilitated or immunocompromised patients. However, there have been several instances in which laboratory technicians and nursing staff were found to have been contaminated with the CHIKV from handling blood from infected patients (Cordel et al., 2006).

The risk of serious clinical consequences is believed to be high (Petersen & Busch, 2010). However, recent preliminary data (ANSM, 2014; Busch, 2015) suggest that transfusion-borne transmission of CHIKV has no major clinical consequences in blood component recipients, including those receiving platelets.

The SHC has no knowledge about there being any detailed studies available on the partitioning of the virus in blood, though the CHIKV is capable of binding preferentially to platelets (Larke & Wheelock, 1970).

CHIKV is able to maintain a man-mosquito-man infection cycle without an animal reservoir.

For the CHIKV, the kinetics of post-infection viraemia go through an acute phase that can last for up to 20 days after infection. The symptoms appear quickly, as soon as the viraemia goes up, sometimes a day later. About 25 % of infected donors never display any visible symptoms.

The **infectious load reaches its peak** at around 5.10^7 $TCID_{50}$/mL plasma (*i.e.* **7.70 $\log_{10}$**).

It is unclear what the lowest viral load is that is infective when transfusing platelets. No clinical consequences have been described to date following a transfusion-borne transmission of contemporary CHIKV strains.

[6] In the beginning of the epidemic wave, some 500 other platelet donations were tested by RT-PCR and two of them turned out to be positive, though no details were given about the viral load (Brouard et al., 2008). With the number of symptomatic cases having dropped dramatically since April 2006, maintaining the stricter donor exclusion criteria probably resulted in there being no additional contaminated donations.

5. Examining the appropriateness and efficacy of the techniques

Until now, the pathogen reducing potential of PRT methods in platelet concentrates has been estimated with highly specialised biological assays based on internationally standardised cell culture methods (ICH, 1999). These assays have been standardised as part of the industrial manufacturing process of plasma-derived blood products. Among other things, carrying out a comprehensive assessment of the efficacy of the various methods is concerned with the manner in which the experiments are conducted and interpreted (choice of viral strains, methods used for inoculation, cell culture, virus detection, etc.). Attention is paid to the threats that certain viruses may pose to the health of the staff conducting the viral clearance studies.

The plasma-derivative manufacturing process typically involves several physico-chemical steps, including specific steps that may each result in a reduction rate of up to 6 logs or more for enveloped viruses with a low to medium resistance to treatment. As regards WNV, Kreil et al. (2003) and Jakubik et al. (2004) have shown that each specific manufacturing step displays a reduction rate of ≥ 5 or 6 logs. It is important to note that these manufacturing processes involve virus removal steps (precipitation, filtration, etc.) that significantly increase the safety margin of inactivated products (Dichtelmüller et al., 2011). The viral loads are considerably diluted by pooling many plasma donations: according to the assessment made by Pfleiderer et al. (2008), this load is $< 10^3$ gEq WNV/mL plasma. As a result, there is no known case in which a recipient of plasma derivatives was infected with the WNV in spite of the fact that no screening is performed on the source plasma.

As regards CHIKV, the nucleocapsid is more strongly anchored to the structural proteins of the envelope than is the case for the WNV, which is believed to make the CHIKV more resistant to PRT methods. Indeed, Leydold et al. (2012) have just recently shown that this virus is often 10-15 times more resistant to physico-chemical treatment than the WNV. Still, certain steps of the plasma-derivative manufacturing process were found to display reduction rates of ≥ 6 logs. The viral loads in plasma pools that may also contain donations contaminated with CHIKV will also be diluted, but the SHC has no knowledge of any studies having been conducted on this subject.

The traditional physico-chemical pathogen reduction method — viz. the "solvent-detergent" method — is also used to inactivate viruses in plasma for transfusion. There are currently other PRT processes available that have been designed for use on individual plasma samples as well as on platelet concentrates. These methods target the nucleic acids of pathogens, especially by using visible or ultraviolet light, as well as, in many cases, an additive (Rock, 2011; Tsen et al., 2014). In such cases, there are significantly less simultaneous virus removal steps and there is no significant dilution of the viral load, for example during the treatment of apheresis platelets from a single donor.

As for identifying the infection rate in viral clearance studies, Farshid (2002) and Dichtelmüller et al. (2011) enumerate the main factors that may have an impact on whether or not real values of viral reduction are achieved. In actual practice, the dynamic range of the test can be dependent on or limited by:

- the titre and volume of the inoculum used;
- sample dilution to avoid cytotoxicity;
- the volume of the sample spread on the cell cultures used.

Dichtelmüller et al. (2011) indicate that, depending on the inoculum and the volume of the sample spread on the cell cultures by different laboratories, even a 6 $\log_{10}$ reduction does not by any means rule out any residual infections.

This is why some health authorities require a 3 to 5 $\log_{10}$ reduction of the viral load as a safety margin for plasma (Farshid, 2002). Similarly, Epstein (2010) advises a safety margin of at least 3 $\log_{10}$ of the infectious load for the reduction rates set for platelets.

Moreover, the viral load (see Figure 1) is expressed per millilitre of plasma: it therefore does not take into account defective viruses or viral particles that potentially aggregate onto cellular blood components (Flaujac et al., 2010). This means that the estimated infectious loads are representative for blood components such as plasma (Rios et al., 2007; Chancey et al., 2012), but that they could be higher for platelets (Lee et al., 1993; Lee et al., 1998).

Apart from this preliminary *in vitro* assessment, the details about the preparation of platelet concentrates, such as centrifugation parameters, the version of the medical device used to obtain the results, etc. should also be taken into account. As there are quite a few alternative ways in which platelet concentrates can be prepared, the latter are in fact likely to be affected differently by the same pathogen reduction treatment.

Last but not least, we must not underestimate the donor-specific variations that affect platelet concentrate samples — e.g. the plasma lipid content, the degree of platelet aggregation (van der Meer et al., 2015), etc.

Indeed, the routine implementation of these techniques sometimes shows significant discrepancies in terms of their efficacy (cf. SHC, 2011; CSS 2011b; Müller et al., 2011).

Given the discrepancies observed between the various parameters, especially those that pertain to the routine preparation process, CSS advises that **a safety margin of at least 3 $\log_{10}$** of the infectious load be implemented for the reduction rates that pertain to enveloped viruses in platelet concentrates.

6. Epidemic outbreaks of the West Nile and chikungunya viruses

6.1. WNV epizootics

WNV was first isolated in 1937 in the West Nile region of Uganda in a woman suffering from a high fever. It was found to be a virus that is transmitted to birds through mosquito bites in Egypt and Israel in the 1950s. Mammals, including horses and humans, may also be infected when mosquitoes thrive. Many mosquitoes pass WNV, but it is especially the most common mosquito in temperate regions, viz. *Culex pipiens*, that is responsible for the rapid colonisation of the other continents, to which international transport and trade have contributed. Birds migrating from Africa and the importing of the house sparrow too have been claimed to have had their share of responsibility in this spread. Crows are birds that easily succumb to WNV.

Before the 1990s, the WNV was endemic in Africa (lineage 1A and lineage 2), Australia (lineage 1B), the Middle East and India (lineage 1C) and any occasional infection in human beings was considered benign. Since the 1990s, a more invasive 1A strain with potentially severe clinical consequences (fatal encephalitis) has begun to spread (Artsob et al., 2009). Only small outbreaks were observed in Europe (Kilpatrick, 2011) but three major epidemics occurred in Bucharest, Volgograd and Israel, resulting in dozens of fatal cases (Reiter, 2010). In 1999, WNV emerged in

North America, triggering a dramatic and unprecedented epizootic: within four years, WNV had become ubiquitous in the US and Canada with epidemics every summer from July until late October, causing some patients to suffer from neuroinvasive West Nile disease. By 2009, some 1.8 million people had been infected in the US, with 12,852 reported cases of meningoencephalitis and 1,308 fatal cases (Artsob et al., 2009). Since then, this WNV-strain has also invaded Central and South America.

Transfusion-borne transmission has been shown to occur in the USA (Pealer et al., 2002) and American blood donations are screened by means of NAT that have been validated to detect WNV lineage 1A. The presence of infections in travellers returning from America has been confirmed by European laboratories (Charles et al., 2003; Prick et al., 2003; Maillo et al., 2008), which prompted the European authorities to establish a temporary exclusion criterion for blood donation.

Since 2008, new WNV outbreaks have occurred in Europe as extensive epizootics raged in southern Russia and epidemic outbreaks appeared in the Middle East and the Maghreb countries (ECDC, 2011). An "affected area" is an area in which there in an ongoing risk of the virus being transmitted to humans (Domanovic & Giesecke, 2012). It is important to note that the outbreaks of human neuroinvasive cases in Russia, Romania, Greece, Italy, Sardinia and Serbia were caused by WNV lineage 2 (Hernández-Triana et al., 2014). At the same time, Venter & Swanepoel (2010) confirmed that the cases of meningoencephalitis that occurred in South Africa were caused by lineage 2, which had, until then, been considered innocuous. This lineage originated in sub-Saharan Africa and Madagascar but had already been discovered by chance in Hungarian birds of prey in 2004, before being found in Austrian birds as well.

Genomic screening with NAT could quickly be implemented in Italy and Greece because different tests had already been amply validated during the North American epizootic. Nevertheless, the screening technique has to be adjusted for the various WNV-strains circulating in Europe to be detected effectively (cf. Linke et al., 2007), whilst taking into account any interfering viruses (Gaibani et al., 2010).

6.2. CHIKV epidemics

CHIKV was first isolated in 1953 when a fever epidemic prevailed on the Makonde Plateau in Tanzania. The disease causes very severe joint pain associated with stiffness, hence the reason why its original name means "to become contorted". The unexpected chikungunya epidemic that raged on the islands in the Indian Ocean islands in 2006 propelled this "emerging" disease to the forefront of international health concerns. However, in light of recent knowledge on the clinical picture that is suggestive of chikungunya, a number of "dengue" epidemics that emerged over the last 250 years, might, in hindsight, be attributed to chikungunya instead (Carey, 1971; Halstead, 2015).

