Residual infectious risks in blood transfusion
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Citation for published version (APA):
General introduction
GENERAL PRINCIPLES OF BLOOD SAFETY

Although the risk of transmitting infections via transfusion is very small, transmission still occurs [1,2]. Requirements for transmission through transfusion are the presence of a viable agent in blood or blood components, and the possibility of acquiring infection via parenteral exposure to the agent. The most important tools to reduce the risk of transfusion transmitted infections (TTIs) are a combination of appropriate procedures for donor recruitment, donor education, donor selection and the testing of donated blood and blood components [3], see figure 1.

**Donor selection**

To safeguard the health of both donors and recipients, voluntary unpaid blood donors are preferable. Subsequent donor selection consists of a questionnaire, an interview and a limited physical check. A history of exposure to HIV, viral hepatitis, other blood-borne or sexually transmitted infections and certain endemic infections trigger measures for the safety of blood recipients. Donors who are at risk for a TTI often are temporarily or permanently deferred for donation; sometimes additional testing is initiated.

**Donor and donation screening**

All Western blood donations are routinely screened for the major TTIs. Since 1973 all blood donations are tested for HBsAg in the Netherlands. The implementation of hepatitis B surface antigen (HBsAg) screening reduced the risk of post transfusion hepatitis substantially [4,5]. In 1976 TPHA screening was implemented for syphilis. Before this date syphilis was tested using a rapid plasma reagin (RPR) technique, but the exact date of implementation in the Netherlands is not documented. Since the emergence of HIV in the early 1980s the importance of donor selection and sensitive blood screening has increased. Growing knowledge of the epidemiology and risks of TTIs led to the implementation of additional screening tests, see figure 2. For some TTIs the screening of donations is prescribed by national or international law [6].

In the Netherlands all blood donations are currently tested for human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV) and *Treponema Pallidum* (TP). Routine screening for presence of human T-lymphotropic virus (HTLV) was changed from the screening of all donations to the screening of new donors only in July 2013. The routine screening of blood donations involves the serological testing for anti-HIV 1/2, HBsAg, anti-HBc, anti-HCV, anti-HTLV I/II (new donors only) and anti-TP. Over the years the serological tests improved regarding sensitivity and specificity. The introduction of nucleic acid amplification technology (NAT) for detection of HCV RNA, HIV RNA and HBV DNA further reduced the risk of TTI by detecting infections in an earlier phase [7-9].
Figure 1. Stages of blood safety interventions with yields and limitations regarding the risk of infection.

# Post-transfusion information (PTI), * Post-donation information (PDI), including seroconversions in repeat donors.
Additional selective serological screening includes: the testing for antibodies against malaria of donors with a history of malaria, and of certain donors who travelled to a malaria endemic area; and the screening for parvovirus B19- and CMV-antibodies of blood products available for immunocompromised recipients. Additional plasma pool screening takes place for plasma derived medical products, including the testing for HAV RNA and Parvo-B19 DNA. Plasma used for the production of Solvent Detergent plasma is tested for presence of HEV RNA.

An aliquot of 0.9 ml of each donation is kept in storage at -80°C for a period of 2 years. This sample can be retrieved for additional testing, for example after notification of an infected donor or recipient.

Leukoreduction

Removal of leukocytes from blood or blood components through filtration (with a current efficiency of 99.99%) has several advantages. Pre-storage leukoreduction reduces the risk of non-hemolytic febrile transfusion reactions by lowering the cytokine production, human leukocyte antigen (HLA) alloimmunisation and refractoriness to allogeneic platelet transfusions [10]. Leukoreduction also reduces the transmission risk of intracellular pathogens in leukocytes, such as cytomegalovirus, Epstein-Barr virus, human herpesvirus-8 and HTLV [10-13]. A study of Proctor at al. suggests that *anaplasma phagocytophylum* may be removed during leukoreduction thereby reducing, but not eliminating the likelihood of transfusion transmission [14]. Universal leukoreduction of blood products was introduced in 2001 as a preventive blood safety measure for variant Creutzfeldt-Jacob disease [15]. The European standard for leukodepleted blood components prescribes a residual level below 1x10⁶ leukocytes per unit [16].