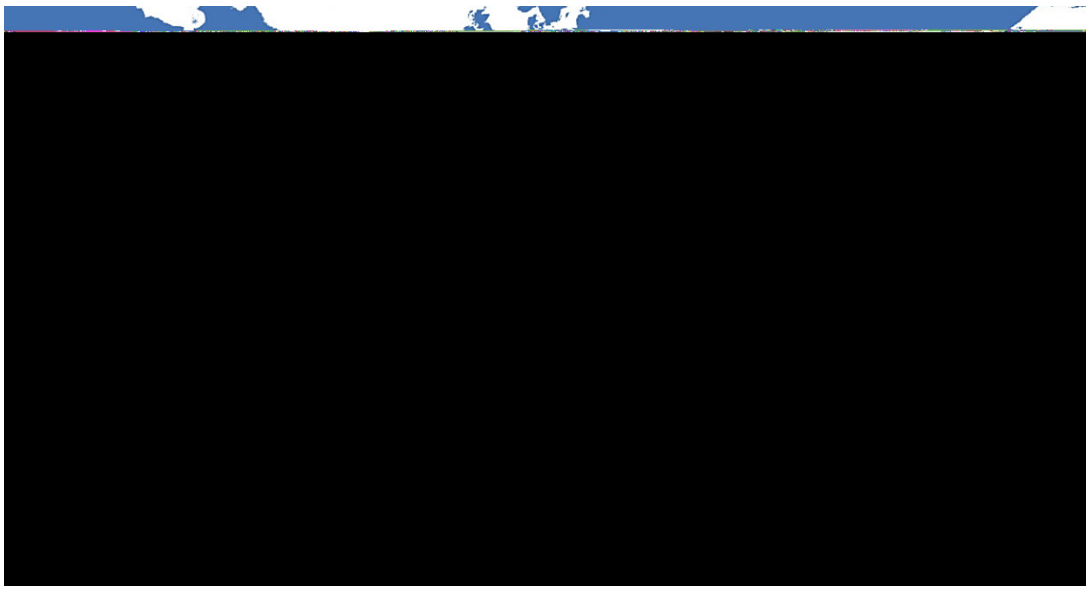
CHIKV is transmitted through tropical mosquito bites and is maintained in a complex zoonotic cycle in Africa and Asia that especially involves monkeys. At 40 – 50 year intervals, it enters into an urban human-mosquito-human cycle that triggers explosive and extensive pandemics. The main mosquito involved in spreading this virus is the *Aedes aegypti* mosquito, which is the vector of yellow fever and dengue. Its range covers tropical and subtropical regions (cf. Figure 4).

In the last 50 years, there has been a 30-fold increase in the incidence of dengue. In the current decade, it has expanded from urban sites to rural areas. As a result, viruses are now intensely spread by this mosquito up to the potential geographical limits for its year-round survival in the northern (Mexico, Nepal) and southern hemispheres (northern Argentina, Swaziland). In Europe, *A. aegypti* is endemic on the island of Madeira.

Since 2004, *Aedes albopictus* (the "tiger mosquito") has caused CHIKV to rapidly colonise other continents from the Kenyan coastline and the islands in the Indian Ocean. In Africa and South America, the tropical and subtropical range is currently still more limited than that of the *A.aegypti* mosquito, but this mosquito has already invaded the temperate regions, to which international transport and trade have contributed. For the past few decades, it has been colonising such regions as the East Coast of the USA (beyond Chicago), the Mediterranean coast from Spain to Greece and has been working its way down the Australian East Coast (beyond Sidney). Conversely, the east coast of China, South Korea and Japan belong to its native range.

In temperate regions, *A. albopictus* goes into diapause during the winter and is unable to pass the CHIKV during the following spring. However, in regions in which there is no clear winter, female mosquitoes remain active throughout the year.

Figure 4. Areas in which there is a risk of CHIKV- transmission by the *Aedes aegypti* mosquito (addition of 10 °C isotherms on the distribution map drawn up by Kraemer et al., 2015). The areas with malaria transmission lie inside those in which this mosquito is endemic, except for Kurdistan, Yemen and southern Iran/Afghanistan (marked by ||| on the map). The January and July isotherms show the potential geographical limits in the northern and southern hemispheres for the year-round survival of this tropical mosquito.



A distinction is drawn between three viral lineages: a West African lineage, an East, South and Central African lineage, and an Asian lineage. They are mainly spread by the *A. aegypti* mosquito.

Prior to the explosive epidemic that raged in Reunion Island in 2006, CHIKV infections were considered debilitating, causing joint and muscle pain that cleared after a few weeks, without being life-threatening. However, a mutation in a surface protein is believed to have allowed the CHIKV to be efficiently transmitted by the *A. albopictus* mosquito in the Indian Ocean islands, as well as to be responsible for the occurrence of severe clinical complications. This lineage has arrived in India and South-East Asia, where it is now established next to the native Asian lineage. This strain is more virulent, causing neurological complications and excess mortality (Mavalankar et al., 2008; Robin et al., 2008). In 2007, this CHIKV lineage was imported into the north-east of Italy, where some 250 people showed symptoms during the summer of 2007, which in turn adversely affected the country's blood supply (Liumbruno et al., 2008). Other outbreaks occurred in Gabon.

The Asian lineage has started to spread in China and Oceania (Yap Island, Polynesia) and has triggered a dramatic spread in America, affecting some ten million people in the Caribbean and Central and South America in the course of 2014. The disease was first recognised in late 2013 in the French Antilles, a well-supervised French overseas territory. The cosmopolitan spread of this new viral strain was preceded by a large-scale epidemic in the Philippines in 2012 (Tan et al., 2015).

This vast epidemic is still ongoing with explosive outbreaks occurring near the boundaries of the *A. aegypti* mosquito's range, viz. in Mexico as well as in Paraguay and Peru. A small outbreak of this lineage hit Florida last year (11 individuals with symptoms).

In Brazil, the East, South and Central African lineage also took root in the state of Bahia on the Atlantic coast during the summer of 2014, but it does not display the mutation that enables it to be passed by the tiger mosquito. Another small outbreak of the East, South and Central African lineage appeared in 2014 around Montpelier in France (12 patients with symptoms).

As was the case with the yellow fever virus, CHIKV will be able to establish a zoonotic cycle involving New World monkeys and to remain on the continent from this reservoir. No such cycle seems to have ever taken root in Asia, where the CHIKV exclusively circulates via human transmission in the vicinity of dwellings.

The presence of infections in travellers returning from areas affected by the CHIKV has been confirmed in European and American laboratories (Parola et al., 2006; Panning et al., 2008; Gibney et al. 2011; see Fig. 5). The number of thus detected symptomatic cases is clearly proportional to the occurrence of large epidemics in tourist regions like Reunion Island in 2006 and the Caribbean in 2014 (see Figure 5).

Figure 5. Fluctuation in the number of likely and confirmed cases of CHIKV infection in travellers returning to Belgium per year in which the virus spread in the regions visited (Van den Bossche et al., 2015; M. Van Esbroeck, *pers. comm.*).

* Number of infections identified between January and June 2015.

It was suspected that CHIKV could be transmitted via transfusion, which, as a precautionary measure, resulted in the implementation of periods of temporary deferral from blood donation during the epidemic on the islands of the south-west Indian Ocean and in the north-east of Italy (cf. SHC 2007b). Most of the other regions in which CHIKV prevailed had already been excluded by the deferral criteria for malaria (see Figure 4). In actual fact, the Asian CHIKV lineage has just recently been shown to have been transmitted through the transfusion of contaminated blood components (ANSM, 2014; Busch, 2015). However, based on the preliminary data available, this does not seem to have any major consequences for the recipients.

6.3. Strategies to reduce the risk of contamination linked to travellers

The transmission of CHIKV and WNV strains is characterised by the appearance of outbreaks and a seasonal fluctuation of the epidemics, which may sometimes occur unexpectedly. It follows that there are a few impediments to setting up strategies for excluding travellers returning from affected areas (Lieshout-Krikke et al., 2013; Seed et al., 2014; Petersen & Epstein, 2015).

- delimiting the affected areas is inherently complex;
- it remains unclear for how long a deferral period for blood donation should ideally be implemented[7];
- it is possible that by the time the safety measures have been set up, the greatest threat of a new epidemic has already passed.

These impediments, as well as the cumbersomeness of constantly having to adjust the strategy to new outbreaks, have prompted Belgium to adopt a common exclusion criterion for all blood

[7] e.g. in 2012, the US WNV-epizootic season lasted one month longer than in other years.

donors who have recently travelled overseas (SHC, 2007). The growing number of areas in Europa affected by the WNV, as well as the occurrence of small outbreaks of dengue and chikungunya, make it more complicated to manage the strategies aimed at reducing the risks of infection.

In all these situations, PRT can contribute towards reducing the risk of transfusion-borne transmission of viral strains in the absence of pathogen-specific screening. Moreover, the validated PRT methods may confer significant protection when the viral load in the donor does not exceed the efficiency limit. Still, for the time being, the most frequently transfused blood component, viz. red blood cell concentrates, cannot be treated effectively with PRT yet (see section 7). That is why blood donations are increasingly subjected to nucleic acid testing in affected areas as a means to protect the overall blood supply against the risk of contamination (Seed, 2014). In low-prevalence areas (*i.e.* in which there are only a minimal number of neuroinvasive cases), WNV-transmission will be under-reported due to the lack of WNV-screening that is representative of the population — e.g. in blood donors. When such a monitoring programme was implemented in eastern Austria, Jungbauer et al. (2015) were able to identify one blood donor infected with lineage 2 in August 2014. Some 67,800 blood donations have been tested in that area to date.

NAT screening was also implemented during the 2012 season in England and North Wales, targeting travellers who were "at risk" of having been infected with WNV (NHSBT, 2013). Since no donor was found to have a confirmed positive screening result for WNV, the English blood services were not compelled to defer 30,000 blood donations in accordance with the exclusion criteria that have been set for this virus. This number of donations amounted to 1.47 % of all tested donations, *i.e.* it was roughly equivalent to the number of donations screened for hepatitis B based on anti-HBc antibodies or for malaria based on anti-malaria antibodies.

In the Netherlands, some 28,000 donors are deferred each year upon returning from a stay in countries at risk for Crimean-Congo fever, dengue, malaria, visceral leishmaniasis, CHIKV and WNV viruses (Lieshout-Krikke et al., 2013). This amounts to 2.7 % of all donors. Lieshout-Krikke et al. (2013) believe that the number of infected travellers returning from areas newly affected by WNV (areas in Greece or Italy) or CHIKV (Thailand) is very low. Some 5,500 Dutch donors were excluded for 4 weeks after travelling to the affected areas in Italy and Greece. Since traveller destinations and habits (tourism, business travel) differ from one country to another, a comprehensive analysis still needs to be conducted for Belgium.

Each proposal to adapt the exclusion periods for blood donation for a given pathogen must take into account any deferral periods that are in place for other pathogens that prevail in the affected areas. The CHIKV is transmitted by mosquitoes, the main species of which are widespread in tropical and subtropical countries. These countries are mainly situated in the area where malaria is endemic (see Figure 4). Given the fact that malaria is one of the infectious diseases that must be ruled out for all blood donors, donor candidates arriving from countries in which malaria is endemic are excluded from donating blood for a 6-month period (see Royal Decree of 1 February 2005, Appendix 2., a) Infections). Moreover, there may be cases of chikungunya and malaria co-infection (Raut et al., 2015). When an exclusion period for a given pathogen has been shortened, there must be increased vigilance as regards the other pathogens for which there is a confirmed transfusion risk[8]. For example, in the USA, which is an area in which WNV is endemic, there is a

[8] It should be noted that the risk of transfusion-borne transmission is sometimes considered to be greater for some blood components, e.g. platelet concentrates in the event of Chagas disease (Cancino-Faure et al., 2015).

serious risk of transfusion-borne transmission of visceral babesiosis (Leiby, 2006; Goss et al., 2012; Gray & Herwaldt, 2015). Chikungunya epidemics often overlap with dengue epidemics and some 3 % of chikungunya-dengue co-infections were found in an epidemic area or in travellers returning from these regions (Pialoux et al., 2007; Kalawat et al., 2011). On the other hand, a 50 % co-infection rate has been reported by Saswat et al. (2015). The extent of the risk posed by dengue for transfusion is currently being investigated (Semenza & Menne, 2009; Arellanos-Sotos et al., 2015). Conversely, since 15 % of the fatalities that were directly attributed to CHIKV have in fact been linked to co-infection (INS, 2015), people infected with these two viruses could show severe symptoms and thus be excluded from donating blood on this basis.

WNV has spread on all continents and circulates in temperate regions. In Europe, the areas that are at risk or are affected by the WNV have expanded in recent years. During the last decade, CHIKV entered into a pandemic phase and is now present in all tropical and subtropical regions with incursions into temperate regions. The number of travellers infected with these viruses is proportional to the intensity of the epidemics as well as to travel destinations and habits (season, altitude, length of stay, etc.).

The appropriateness of shortening a deferral period for blood donation for a given pathogen depends on the reduction performance for any other pathogen for which there is a mandatory exclusion period and which the donor may have been co-infected with. Should the deferral periods be shortened after PRT, there would need to be increased vigilance as regards pathogens that entail a confirmed risk for transfusion and that cannot be entirely eliminated by means of the method implemented.

7. The usefulness of pathogen reduction methods for platelet concentrates

In Belgium, pathogen reduction in platelet concentrates has been authorised by Royal Decree since February 2005 as an alternative to the detection of bacterial contamination to extend the shelf life of platelets from 5 to 7 days. More recently, the SHC advised that pathogen-reduced platelet concentrates should not be kept for more than five days after collection (cf. SHC, 2011b). This shorter shelf life was introduced by Circular in November 2009 (FAMHP, 2009). Moreover, the Royal Decree of June 2009 states that all platelet concentrates must undergo a validated pathogen reduction method (*"tous les concentrés plaquettaires doivent subir une méthode de réduction des pathogènes validée*"). This Decree entered into force on 1 July 2015. The legislation therefore concerns both apheresis platelet concentrates (*i.e.* selective removal using a cell separator) as well as those obtained from buffy coats, viz. by fractionation of whole blood into plasma, platelets and erythrocytes (SHC, 2010).

Since deferrals from blood donation that target pathogens affect all blood components collected from the potential donor, and since, to date, there is no validated PRT method for red blood cell concentrates (cf. SHC, 2008), the advisory report does not apply to concentrates prepared from whole blood (see Table 1). During the manufacture process of platelet concentrates, other blood components too are collected by means of multicomponent apheresis. Due to the increasing demand for plasma, very few single-apheresis platelet concentrates were collected in 2008 and 2009; instead, double apheresis was used to collect both platelets and plasma.

Since 2010, this situation has turned around, with over 50 % of apheresis platelets collected by means of plateletpheresis in 2013. For double-apheresis platelets, the exclusion periods must be shortened in accordance with the most stringent reduction rate for the collected component.

Table 1. Distribution of the number of platelet concentrates prepared each year in Belgium per collection method used (FAMHP, 2013; FAMHP, 2015; L. Muylle, *pers. comm.*).

Origin of the platelets	Year of collection					
	2008	2009	2010	2011	2012	2013
Whole blood	40,049	41,100	32,971	32,621	33,437	33,040
Double apheresis (platelets + plasma$^{\Delta}$)	22,614	25,079	15,314	15,013	13,471	15,558
Plateletpheresis	4,522	1,886	12,133	13,710	15,543	17,009
Collection total$^{\lozenge}$	65,030	68,910	69,328	68,966	69,447	68,800

$^{\Delta}$ A portion of this plasma may be intended for industrial fractionation.
$^{\lozenge}$ Some apheresis procedures yield two platelet concentrates.

Physico-chemical methods for pathogen reduction in plasma are not used to inactivate pathogens in platelet concentrates, as these methods also damage cell components (e.g. the solvent-detergent method purposefully damages the cell-membrane lipid layer). The PRT processes that have been considered so far for use on platelet concentrates use light rays to damage the nucleic acids of pathogens, both with and without a photoactivatable additive (Mohr & Redecker-Klein, 2003; Mohr et al., 2009; Salunkhe et al., 2015). The pathogen-reducing potential of these PRT methods has been estimated by means of the same cell culture methods as those used in the industrial manufacturing of plasma-derived blood products.

The viral reduction efficacy should be established under small-scale laboratory conditions that are representative of the working of the commercial unit in blood establishments (Friedman & Stromberg, 1993; Friedman et al., 1995; Farshid et al., 2005; Dichtelmüller et al., 2011). Approaches that include non-compliant manufacturing steps (e.g. washing platelets)[9] or omit certain steps are not acceptable. The extent to which the product ingredients — such as e.g. the preservation solutions, residual leukocytes, the platelets themselves — interfere with the assays used to determine the infectious virus titre must be assessed separately, as these factors may have an adverse effect on the indicator cells (ICH, 1999).

As regards CHIKV, it is also important to draw attention to the fact that this virus is able to bind preferentially to platelets (Larke & Wheelock, 1970). Moreover, Chernesky & Larke (1977) pointed out that when this virus can surround itself with large platelet aggregates, this adversely affects the heat inactivation of the virus. The validation of the PRT-methods in platelets must take into

[9] For example, Sawyer & Dupuis (2006) or Cazenave et al. (2007).

account this feature (cf. SHC, 2007), given the fact that the light may be prevented from penetrating into the platelet concentrates sufficiently for these techniques to be effective. In short, the infectious load may be turn out to be higher in platelets than in plasma.

Many studies have shown that the reduction performance of these PRT methods depends on the preservation solution used during the shelf life — *i.e.* either autologous plasma or a blend of about 1/3 plasma and 2/3 of a platelet storage solution (SHC, 2010). Several studies have indeed shown that plasma proteins prevent the UV rays from penetrating properly (Terpstra et al., 2008; Mohr et al., 2009b; Störmer et al., 2010; Yomtovian & Jacobs, 2010). Similarly, for many pathogens, the reported reduction values are lower in 100 % plasma[10] than those obtained in experiments using the same amount of energy delivered to platelets resuspended in an additive solution (cf. Irsch & Lin, 2011; Marschner & Goodrich, 2011; Seltsam & Müller, 2011). Moreover, the reduction rates are typically obtained by analysing four to six samples and are expressed as mean values (Lin et al., 2005; Goodrich et al., 2006; Mohr et al., 2009b; Tsetsarkin et al., 2013). When the standard deviation is mentioned, it is about 0.5 $\log_{10}$ for studies with platelets in an additive solution, whereas the standard deviation for studies with platelets in autologous plasma can exceed 1 $\log_{10}$ (cf. Tsetsarkin et al., 2013).

In addition, the initial virus titre that can be obtained experimentally to inoculate platelets in an additive solution is almost 1 $\log_{10}$ lower than that for platelets in autologous plasma. However, for CHIKV, the higher infectious titres that can be obtained in plasma do set limits to the photochemical or photodynamic techniques: an infectious load over ca. $10^{6,5}$ to 10^{7} $TCID_{50}$/mL[11] (Sawyer et al., 2007; Tsetsarkin et al., 2013) or over 10^{6} $TCID_{50}$/mL (Rossini et al., 2011) stands in the way of achieving the viral reduction values under these experimental conditions.

In short, the reduction performance of these PRT methods in platelet concentrates will approximately equal the lowest reduction rate achieved in an additive solution.

As there is no validated PRT method for red blood cell concentrates available to date, this advisory report does not apply to concentrates prepared from whole blood. For double-apheresis platelets, the exclusion periods must be shortened in accordance with the most stringent reduction rate for the collected component.

The reduction performance of PRT methods in platelet concentrates will approximately equal the lowest reduction rate achieved in an additive solution.

[10] In fact, this precisely does not seem to be the case for the WNV and CHIKV, for which the reduction performance announced by Irsch & Linn (2011) is about 1 $\log_{10}$ higher for platelets in autologous plasma than those in an additive solution.

[11] Sawyer et al. (2007) show that a titre of $10^{5,7}$ to $10^{7,3}$ $TCID_{50}$/mL inoculated in plasma resulted in one or two residual plaques and Tsetsarkin et al. (2013) observed a residual viral load of less than 1.3 $\log_{10}$ in two of the four reproduced experiments.