Measures against bacterial contamination

The majority of transfusion transmitted bacterial infections (TTBIs) concern bacteria derived from the skin of the donor. The use of the diversion bag (which removes the first aliquot of the donation) and the use of appropriate skin disinfection before blood collection are effective in reducing the rate of bacterial contaminated blood components [17-19]. The use of diversion bags reduced the bacterial contamination rate of pooled 5-donor platelet concentrates from 0.95 to 0.50 %. The new disinfection method (double-swab with isopropyl alcohol) further reduced the rate to 0.37 % [19].

![Figure 2. Introduction of screening tests in the Netherlands.](image-url)
Due to their storage at room temperature platelet components are more sensitive for contamination. The risk of bacterial contamination led to the introduction of bacterial screening of platelet concentrates by aerobic and anaerobic culturing, for example using the BacT/ALERT culturing system (BioMérieux, Boxtel, the Netherlands). Due to their short shelf life, platelet concentrates are released with a "negative to date" approach, with continuation of culturing until 7 days. Positive bacterial screening results in a recall of issued blood components (platelets and other derived blood components) and active surveillance of transfusion reactions in transfused patients [20].

Pathogen reduction
For several blood components pathogen inactivation or reduction techniques are available or being developed. Most of the technologies are designed to kill pathogens or leukocytes with nucleic acids as targets [21]. The robustness of these technologies and their effect on blood components differ significantly. For photo-inactivation, the dose and wavelength determine the extent of loss of blood component functionality. In several countries pathogen reduction has been adopted to reduce bacterial contamination in platelet concentrates and plasma products. However, reduction of non-lipid enveloped viruses and bacterial spores by these techniques is limited [21-24]. For red blood cell concentrates and for whole blood pathogen reduction techniques are not yet available. Solvent Detergent (SD) treatment, filtration, low pH treatment and pasteurization for pathogen inactivation in plasma inactivate lipid-enveloped pathogens, but these techniques cannot be used on cellular blood products [21,25].

Storage
Red blood cell concentrates (RBCC) are stored at 2-6°Celsius which limits the survival of certain pathogens. Experiments have shown that Treponema Pallidum does not survive more than a few days in stored RBCC [26]. The spirochete Borrelia burgdorferi does survive under standard storage conditions, but surprisingly transmission through transfusion of this agent has never been demonstrated. A viability study of Babesia divergens in stored RBCC showed a significant but incomplete decrease of the parasite level during storage at 4° Celsius [27]. Plasmodium species can survive for at least 3 weeks in refrigerated blood [28].

Plasma products are stored for a maximum period of 2 years at a storage temperature of -30°Celsius or lower. For this reason fresh frozen plasma can be quarantined until the donor returns to the blood center and again tests negative for TTIs. This measure effectively prevents transmission via donations in the very early ("window") phase of infection [29].

EMERGING AGENTS
Although the transfusion risk of the major TTIs (HIV,HCV, HBV) declined, the increasing risk of emerging infectious diseases (EIDs) is of concern to blood safety [7]. Not only the
appearance of new outbreaks of EIDs, but also the increased mobility of donors, and the awareness of these infections and insights in risks for blood transfusion attribute to this concern.

**General aspects of emerging infectious diseases**

An emerging infectious disease is an infectious disease that has newly appeared in a population, or that has been known for some time but is rapidly increasing in incidence or geographic range, or threatens to emerge in the near future [30-32]. Emerging infectious diseases include:

- Newly identified disease caused by a previously known, unknown or unrecognized microorganism (e.g. AIDS, vCJD).
- Known diseases found to be caused by a microorganism (peptic ulcer caused by *Helicobacter pylori*).
- Diseases and microorganisms found in new geographic areas (WNV in United States).
- Microorganisms developing resistance to antimicrobial agents (vancomycin-resistant enterococci (VRE), methicillin resistant *Staphylococcus aureus* MRSA).
- Microorganisms of animals that extend their host range to humans (influenza).
- Microbial evolution resulting in a change of virulence or other characteristics (*Escherichia coli* O157:H7).
- Known diseases that have markedly increased in incidence (Ebola hemorrhagic fever).
- Organisms that have been deliberately modified to cause harm (resistant *Bacillus anthracis*).

Socio-economic, environmental and ecological factors are involved in the (re-) emergence of infectious diseases [30,33]. EIDs arise from all classes of agents. EIDs may arise from human activities (outdoor activities and travelling), extensive farming and international transportation. Changes in the local climate, due to heavy rainfall or global warming, optimizing the habitat and thus expansion of vectors, can cause an outbreak. In addition more efficient surveillance and sensitive diagnostic techniques stimulate the reporting of EIDs. New molecular techniques generate data on known and unknown viruses. “New viruses” need careful interpretation, especially in absence of apparent pathogenicity in humans, as illustrated by the erroneous reports on XMRV [34-37].

The major EIDs with relevance for the Dutch blood transfusion service are summarized in table 1.

**Zoonotic infections**

Zoonotic infectious diseases are infectious diseases in animals that can be transmitted to humans via natural ways. Zoonoses can be transmitted from animals to humans via air, contact, a bite, saliva and contaminated water and food. Human pathogens are for 60-70% zoonotic. Zoonotic infections represent at least 60% of all EIDs [33]. Increased
contact between humans and animals, either in wildlife or via extensive or intensive animal farming, is an important factor leading to new zoonotic human infections.

Recently three zoonoses emerged with serious consequences for blood safety in the Netherlands: variant Creutzfeldt-Jakob disease, Q fever and porcine hepatitis E virus:

**Variant Creutzfeldt-Jakob disease**

Since the first cases of variant Creutzfeldt-Jakob disease (vCJD) were reported in 1995/1996 in patients, exposed to the bovine spongiform encephalopathy (BSE) agent, a total of 229 vCJD cases have been reported worldwide (figure 3) [67,68]. The highest number (177 cases) occurred in the United Kingdom, followed by 27 cases in France, 5 in Spain, 4 in Ireland, 4 in the United States, 3 in the Netherlands, 2 cases each in Portugal, Italy and Canada, and 1 case each in Japan, Taiwan and Saudi Arabia (November 2015). In the period 1997 to 2014, 88 BSE cases were found in Dutch cattle with the last case in 2011 [69]. Following active BSE surveillance and control measures in cattle the BSE and vCJD epidemic declined rapidly since 2000.

So far five cases of transmission through blood components have been reported of whom four probable cases (3 clinical infections and a sub- or pre-clinical infection) were associated with non-leukodepleted RBCC. The fifth case was a hemophiliac treated with vCJD implicated plasma derivatives and found to have PrP\textsuperscript{Sc} in his spleen at post-mortem after death unrelated to vCJD [15,46-49]. To date no transmissions of vCJD has been demonstrated through tissue or organ transplantation from infected humans or through contam-