8. The virus-reduction performance of PRT-methods for WNV and CHIKV in platelet concentrates

The currently published data for three commercial PRT systems generally mention reduction rates of 4.5 $\log_{10}$ $TCID_{50}$/mL or higher[12] for WNV (Ruane et al., 2004; Lin et al., 2005; Gallian et al., 2006; Mohr et al., 2009b). These data further demonstrate that WNV is highly sensitive to light-exposure (Mohr et al., 2004), but the SHC has no knowledge of independent comparative studies evaluating these methods under standardised experimental conditions.

Gallian et al. (2006) hold that applying photochemical PRT on platelet concentrates is as effective on European WNV strains as it is on the American strains (see section 6.1.). However, the analysed WNV strain still belongs to lineage 1A, and displays a 3.7 % divergence in the viral RNA sequence, whereas the genetic distance is about 4x greater for lineage 2. Although the SHC does not expect these different West-Nile viruses to show a significantly greater resistance to PRT methods, the equivalence of the reduction rate has not been experimentally validated to date.

As regards CHIKV, the published reduction rates for both commercial PRT systems are approximately 2 – 3.5 $\log_{10}$ or 5 – 6 $\log_{10}$ $TCID_{50}$/mL, respectively (Sawyer et al., 2007; Sawyer et al., 2009; Rossini et al., 2011; Tsetsarkin et al., 2013; Vanlandingham et al., 2013). These studies quantified the reduction rate immediately after the inactivation.

The SHC points out that Tan et al. (2013) assessed the reduction performance of these two methods simultaneously and over a storage period of up to five days. These authors showed that the reduction efficacy was the same for both photochemical methods, which reduced the infectious viral loads from $10^{4,4}$ PFU/mL to the detection limits (*i.e.* 3.75 $\log_{10}$ PFU/mL). However, the SHC emphasises that the PFU titrations are based on counting that is difficult to standardise and that identifying a cytopathic effect with the $TCID_{50}$ limiting dilution methods is more reliable. Tsetsarkin et al. (2013) emphasise the need to include heparin in the dilutions to prevent loss of sensitivity in the $TCID_{50}$ assay when the anticoagulant of the blood component comes into contact with the divalent cations in the culture medium. Vanlandingham et al. (2013) do not mention the use of heparin. As regards the PRT methods based on UV-rays only, *i.e.* with no photoactivatable additive, it would be surprising to find that CHIKV displays a greater photosensitivity, given the fact that its nucleocapsid is more strongly anchored to the viral envelope protein than is the case for the WNV.

The pathogen reducing performance of PRT methods in platelet concentrates has often been assessed by means of the same assays as those used to determine the infectious virus titre in the industrial manufacture of plasma-derived blood products. Although the cell culture methods used display significant detection limits, implementing several virus inactivation/removal steps greatly increases the safety margin for inactivated plasma products. Expressing the reduction factors as logarithmic reductions in titre implies that the residual infectivity may be greatly reduced, but it will never be brought down to zero (ICH, 1999). Thus, a reduction factor that is identical to the infectious units in the concentrate leaves one infectious unit per mL, which amounts to 300 infectious units per platelet concentrate. If no additional inactivation or removal steps[13] are applied during the preparation of platelet concentrates, these residual infectious units

[12] According Seltsam & Müller (2011), irradiation with UV-rays only (Mohr et al, 2009b) is less efficient, the reduction rate being about 3.5 – 4 $\log_{10}$.

[13] Mohr et al. (2004) and Tan et al. (2013) had provided evidence that both CHIKV and WNV are photosensitive by simply exposing them to daylight: for WNV, this results in a 60 – 70 % degradation and, for CHIKV, 27 %. This is a significant portion of the reduction rate attributed to PRT.

may be transmitted by transfusion. It is unclear what the lowest viral load is that should be considered to be infective when transfusing platelets and, to date, no (severe) clinical consequences have been described as a result of transfusion-borne CHIKV transmission (see section 4). However, platelet concentrates contaminated with WNV remain infectious if there is viral RNA present that is not reactive to individual NAT screening (see section 3; Kelly et al., 2013). The SHC therefore believes that, in order to prevent the transfusion-borne transmission of these viruses, validated PRT-methods should fully reduce the residual infectious loads in blood components.

In order to fully eliminate WNV during the viraemic phase, the PRT methods should reduce the infectious load by at least 6.5 $\log_{10}$ $TCID_{50}$/mL, taking into account a safety margin of 3 $\log_{10}$ (see sections 3 and 5). For CHIKV, this should be at least 10.7 $\log_{10}$ (see sections 4 and 5). Given the efficiency limits of the PRT methods used in platelet concentrates, an exclusion period covering the acute phase of a WNV or CHIKV infection remains of paramount importance to prevent their transfusion-borne transmission.

The SHC takes the view that a reduction performance of at least 3.7 $\log_{10}$ $TCID_{50}$/mL of the infectious load, safety margin included, reduces the amount of WNV and CHIKV after the viraemic phase, *i.e.* from the 12th day following the infection for WNV-strains, and from the 21st day for the CHIKV strains. Many PRT methods used for platelet concentrates display a reduction performance for WNV and CHIKV that exceeds this reduction rate.

Still, one should keep in mind that there is a fundamental difference between pathogen inactivation in cell-free plasma and pathogen reduction in a (cellular) platelet preparation. Any pathogens in the plasma that may not have been inactivated after treatment for transfusion are unable to multiply in sufficient numbers to cause morbidity and mortality in patients because the plasma is frozen before being stored under cold conditions and used within less than six hours after thawing (SHC, 2010). However, the situation is very different for platelet concentrates treated by means of methods that only target nucleic acids: not only is it possible for some non-inactivated bacteria to proliferate for several days at room temperature, but some viruses can then also replicate using the translation machinery in platelets or other remaining cells (e.g. $<10^6$ residual leukocytes).

Indeed, in a previous advisory report (SHC, 2011), the SHC pointed out that applying a photochemical method to platelet concentrates was not sufficient to make them entirely safe from any bacteria present at low yet clinically significant levels. Kwon et al. (2014) and Schmidt et al. (2015) also described the proliferation of bacteria in platelet concentrates after treatment with another photochemical method. It is plausible that the shade caused by the formation of aggregates as well as the platelets themselves may prevent the light from penetrating into the platelet concentrates sufficiently for the pathogens to be fully eliminated.

The SHC has no knowledge of any studies on the viral contamination that may remain after treatment of the platelet concentrates[14] with PRT methods that cover the entire storage period. It is probably not entirely clear yet what the consequences are of the existence of a translation machinery in platelets and/or residual leukocytes.

[14] Mohr et al. (2004) used nucleic acid testing to monitor the extent to which WNV was inactivated in plasma for transfusion. RNA could not be fully eliminated. Still, the short amplified sequence does not allow to deduce that any live viruses remain. Conversely, Aytay et al. (2004) have developed an alternative nucleic acid amplification method. These amplification techniques may not, however be used for all PRT methods to measure the residual infectivity (cf. Sagripanti et al., 2011).

In their assessment of a PRT method, Mather et al. (2003) found WNV in peripheral mononuclear cells and showed that there were productive infections in monocytes (see also Garcia-Tapia et al., 2006; Lanteri et al., 2014). Moreover, Bai et al. (2010) point out that WNV does not replicate in neutrophils. Monocytes make up 2 to 10 % of all leukocytes in the human body, whilst neutrophils are the most abundant leukocytes (40 % to 75 %) in mammals. Though it is not entirely clear yet which blood cells play a part in CHIKV infections, Her et al. (2010) have shown that monocytes too may be involved. Moreover, CHIKV is able to bind preferentially to platelets (Larke & Wheelock, 1970; Chernesky & Larke, 1977). Just recently, Sutherland et al. (2014) and Simon et al. (2015) provided proof-of-principle that infectious viruses may be produced in platelets stored in accordance with blood-bank procedures by quantifying the dengue virus throughout the storage period, in this case 7 days, these authors found that, at the end of this period, they contained up to four times more viral RNA.

As a result, the SHC advises that independent comparative studies be conducted to determine the extent to which the infectivity of the WNV and CHIKV persists in platelet concentrates that have been subjected to PRT treatment and are stored for up to 7 days. For any thus validated PRT method, the exclusion period may be shortened and end after the viraemic phase.

In order to inactivate WNV **during the viraemic phase,** PRT methods need to yield a full elimination of **at least 6.5 $\log_{10}$ $TCID_{50}$/mL** of the infectious load, taking into account a safety margin of 3 $\log_{10}$ (see sections 3 and 5). For CHIKV, **at least 10.7 $\log_{10}$ $TCID_{50}$/mL** must be achieved.

A reduction performance of **at least 3.7 $\log_{10}$ $TCID_{50}$/mL** of the infectious load, safety margin included, should reduce the amount of WNV and CHIKV **after the viraemic phase**, *i.e.* from the 12th day following the infection for WNV-strains, and from the 21st day for CHIKV strains.

The SHC takes the view that the validated PRT-methods should **fully** reduce the residual infectious loads and advises that independent comparative studies be conducted to determine the extent to which the infectivity of the WNV and CHIKV strains persists in platelet concentrates that have been subjected to PRT treatment and are stored for up to 7 days.

CONCLUSIONS

The appropriateness of shortening the deferral periods for blood donation following the implementation of pathogen reduction technology on platelet concentrates against the chikungunya and West-Nile strains depends on a significant number of factors. In its assessment, the SHC focused on the following points in particular: the viral load that could be reduced by applying PRT, the duration of (maximum) viraemia in these donors, the proportion of asymptomatic infected donors, the lowest infectious load, the severity of post-transfusion clinical disease, as well as the possibility of achieving an equivalent reduction for any of the other components collected from the same donor or for another pathogen present in the same donor through co-infection.

As there is no validated PRT method for red blood cell concentrates available to date, this advisory report does not apply to concentrates prepared from whole blood. For double-apheresis platelets, the exclusion periods must be shortened in accordance with the most stringent reduction rate for the collected component.

It is unclear what the lowest viral load is that should be considered infective when transfusing platelets, but it may lie below the detection limit achieved with individual nucleic acid testing. So far, no clinical consequences have been described following the transfusion-borne transmission of contemporary CHIKV strains.