![Figure 3. Number of reported BSE and vCJD cases worldwide (Source: ECDC - TESSy and EC Health and Consumers; http://ecdc.europa.eu/en/healthtopics/Variante Créutzfeldt-Jakob_disease(vCJD)/Pages/factsheet_health_professionals.aspx).](image-url)
Table 1. Emerging infectious diseases with relevance for the Dutch transfusion service.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Agent</th>
<th>Proportion of infected Dutch donors or donations</th>
<th>Asymptomatic course of infection (%)</th>
<th>Chronic infection (%)</th>
<th>Blood-borne transmission demonstrated</th>
<th>Consequences for recipient</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zoonotic infections in the Netherlands</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Hepatitis E (genotype 3)</td>
<td>Non-enveloped RNA hepevirus</td>
<td>≥ 1/1000</td>
<td>≥ 70%</td>
<td>Rare, only in immune suppressed patients</td>
<td>Case reports; Transmission rate of 42% in UK</td>
<td>Varies from asymptomatic or mild self-limiting infection to chronic infection or fatal cirrhosis</td>
<td>38,39,40,41</td>
</tr>
<tr>
<td>Q fever</td>
<td>Intra-cellular gram negative bacterium, Coxiella burnetii</td>
<td>0.3% donations C. burnetii/DNA positive during outbreak</td>
<td>≥ 60%</td>
<td>1-2%</td>
<td>1 case report; 1 possible case during Dutch outbreak</td>
<td>Unknown</td>
<td>42,43,44,45,73</td>
</tr>
<tr>
<td>vCJD</td>
<td>Abnormally folded prion protein (PrP&lt;sup&gt;Sc&lt;/sup&gt;)</td>
<td>Not known</td>
<td>Not known</td>
<td>Not known</td>
<td>5 highly likely UK cases observed</td>
<td>Fatal</td>
<td>46,47,48,49</td>
</tr>
<tr>
<td>Major (vector-borne) EIDs for returning travellers</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chagas disease (T. cruzi)</td>
<td>None</td>
<td>Majority</td>
<td>Low transmission rate, only associated with PLTs or whole blood</td>
<td>Can be severe and life-threatening in acute phase of infection, especially in immunosuppressed patients. Long-term sequelae of chronic infection, such as cardiomyopathy and gastro-intestinal disease</td>
<td>Unknown</td>
<td></td>
<td>50,51,52,53</td>
</tr>
<tr>
<td>Chikungunya Fever</td>
<td>Enveloped RNA alphavirus</td>
<td>Not applicable</td>
<td>15%</td>
<td>Not reported</td>
<td>Theoretically possible</td>
<td>Unknown</td>
<td>54,55</td>
</tr>
<tr>
<td>Crimean-Congo Hemorrhagic Fever</td>
<td>Enveloped RNA nairovirus</td>
<td>Not applicable</td>
<td>Unknown</td>
<td>No</td>
<td>Not reported</td>
<td>Unknown</td>
<td>56,57</td>
</tr>
<tr>
<td>Dengue</td>
<td>Enveloped RNA flavivirus</td>
<td>Not applicable</td>
<td>75%</td>
<td>No</td>
<td>Case reports</td>
<td>Asymptomatic or mild self-limiting infection</td>
<td>58,59,60,61</td>
</tr>
<tr>
<td>Ross River Fever</td>
<td>Enveloped RNA alphavirus</td>
<td>Not applicable</td>
<td>55-75%</td>
<td>No</td>
<td>Possible (one case report)</td>
<td>Unknown, but possibly asymptomatic or mild self-limiting infection</td>
<td>62,63</td>
</tr>
<tr>
<td>West Nile Fever</td>
<td>Enveloped RNA flavivirus</td>
<td>Not applicable</td>
<td>80%</td>
<td>No</td>
<td>Several cases reported in USA</td>
<td>Varies from asymptomatic or mild self-limiting infection to fatal neuro-invasive disease</td>
<td>64</td>
</tr>
<tr>
<td>Zikavirus</td>
<td>Enveloped RNA flavivirus</td>
<td>Not applicable</td>
<td>82%</td>
<td>No</td>
<td>Theoretically possible</td>
<td>Unknown</td>
<td>65,66</td>
</tr>
</tbody>
</table>
inated surgical instruments. However, vigilance is still needed because of the theoretical risk of secondary vCJD transmissions from human to human, for example via blood and blood products, organs and tissues, or via contaminated surgical instruments and medical devices [70]. The finding of the vCJD protein in 1:2000 removed appendices in the UK suggests that a part of the British population carries the agent [71].

**Q fever**

Q fever is a zoonosis with a worldwide distribution. From 2007 through 2009 the Netherlands experienced the world’s largest Q fever outbreak, with more than 3500 notified human cases (figure 4). The sources of infection were dairy goat farms where abortion waves occurred, caused by *Coxiella burnetii*. Humans became infected by inhalation of spore-like form of *Coxiella burnetii* [72]. Transmission through transfusion has been reported once in 1977 [42]. The universal leukoreduction of blood components may reduce the intracellular presence of *Coxiella burnetii*.

A recent Dutch lookback study of the transfused blood components from donors with a confirmed *Coxiella burnetii* infection within 3 weeks before to 3 weeks after blood donation could not demonstrate transmission through transfusion, but in this study a limited number of recipients (11/18) were tested and the recipients lived in the same area as the associated donors. Unfortunately, for one donation tested positive for *Coxiella burnetii* DNA in a repository sample the associated recipient was not tested [45]. A study among Dutch blood donors living in the area with the highest incidence during the Q fever outbreak found that 3 of the 1004 (0.3%) tested donations contained *Coxiella burnetii* DNA.

![Figure 4. Reported human Q fever cases in the Netherlands (1-1-2007 till 16-6-2010) (Source: RIVM; http://qmrawiki.canr.msu.edu/images/Q_fever_pic_1v1.jpg).](http://qmrawiki.canr.msu.edu/images/Q_fever_pic_1v1.jpg)
lookback exercise performed among the recipients of these *Coxiella burnetii* DNA positive donors resulted in one possible transmission, but no final conclusion could be drawn. Antibodies to *Coxiella burnetii* (phase 2 IgG titer ≥ 64) were found in 12.2% donors and 10/66 sample pairs demonstrated seroconversion [73]. It is estimated that 1-2% of infected persons develop chronic infection. A seroprevalence study performed after the Q fever outbreak did not find asymptomatic chronically infected Dutch donors [74].