The SHC takes the view that the validated PRT-methods should fully reduce the residual infectious loads and advises that independent comparative studies be conducted to determine to what extent the infectivity of the WNV and CHIKV strains persists in platelet concentrates that have been subjected to PRT treatment and are stored for up to 7 days.

In order to inactivate WNV during the viraemic phase, PRT methods should eliminate at least 6.5 $\log_{10}$ $TCID_{50}$/mL of the infectious load, taking into account a safety margin of 3 $\log_{10}$. For CHIKV, at least 10.7 $\log_{10}$ $TCID_{50}$/mL must be achieved. A reduction performance of at least 3.7 $\log_{10}$ $TCID_{50}$/mL of the infectious load (safety margin included) should make it possible to reduce WNV and CHIKV after the viraemic phase.

REFERENCES

- Andonov A. Current relevance of arbovirus infections in transfusion medicine. ISBT Science Series 2010;5:101-6.
- Andrade CC, Maharaj PD, Reisen WK, et al. North American West Nile viral genotype isolates demonstrate differential replicative capacity in response to temperature. J Gen Virol 2011;92:2523-33.
- Añez G, Jiang Z, Heisey DAR, et al. Collaborative study for the characterization of a chikungunya virus RNA reference reagent for use in nucleic acid testing. Vox Sang 2015;109:312-8.
- Angelini MF, Fressy P, Rasonglès P. Epidémie de Chikungunya à la Réunion et transfusion sanguine. Le Bulletin de l'Hemovigilance de l'Afssaps 2006;13:9-10.
- ANSM. Agence nationale de sécurité des médicaments et des produits de santé. Compte-rendu de séance du 18/06/14. Comité technique d'hémovigilance – CT032014033. Saint-Denis: ANSM; 2014. [accessed 2014 Septembre 30]. Available from: http://www.ansm.sante.fr/var/ansm_site/storage/original/application/b12e3aab320d5838e3e908142e6d453d.pdf
- Appassakij H, Khuntikij P, Kemapunmanus M, et al. Viremic profiles in asymptomatic and symptomatic chikungunya fever: a blood transfusion threat? Transfusion 2013;53:2567-74.
- Arellanos-Soto D, Cruz VBI, Mendoza-Tavera N, et al. Constant risk of dengue virus infection by blood transfusion in an endemic area in Mexico. Transf Med 2015;25:122-4.
- Artsob H, Gubler DJ, Enria DA, et al. West Nile Virus in the New World: Trends in the Spread and Proliferation of West Nile Virus in the Western Hemisphere. Zoonoses and Public Health 2009;56:357-69.
- Aytay S, Ohagen A, Busch MP, et al. Development of a sensitive PCR inhibition method to demonstrate HBV nucleic acid inactivation. Transfusion 2004;44:476-84.
- Baba M, Logue CH, Oderinde B, et al. Evidence of arbovirus co-infection in suspected febrile malaria and typhoid patients in Nigeria. J Infect Dev Ctries 2013;7:51-9.
- Bai F, Kong KF, Dai J, et al. A paradoxical role for neutrophils in the pathogenesis of West Nile virus. J Inf Dis 2010;202:1804-12.

- Beltrame A, Angheben A, Bisoffi Z, et al. Imported Chikungunya Infection, Italy. Emerg Infect Dis 2007;13:1264-6.
- Biggerstaff BJ, Petersen LR. Estimated risk of West Nile virus transmission through blood transfusion during an epidemic in Queens, New York City. Transfusion 2002;42:1019-26.
- Borgherini G, Poubeau P, Jossaume A, et al: Persistent arthralgia associated with chikungunya virus: A study of 88 adult patients on Reunion Island. Clin Infect Dis 2008;47:469-75.
- Brouard C, Bernillon P, Quatresous I, et al. Estimated risk of Chikungunya viremic blood donation during an epidemic on Reunion Island in the Indian Ocean, 2005 to 2007. Transfusion 2008;48:1333-41.
- Busch MP, Kleinman SH, Tobler LH, et al. Virus and antibody dynamics in acute West Nile virus infection. J Infect Dis 2008;198:984-93.
- Busch MP, Murthy KK, Kleinman SH, et al. Infectivity in chimpanzees (Pan troglodytes) of plasma collected before HCV RNA detectability by FDA-licensed assays: implications for transfusion safety and HCV infection outcomes. Blood 2012;119:6326-34.
- Busch MP. CHIKV Crash Lands in the Americas in 2014 – Blood Safety Implications and Actions [Présentation de diapositives]. Presented at the 22nd International Workshop on "Surveillance and Screening of Blood Borne Pathogens" of the International Plasma Fractionation Association/Paul-Ehrlich-Institut (IPFA/PEI); 2015 May 20-21; Prague, Czech Republic.
- Campbell GL, Mafin AA, Lanciotti RS, et al. West Nile virus. Lancet Infect Dis 2002;2 :519-29.
- Cancino-Faure B, Fisa R, Riera C, et al. Evidence of meaningful levels of *Trypanosoma cruzi* in platelet concentrates from seropositive blood donors. Transfusion 2015;55:1249-55.
- Carey DE. Chikungunya and dengue: a case of mistaken identity. J Hist Med Allied Sci 1971;26:243-62.
- Caridian. Mirasol Pathogen Reduction Technology: Proven effective against a broad range of disease-causing agents. [accessed 2010 Dec 15]. Available from: http://www.caridianbct.com/location/emea/Documents/306690151A-web.pdf, 2010
- Carletti F, Bordi L, Chiappini R, et al. Rapid Detection and Quantification of Chikungunya Virus by a One-Step Reverse Transcription–Polymerase Chain Reaction Real-Time Assay. Am J Trop Med Hyg 2007;77:521-4.
- Caron M, Paupy C, Grard G, et al. Recent introduction and rapid dissemination of Chikungunya virus and Dengue virus serotype 2 associated with human and mosquito coinfections in Gabon, central Africa. Clin Infect Dis 2012;55:e45-53.
- Cazenave J-P, Rasonglès P, Isola H, K et al. Photochemical Pathogen Inactivation for Preparation of Platelet Components During an Epidemic of Chikungunya Virus. Presented at the Annual Meeting of the American Association of Blood Banks (AABB); 2006 Oct 21-24; Miami Beach, Florida, USA. Available from: http://www.interceptbloodsystem.com/poster_pdfs_2006/AABB-2006_Corash.pdf, 2006
- CDC. Centers for Disease Control and Prevention. Laboratory-acquired West Nile Virus infections – United States, 2002. Morb Mortal Wkly Rep 2002;51:1133-5.
- Cerus. Viruses tested using the INTERCEPT Blood System for platelets. [accessed 2010 Dec 15]. Available from: http://www.interceptbloodsystem.com/resource-center/technical-data-sheets/intercept-blood-system-for-platelets/viruses.html
- Charles PE, Zeller H, Bonnotte B, et al. Imported West Nile Virus Infection in Europe. Emerg Infect Dis 2003;9:750.
- Chernesky MA, Larke RPB. Contrasting effects of rabbit and human platelets on chikungunya virus infectivity. Can J Microbiol 1977;23:1237-44.
- Chiu CY, Bres V, Yu G, et al. Genomic Assays for Identification of Chikungunya Virus in Blood Donors, Puerto Rico, 2014. Emerg Infect Dis 2015;21:1409-13.
- Chusri S, Siripaitoon P, Silpapojakul K, et al. Kinetics of Chikungunya Infections during an outbreak in Southern Thailand, 2008-2009. Am J Trop Med Hyg 2014;90:410-7.

- Cordel H, Quatresous I, Paquet C, et al. Imported cases of chikungunya in metropolitan France, April 2005 - February 2006. Euro Surveill 2006;11:2944.
- Custer B, Kamel H, Kiely NE, et al. Associations between West Nile virus infection and symptoms reported by blood donors identified through nucleic acid test screening. Transfusion 2009;49:278-88.
- Dichtelmüller HO, Biesert L, Fabbrizzi F, et al. Contribution to safety of immunoglobulin and albumin from virus partitioning and inactivation by cold ethanol fractionation: a data collection from Plasma Protein Therapeutics Association member companies. Transfusion 2011;51: 51:1412-30.
- Dodd RY, Foster GA, Stramer SL. Keeping blood transfusion safe from West Nile virus: American Red Cross experience, 2003 to 2012. Transfus Med Rev 2015;29:153-61.
- Domanovic D, Giesecke J. How to define an area where transmission of arthropod-borne disease is occurring?. Euro Surveill 2012;17:20171.
- ECDC. European Centre for Disease Prevention and Control. Chikungunya in Italy. Stockholm: ECDC; 2007.
- ECDC. European Centre for Disease Prevention and Control. West Nile virus infection outbreak in humans in Central Macedonia, Greece: July–August 2010. Stockholm: ECDC; 2010.
- ECDC. European Centre for Disease Prevention and Control. Meeting report: Expert consultation on West Nile virus infection. Stockholm: ECDC; 2011.
- EDQM. Council of Europe. European Directorate for the Quality of Medicines & Health Care. Guide to the preparation, use and quality assurance of blood components. 16th edition. Strasbourg: Council of Europe Publishing; 2011.
- Epstein J. Alternative strategies in assuring blood safety: An Overview. Biologicals 2010;38:31-5.
- Epstein JS, Vostal JG. FDA approach to evaluation of pathogen reduction technology. Transfusion 2003;43:1347-50.
- FAMHP. Federal Agency for Medicines and Health Products. WNV en Hongrie, Italie et Roumanie (complément de la note d 'information du 23/08/2010) [Note d'information]. Brussels: FAMHP; 2010.
- FAMHP. Federal Agency for Medicines and Health Products. Réduction de pathogènes de concentrés plaquettaires. Circular no. 491. Bruxelles: FAMHP; 2009. [accessed 2009 November 20]. Available from: http://www.fagg-afmps.be/fr/binaries/Circ%20491_tcm291-81203.pdf
- FAMHP. Federal Agency for Medicines and Health Products. Total de l'activité des Etablissements de transfusion de Belgique. Brussels: FAMHP; 2012. [accessed 2015 June 26]. Available from: http://www.fagg-afmps.be/fr/binaries/Sang%202004%20-%202011_tcm291-73802.pdf
- FAMHP. Federal Agency for Medicines and Health Products. Hémovigilance en Belgique - Rapport annuel 2013. Brussels: FAMHP; 2015. [accessed 2015 June 26]. Available from: http://www.fagg-afmps.be/fr/binaries/RAPPORT_ANNUEL_%202013_fr_Final_tcm291-267916.pdf
- Farcet MR, Kindermann J, Modrof J, et al. Inactivation of hepatitis A variants during heat treatment (pasteurization) of human serum albumin. Transfusion 2012;52:181-7.
- Farshid M. Evaluation of Viral Clearance Studies [Présentation de diapositives]. In: Update on Implementation of Revised Guidance on Blood Donor Deferrals for Risk of CJD and vCJD. Meeting of the Transmissible Spongiform Encephalopathies Advisory Committee: June 26, 2002. US Food and Drug Administration/Center for Biologics Evaluation and Research. [accessed 2012 Nov 23]. Available from http://www.fda.gov/OHRMS/DOCKETS/ac/02/slides/3868S1_11.ppt
- Farshid M, Taffs RE, Scott D, et al. The clearance of viruses and transmissible spongiform encephalopathy agents from biologicals. Curr Opin Biotechnol 2005;16:561-7.
- Flaujac C, Boukour S, Cramer-Borde E. Platelets and viruses: an ambivalent relationship. Cell Mol Life Sci 2010;67:545-56.

- Friedman LI, Stromberg RR. Viral inactivation and reduction in cellular blood products. Rev Fr Transfus Hemobiol 1993;36:83-91.
- Friedman LI, Stromberg RR, Wagner SJ. Reducing the infectivity of blood components - what we have learned. Immun Invest 1995;24:49-71.
- Gaibani P, Pierro AM, Cavrini F, et al. False-positive Transcription-Mediated Amplification assay detection of West Nile virus in blood from a patient with viremia caused by an Usutu virus infection . J Clin Microbiol 2010;48:3338-9.
- Gallian P, Vignoli C, Dombey AM, et al. Inactivation of a European strain of West Nile virus in single-donor platelet concentrate using the INTERCEPT blood system. Vox Sang 2006;91:345-7.
- Gallian P, de Lamballerie X, Salez N, et al. Prospective detection of Chikungunya virus in blood donors, Caribbean 2014. Blood 2014;123:3679-81.
- Garcia-Tapia D, Loiacono CM, Kleiboeker SB. Replication of West Nile virus in equine peripheral blood mononuclear cells. Vet Immunol Immunopath 2006;110:229-44.
- Gea-Banacloche et al. West Nile Virus: Pathogenesis and Therapeutic Options. Ann Int Med 2004;140:545-53.
- Gérardin P, Barau G, Michault A, et al. Multidisciplinary Prospective Study of Mother-to-Child Chikungunya Virus Infections on the Island of La Réunion. PLoS Med 2008;5(3):e60.
- Gibney KB, Fischer M, Prince HE, et al. Chikungunya fever in the United States: a fifteen year review of cases. Clin Infect Dis 2011;52:e121-6.
- Goodrich RP, Edrich RA, Goodrich LL, et al. The Antiviral and Antibacterial Properties of Riboflavin and Light: Applications To Blood Safety and Transfusion Medicine. Chapter 5. In: Silva E, Edwards AM, editors. Flavins. Photochemistry and Photobiology. Cambridge: RSC Publishing; 2006. p. 83-113.
- Goodrich RP, Custer B, Keil S, et al. Defining “adequate” pathogen reduction performance for transfused blood components. Transfusion 2010;50:1827-37.
- Goss C, Giardina P, Simon MS, et al. Increasing rate of babesiosis in transfused patients at a New York City hospital [abstract]. Transfusion 2012;52(Suppl.3):38A.
- Gould LH, Osman MS, Farnon EC, et al. An outbreak of yellow fever with concurrent chikungunya virus transmission in South Kordofan, Sudan, 2005. Trans R Soc Trop Med Hyg 2008;102:1247-54.
- Gould EA, Gallian P, De Lamballerie X, et al. First cases of autochthonous dengue fever and chikungunya fever in France: from bad dream to reality! Clin Microbiol Infect 2010;16:1702-4.
- Gray EB, Herwaldt BL , Babesiosis National Surveillance: Data Uniformity, Completeness, and Timeliness, 2011–2013. Presented at the Annual Conference of the Council of State and Territorial Epidemiologists (CSTE); 2015 Jun 14-18; Washington, USA. Available from: https://cste.confex.com/cste/2015/webprogram/Paper4847.html
- Halstead SB. Reappearance of Chikungunya, Formerly Called Dengue, in the Americas. Emerg Infect Dis 2015;21:557-61.
- Her Z, Malleret B, Chan M, et al. Active infection of human blood monocytes by Chikungunya virus triggers an innate immune response. J Immunol 2010;184:5903-13.
- Hernández-Triana LM, Jeffries CL, Mansfield KL, et al. Emergence of west nile virus lineage 2 in europe: a review on the introduction and spread of a mosquito-borne disease. Front Public Health 2014;2:271.
- Ho PS, Mary Mah Lee Ng MML, Justin Jang Hann Chu JJH. Establishment of one-step SYBR green-based real time-PCR assay for rapid detection and quantification of chikungunya virus infection. Virol J 2010;7:13.
- Hoarau JJ, Jaffar Bandjee MC, Krejbich Trotot P, et al. Persistent chronic inflammation and infection by Chikungunya arthritogenic alphavirus in spite of a robust host immune response. J Immunol 2010;184:5914-27.

- ICH. International Conference on Harmonization. Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin. Geneva: ICH; 1999. [accessed 2012 September 11]. Available from http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q5A_R1/Step4/Q5A_R1__Guideline.pdf
- INS. Instituto Nacional de Salud. Dirección de Vigilancia y Análisis del Riesgo en Salud Pública. Boletín epidemiológico semanal. Semana epidemiológica número 27 de 2015. Bogota (Colombie): INS; 2015. [accessed 2015 July 23]. Available from http://www.ins.gov.co/boletin-epidemiologico/Boletn%20Epidemiolgico/2015%20Boletin%20epidemiologico%20semana%2027.pdf
- Irsch J, Lin L. Pathogen Inactivation of Platelet and Plasma Blood Components for Transfusion Using the INTERCEPT Blood System™. Transfus Med Hemother 2011;38:19-31.
- Jean CM, Honarmand S, Louie JK, et al. Risk factors for West Nile virus neuroinvasive disease, California, 2005. Emerg Infect Dis 2007;13:1918-20.
- Josseran L, Paquet C, Zehgnoun A, et al. Chikungunya disease outbreak, Reunion Island. Emerg Infect Dis 2006;12:1994-5.
- Jakubik JJ, Vicik SM, Tannatt MM, et al. West Nile Virus inactivation by the solvent/detergent steps of the second and third generation manufacturing processes for B-domain deleted recombinant factor VIII. Haemophilia 2004;10:69-74.
- Jungbauer C, Hourfarb MK, Stiasnyc K, et al. West Nile virus lineage 2 infection in a blood donor from Vienna, Austria, August 2014. J Clin Virol 2015;64:16-9.
- Kalawat U, Sharma KK, Reddy SG. Prevalence of dengue and chickungunya fever and their co-infection. Indian J Pathol Microbiol 2011;54:844-6.
- Kelly S, Te TN, Brown JA, et al. Fatal West Nile virus infection after probable transfusion-associated transmission--Colorado, 2012. MMWR 2013;62:622-4.
- Kilpatrick AM. Globalization, land use, and the invasion of West Nile virus. Science 2011;334:323-7.
- Kraemer MU, Sinka ME, Duda KA, et al. The global distribution of the arbovirus vectors *Aedes aegypti* and *Ae. albopictus*. eLife 2015;10.7554/eLife.08347.
- Kreil TR, Berting A, Kistner O, et al. West Nile virus and the safety of plasma derivatives: verification of high safety margins, and the validity of predictions based on model virus data. Transfusion 2003;43:1023-8.
- Kwon SY, Kim IS, Bae JE, et al. Pathogen inactivation efficacy of Mirasol PRT system and Intercept blood system for non-leucoreduced platelet-rich plasma-derived platelets suspended in plasma. Vox Sang 2014;107:254-60.
- Lai L, Lee TH, Tobler L, et al. Relative distribution of West Nile virus RNA in blood compartments: implications for blood donor nucleic acid amplification technology screening. Transfusion 2011;52:447-54.
- Lanciotti RS, Kerst AJ, Nasci RS, et al. Rapid detection of West Nile virus from human clinical specimens, field-collected mosquitoes, and avian samples by a TaqMan reverse transcriptase-PCR assay. J Clin Microbiol 2000;38:4066-71.
- Lanciotti RS, Kosoy OL, Laven JJ, et al. Chikungunya virus in US travelers returning from India, 2006. Emerg Infect Dis 2007;13:764-7.
- Lanteri MC, O'Brien KM, Purtha WE, et al. Tregs control the development of symptomatic West Nile virus infection in humans and mice. J Clin Invest 2009;119:3266-77.
- Lanteri MC, Lee TH, Wen L, et al. West Nile virus nucleic acid persistence in whole blood months after clearance in plasma: implication for transfusion and transplantation safety. Transfusion 2014;54:3232-41.
- Larke B, Wheelok F. Stabilization of Chikungunya virus infectivity by human blood platelets. J Inf Dis 1970;122:523-31.
- Lee TH, Stromberg RR, Henrard D, et al. Effect of platelet associated virus on assays of HIV-1 in plasma. Science 1993;262:1585-6.

- Lee TH, Stromberg RR, Heitman JW, et al. Distribution of HIV type 1 (HIV-1) in blood components: detection and significance of high levels of HIV-1 associated with platelets. Transfusion 1998;38:580-8.
- Leparc-Goffart I, Nougairede A, Cassadou S, et al. Chikungunya in the Americas. Lancet 2014;383:514.
- Leiby DA. Babesiosis and blood transfusion: flying under the radar. Vox Sang 2006;90:157-65.
- Leydold SM, Farcet MR, Kindermann J, et al. Chikungunya virus and the safety of plasma products. Transfusion 2012;52:2122-30.
- Lieshout-Krikke RW, Zaaijer HL, Prinsze FJ. The yield of temporary exclusion of blood donors, exposed to emerging infections abroad. Vox Sang 2013;104:12-8.
- Lin L, Hanson CV, Alter HJ, et al. Inactivation of viruses in platelet concentrates by photochemical treatment with amotosalen and long-wavelength ultraviolet light. Transfusion 2005;45:580-90.
- Lindsey NP, Staples JE, Lehman JA, et al. Medical risk factors for severe West Nile Virus disease, United States, 2008–2010. Am J Trop Med Hyg 2012;87:179-84.
- Liumbruno GM, Calteri D, Petropulacos K, et al. The Chikungunya epidemic in Italy and its repercussion on the blood system. Blood Transfusion 2008;6:199-210.
- Macedo de Oliveira A, Beecham BD, Montgomery SP, et al. West Nile virus blood transfusion-related infection despite nucleic acid testing. Transfusion 2004;44:1695-9.
- Maillo BM, López-Vélez R, Norman F, et al. Importation of West Nile Virus Infection from Nicaragua to Spain. Emerg Infect Dis 2008;14:1171-3.
- Mavalankar D, Shastri P, Bandyopadhyay T, et al. Increased mortality rate associated with chikungunya epidemic, Ahmedabad, India. Emerg Infect Dis 2008;14:412-5.
- Marschner S, Goodrich R. Pathogen Reduction Technology Treatment of Platelets, Plasma and Whole Blood Using Riboflavin and UV Light. Transfus Med Hemother 2011;38:8-18.
- Mather T, Takeda T, Tassello J, et al. West Nile virus in blood: stability, distribution, and susceptibility to PEN110 inactivation. Transfusion 2003;43:1029-37.
- Mohr H & Redecker-Klein A. Inactivation of pathogens in platelet concentrates by using a two-step procedure. Vox Sang 2003;84:96-104.
- Mohr H, Knüver-Hopf J, Gravemann U, et al. West Nile virus in plasma is highly sensitive to methylene blue-light treatment. Transfusion 2004;44:886-90.
- Mohr H, Gravemann U, Bayer A, et al. Sterilization of platelet concentrates at production scale by irradiation with short-wave ultraviolet light. Transfusion 2009;49:1956-63.
- Mohr H, Steil L, Gravemann U, T et al. A novel approach to pathogen reduction in platelet concentrates using short-wave ultraviolet light. Transfusion 2009b;49:2612-24.
- Moro , et al. Long-term chikungunya infection clinical manifestations after an outbreak in Italy: A prognostic cohort study. J Infect 2012;65:165-72.
- Mostashari F, Bunning ML, Kitsutani PT, et al. Epidemic West Nile encephalitis, New York, 1999: results of a household-based seroepidemiological survey. Lancet 2001;358:261-4.
- Müller TH, Montag T, Seltsam AW. Laboratory Evaluation of the Effectiveness of Pathogen Reduction Procedures for Bacteria. Transfus Med Hemother 2011;38:242-50.
- Murray KO, Walker C, Gould E. The virology, epidemiology, and clinical impact of West Nile virus: a decade of advancements in research since its introduction into the Western Hemisphere. Epidemiol Infect 2011;139:807-17.
- Myers RM, Carey DE. Concurrent isolation from patient of two arboviruses, Chikungunya and dengue type 2. Science 1967;157:1307-8.
- Nett RJ, Kuehnert MJ, Ison MG, et al. Current practices and evaluation of screening solid organ donors for West Nile virus. Transpl Infect Dis 2012;14:268-77.
- Ng LC, Lam S, Teo D. Epidemiology of dengue and chikungunya viruses and their potential impact on the blood supply. ISBT Science Series 2009;4:357-67.

- NHSBT. National Health Services Blood and Transplant. Safe supplies: completing the picture, annual review from the NHSBT and PHE epidemiology unit, 2012. London: NHSBT; 2013. [accessed 2015 June 13]. Available from https://www.gov.uk/government/publications/safe-supplies-annual-review
- Linke S, Ellerbrok H, Niedrig M, et al. Detection of West Nile virus lineages 1 and 2 by real-time PCR. J Virol Methods 2007;146:355-8.
- Odelola HA, Oduye O. West Nile virus infection of adult mice by oral route. Arch Virol 1977; 54:251-3.
- Panning M, Grywna K, van Elsbroeck M, et al. Chikungunya fever in travelers returning to Europe from the Indian Ocean Region, 2006. Emerg Inf Dis 2008;14:416-22.
- Parola P, de Lamballerie X, Jourdan J, et al. Novel chikungunya virus variant in travelers returning from Indian Ocean islands. Emerg Inf Dis 2006;12:1493-9.
- Pealer LN, Marfin AA, Petersen LR, et al. Transmission of West Nile virus through blood transfusion in the United States in 2002. NEJM 2003; 349:1236-45.
- Petersen LR, Busch MP. Transfusion-transmitted arboviruses. Vox Sang 2010;98:495-503.
- Petersen LR, Epstein JS. Chikungunya virus: new risk to transfusion safety in the Americas. Transfusion 2014;54:1911-5.
- Petersen LR, Stramer SL, Powers AM. Chikungunya virus: Possible impact on transfusion medicine. Transfus Med Rev 2010;24:15-21.
- Petersen LR, Brault AC, Nasci RS. West Nile Virus: Review of the Literature. JAMA 2013;310:308-15.
- Pfleiderer C, Blümel J, Schmidt M, et al. West Nile Virus and Blood Product Safety in Germany. J Med Virol 2008;80:557–63.
- Pialoux G, Gaüzère BA, Jauréguiberry S, et al. Chikungunya, an epidemic arbovirosis. Lancet Infect Dis 2007;7:319-27.
- Prick JJ, Kuipers S, Kuipers HD, et al. Opnieuw West-Nijlvirus in Nederland: een man met encefalitis na een reis in Canada. Ned Tijdschr Geneeskd 2003;147:978-80.
- Prowse C. Kills 99% of known germs. Transfusion 2010;50:1636-9.
- Ramful D, Carbonnier M, Pasquet M, et al. Mother-to-Child Transmission of Chikungunya Virus Infection. Pediatr Infect Dis J 2007;26:811-5.
- Rasonglès P, Angelini-Tibert MF, Simon O, et al. Transfusion of platelet components prepared with photochemical pathogen inactivation treatment during Chikungunya virus epidemic in Ile de La Réunion. Transfusion 2009;49:1083-91.
- Raut CG, Rao NM, Sinha DP, et al. Chikungunya, Dengue, and Malaria Co-Infection after Travel to Nigeria, India. Emerg Infect Dis 2015;21:908-9.
- Reiter P. West Nile virus in Europe: understanding the present to gauge the future. Euro Surveill 2010;15:19508.
- Rey FA. Dengue virus: Two hosts, two structures. Nature 2013;497:443-4.
- Reusken CBEM, Bakker J, Reimerink JHJ, et al. Underdiagnosis of Chikungunya Virus Infections in Symptomatic Dutch Travelers Returning From the Indian Ocean Area. J Travel Med 2013;20:44-6.
- Rios M, Daniel S, Chancey C, et al. West Nile virus adheres to human red blood cells in whole blood. Clin Infect Dis 2007;45:181-6.
- Rios M, Daniel S, Dayton AI, et al. In vitro evaluation of the protective role of human antibodies to West Nile virus (WNV) produced during natural WNV infection. J Infect Dis 2008;198:1300-8.
- Robin S, Ramful D, Le Seach F, et al. Neurologic manifestations of pediatric Chikungunya infection. J Child Neurol 2008;23:1028-35.
- Robinson MC. An epidemic of virus disease in Southern Province, Tanganyika Territory, in 1952-53; I. Clinical features. Trans R Soc Trop Med Hyg 1955;49:28-32.
- Rock G. A comparison of methods of pathogen inactivation of FFP. Vox Sang 2011;100:169-78.

- Rossini G, Silvestri AR, Govoni M, et al. Photochemical inactivation of Chikungunya virus using the Mirasol Pathogen Reduction Technology (PRT) System in platelets [poster abstract]. Vox Sang 2011;101 (Suppl 1):185-6.
- Ruane PH, Edrich R, Gampp D, et al. Photochemical inactivation of selected viruses and bacteria in platelet concentrates using riboflavin and light. Transfusion 2004;44:877-85.
- Rudolph KE, Lessler J, Moloney RM, et al. Incubation Periods of Mosquito-Borne Viral Infections: A Systematic Review. Am J Trop Med Hyg 2014;90:882-91.
- Sagripanti J-L, Marschall H-J, Voss L, et al. Photochemical inactivation of alpha- and poxviruses. Photochem Photobiol 2011;87:1369-78.
- Salunkhe V, van der Meer PF, de Korte D, et al. Development of blood transfusion product pathogen reduction treatments: A review of methods, current applications and demands. Transf Apheresis Sci 2015;52:19-34.
- Santhosh SR, Parida MM, Dash PK, et al. Development and evaluation of SYBR Green I-based one-step real-time RT-PCR assay for detection and quantification of Chikungunya virus. J Clin Virol 2007;39:188-93.
- Samuel MA, Diamond MS. Pathogenesis of West Nile Virus infection: a balance between virulence, innate and adaptive immunity, and viral evasion. J Virol 2006;80:9349-60.
- Saswat T, Kumar A, Kumar S, et al. High rates of co-infection of Dengue and Chikungunya virus in Odisha and Maharashtra, India during 2013. Infect Genet Evol 2015;35:134-41.
- Sawyer L, Dupuis K. Inactivation of Chikungunya Virus in Plasma and Platelets Using Helinx™ Technology, as Utilized in the INTERCEPT Blood System™. Presented at the XXIX International Congress of the International Society of Blood Transfusion (ISBT); 2006 Sep 2-7; Cape Town, South Africa. Available from: http://www.interceptbloodsystem.com/poster_pdfs_2006/ISBT-2006_Sawyer.pdf
- Sawyer L, Sampson-Johannes A, Dubensky T, et al. Inactivation of Chikungunya Virus in Plasma and Platelets Using the INTERCEPT Blood System. XVII Regional Congress of the International Society of Blood Transfusion (ISBT); 2007 Jun 23-27; Madrid, Spain. Available from: http://www.interceptbloodsystem.com/poster_pdfs_2007/ISBT-2007_Sawyer_a.pdf
- Sawyer L, Higgs S, Tsetsarkin K, et al. Inactivation of Transfusion-Transmitted Vector Borne Pathogens. XIX Regional Congress of the International Society of Blood Transfusion (ISBT); 2009 Mar 21-25; Cairo, Egypt. Available from: http://www.interceptbloodsystem.com/documents/ISBT_2009_Sawyer.pdf
- Schmidt M, Hourfar MK, Sireis W, et al. Evaluation of the effectiveness of a pathogen inactivation technology against clinically relevant transfusion-transmitted bacterial strains. Transfusion 2015;55:*in press*.
- Seed CR. Risk reduction strategies for transfusion-transmissible arboviral infections. ISBT Sci Series 2014;9:268-75.
- Seltsam A, Müller TH. UVC Irradiation for Pathogen Reduction of Platelet Concentrates and Plasma. Transfus Med Hemother 2011;38:43-54.
- Semenza JC, Menne B. Climate change and infectious diseases in Europe. Lancet Infect Dis 2009;9:365-75.
- Sergon K, Yahaya AA, Brown J, et al. Seroprevalence of Chikungunya virus infection on Grande Comore Island, union of the Comoros, 2005. Am J Trop Med Hyg 2007;76:1189-93.
- SHC. Superior Health Council. Avis concernant l'exclusion (temporaire) des donneurs de sang ayant voyagé à l'étranger (Advisory report on the (temporary) exclusion of blood donors having travelled abroad.) Advisory report no 8307. Brussels: SHC; 2007.
- SHC. Superior Health Council. Risk of Chikungunya virus transmission to the Belgian population through blood transfusion or transplantation. Advisory report no 8201. Brussels: SHC; 2007b.
- SHC. Superior Health Council. La réduction des pathogènes dans les concentrés plaquettaires (Pathogen reduction in platelet concentrates). Advisory report no 8390. Brussels: SHC; 2008.

- SHC. Superior Health Council. Bonnes pratiques de transfusion à l'usage des hôpitaux (Good transfusion practices for hospitals). Advisory report no 8381. Brussels: SHC; 2010.
- SHC. Superior Health Council. Applicabilité des méthodes de détection de la contamination bactérienne dans les concentrés plaquettaires et intérêt pour la sécurité transfusionnelle (Usefulness of bacterial-contamination detection methods in platelet concentrates and significance for transfusion safety). Advisory report no 8670. Brussels: SHC; 2011.
- SHC. Superior Health Council. L'efficacité clinique de la réduction des pathogènes en vue d'une implémentation pour les concentrés plaquettaires (The clinical efficacy of pathogen reduction with a view to applying it to platelet concentrates). Advisory report no 8700. Brussels: SHC; 2011b.
- Simon F, Parola P, Grandadam M, et al. Chikungunya infection: An emerging rheumatism among travelers returned from Indian Ocean islands. Report of 47 cases. Medicine 2007;86:123-37.
- Simon AY, Sutherland MR, Pryzdial ELG. Dengue virus binding and replication by platelets. Blood 2015;126:378-85.
- Simon F, Javelle E, Gasque P. Chikungunya virus infections. NEJM 2015;373:93-4.
- Singh SK, Unni SK. Chikungunya virus: host pathogen interaction. Rev Med Virol 2011;21:78-88.
- Shimasaki N, Kiyohara T, Totsuka A, et al. Inactivation of hepatitis A virus by heat and high hydrostatic pressure: variation among laboratory strains. Vox Sang 2009;96:14-9.
- Southam CM, Moore A. Induced virus infections in man by the Egypt isolates of West Nile virus. Am J Trop Med Hyg 1954;3:19-50.
- Staikowsky F, Talarmin F, Grivard P, S et al. Prospective study of Chikungunya virus acute infection in the Island of La Réunion during the 2005-2006 outbreak. PLoS One 2009;4(10):e7603.
- Staples JE, Breiman RF, Powers A. Chikungunya fever: an epidemiological review of a re-emerging infectious disease. Clin Inf Dis 2009;49:942-8.
- Störmer M, Brachert J, Gathof B, et al. Blood-Transfusion-Bacteria-Standards as a Tool for Development and Validation of Bacteria Screening Methods and Pathogen Reduction Technology in Platelet Concentrates [poster]. XXXI International Congress of the ISBT. Berlin, 26 June - 1 July 2010.
- Stramer SL, Fang CT, Foster GA, et al. West Nile virus among blood donors in the United States, 2003 and 2004. N Engl J Med 2005;353:451-9.
- Sutherland MR, Simon AY, Serrano K, et al. Dengue virus persists in stored platelets and red blood cells. Transfus Med Rev 2014;28:167.
- Tan LK, Lam S, Low SL, et al. Evaluation of Pathogen Reduction Systems to Inactivate Dengue and Chikungunya Viruses in Apheresis Platelets Suspended in Plasma. Advances in Infectious Diseases 2013;3:1-9.
- Tan KK, Sy AK, Tandoc AO, et al. Independent Emergence of the Cosmopolitan Asian Chikungunya Virus, Philippines 2012. Sci Rep 2015;5:12279.
- Terpstra FG, van 't Wout AB, Schuitemaker H, et al. Potential and limitation of UVC irradiation for the inactivation of pathogens in platelet concentrates. Transfusion 2008;48:304-13.
- Tobler LH, Bianco C, Glynn SA, et al. Detection of West Nile virus RNA and antibody in frozen plasma components from a voluntary market withdrawal during the 2002 peak epidemic. Transfusion 2005;45:480-6.
- Tobler LH, Cameron MJ, Lanteri MC, et al. Interferon and interferon-induced chemokine expression is associated with control of acute viremia in West Nile virus-infected blood donors. J Infect Dis 2008;198:979-83.
- Tsen S-WD, Kingsley DH, Kibler K, et al. Pathogen Reduction in Human Plasma Using an Ultrashort Pulsed Laser. PLoS ONE 2014;9(11):e111673.

- Tsetsarkin KA, Sampson-Johannes A, Sawyer L, et al. Photochemical inactivation of chikungunya virus in human apheresis platelet components by amotosalen and UVA light. Am J Trop Med Hyg 2013;88:1163-9.
- Van den Bossche D, Cnops L, Meersman K, et al. Chikungunya virus and West Nile virus infections imported into Belgium, 2007-2012. Epidemiol Infect 2015;143:2227-36.
- van der Meer PF, Dumont LJ, Lozano M, et al. Aggregates in platelet concentrates. Vox Sanguinis 2015;108:96-125.
- Vanlandingham DL, Keil SD, Horne KM, et al. Photochemical inactivation of chikungunya virus in plasma and platelets using the Mirasol pathogen reduction technology system. Transfusion 2013;53:284-90.
- Venter M, Swanepoel R. West Nile virus lineage 2 as a cause of zoonotic neurological disease in humans and horses in southern Africa. Vector Borne Zoonotic Dis 2010;10:659-64.
- Weaver SC, Reisen WK. Present and future arboviral threats. Antiviral Res 2010;85:328-45.
- Weiss D, Carr D, Kellachan J, et al. Clinical findings of West Nile virus infection in hospitalized patients, New York and New Jersey, 2000. Emerg Infect Dis 2001;7:654-8.
- Win MK, Chow A, Dimatatac F, et al. Chikungunya fever in Singapore: acute clinical and laboratory features, and factors associated with persistent arthralgia. J Clin Virol 2010;49:111-4.
- Yap G, Pok K-Y, Lai Y-L, et al. Evaluation of Chikungunya diagnostic assays: differences in sensitivity of serology assays in two independent outbreaks. PLoS Negl Trop Dis 2010;4(7):e753.
- Yomtovian R, Jacobs MR. A prospective bonus of platelet storage additive solutions: a reduction in biofilm formation and improved bacterial detection during platelet storage. Transfusion 2010;50:2295-300.
- Yoon I, Maria Theresa Alera MT, Lago CB, et al. High Rate of subclinical Chikungunya virus infection and association of neutralizing antibody with protection in a prospective cohort in the Philippines. PLoS Negl Trop Dis 2105;9(5):e0003764.
- Zou S, Foster GA, Dodd RY, et al. West Nile fever characteristics among viremic persons identified through blood donor screening. J Infect Dis 2010;202:1354-61.

COMPOSITION OF THE WORKING GROUP

The composition of the Committee and that of the Board as well as the list of experts appointed by Royal Decree are available on the SHC website (composition and mode of operation).

All experts joined the working group ***in a private capacity.*** Their general declarations of interests as well as those of the members of the Committee and the Board can be viewed on the SHC website (site: conflicts of interest).

The following experts were involved in drawing up and endorsing this advisory report. This working group was chaired by Ms Véronique DENEYS, the scientific secretary was Mr Roland HÜBNER.

ARIEN Kevin	virology	ITM Antwerp
BENOIT Yves	paediatric haemato-oncology	UGent
BRUSSELMANS Koen	blood and blood products; molecular and cell biology	IPH
DENEYS Véronique	immunology-haematology; transfusion	Etablissement de transfusion sanguine, UCL Mont-Godinne
DE PAEP Rudi	intensive care	UZA

HÜBNER Roland	molecular and cellular biology; transfusion	SHC
LATINNE Dominique	haematological biology	UCL
LOIX Sébastien	anaesthesiology; intensive care	Hôpital Jolimont La Louvière
PEERLINCK Kathelijne	coagulation and blood vessel disorders	KUL
SELLESLAG Dominik	internal medicine, haematology	AZBrugge
SZABO Bertrand	transfusion	Clinique Reine Astrid Malmédy
THOMAS Isabelle	virology	IPH
VAN DER LINDEN Philippe	anaesthesiology	CHU Brugmann
ZACHEE Pierre	haematology	ZNA

The following experts were heard but did not take part in endorsing the advisory report:

GOUBAU Patrick	virology, travel medicine	UCL
LAMBERMONT Micheline	transfusion	Service du Sang, Croix Rouge de Belgique; ULB

The following representative of the Administration was heard:

MUYLLE Ludo	blood, tissues and cells	FAMHP; UA; UZA