**Hepatitis E**

Hepatitis E virus (HEV) is the most common cause of acute viral hepatitis among young adults worldwide. Infection with HEV genotype 1 and 2 is restricted to humans and is transmitted through faecally contaminated drinking water in developing countries (Asia, Africa and Mexico). Mortality is highest in children under 2 years and pregnant women (10-25%) [75]. HEV genotypes 3 and 4 cause zoonotic infections in humans. The primary hosts are pigs, deer and other mammals [75]. Infection with HEV genotype 3 and 4 is linked to the consumption of raw or undercooked pork, deer, boar or shellfish. Spread through faecally contaminated water used for irrigation is another possible route of transmission. Pigs are regarded as the main amplifying host of HEV genotype 3. In the Netherlands, at least 70% of the pigs have been infected with HEV which is in line with the high seroprevalence among pigs in other European countries [76-79].

HEV can be transmitted through blood components and organ transplantation [39]. HEV genotype 3 infection in immunocompetent persons is asymptomatic or causes a mild, self-limiting disease. Immune suppressed patients (solid organ transplant recipients and hematological patients) may develop chronic or fulminant hepatitis or may show delayed viral clearance.

Seroprevalence rates for HEV gt3 antibodies in humans in Europe vary between 5 and 52%. A recent Dutch study found a seroprevalence rate of HEV IgG and HEV IgM respectively 27% and 3.5% among blood donors, with an estimated incidence of 1.1% per year [80]. The seroprevalence increases with age. The viremic period is estimated to be 68 days [81]. In the years 2014 and 2015, the average occurrence of HEV RNA positive Dutch donations was 1 in 762 and is increasing over time [81].

**Arthropod-borne viral infections**

Arthropod-borne infections are transmitted to humans through arthropod vectors, such as mosquitoes, sandflies, spiders and ticks. Vector-borne diseases account for almost 30% of all EID events [33]. Arboviruses are a common threat for travelers [82]. Sofar arbovirus outbreaks occur outside the Netherlands and therefore they are of concern donors regarding travelling abroad.

For WNV humans are a dead end host. For dengue-, chikungunya-, and yellow fever virus humans are an amplifying host and can infect arthropods [54]. The majority of dengue and WNV infections in humans is asymptomatic. Hence apparently healthy but infected donors may donate viremic blood. For only a few arboviruses cases of human-to-human transmission through blood transfusions and organ transplantation have been reported [58,64,83,84].
West Nile fever

The first case of WNV was isolated in the West Nile district of Northern Uganda in 1937 and the first outbreak occurred in Israel in 1951. Outbreaks continued to occur sporadically in the Mediterranean basin, European countries, Russia, South-Africa and India [85]. West Nile virus (WNV) was introduced in New York City in 1999, and rapidly spread through the American Continent [86]. The WNV strain detected in New York City originated in the Middle East. In 2002 in the USA 23 WNV transmissions via blood donations from 16 asymptomatic donors were documented. Especially older and immunosuppressed patients are at risk for fatal neuro-invasive WNV disease [64]. Screening of blood donors for WNV RNA was introduced in endemic areas.

Lineage 1 strains of WNV have been known to be present in several parts of Europe, with low level transmission to humans since several decades. In 2010 WNV lineage 2 emerged with large outbreaks in Southeastern Europe [87,88]. Increased viral activity and the introduction of a new strain indicate that the WNV epidemiology changed since 2010. The vector responsible for WNV circulation in Europe is the common house mosquito Culex pipiens [89]. Currently WNV is endemic in Greece, Balkan countries, Northern parts of Italy, and in wetlands with migratory birds (such as Faro in the Algarve in Portugal, the Costa de la Luz in Spain, and the Rhone delta in France) and it seems to spread to new areas (Austria). The cases in Europe peak in August and September. The European Centre of Disease Control (ECDC) publishes weekly updated lists and maps of WNV affected areas during the WNV season in Europe since 2010 (figure 5 and 6).

Dengue

Dengue is one of the most prevalent arthropod-borne diseases in the world, with an estimation of 50 to 100 million new infections per year [59]. Sporadic autochthonous cases, associated with infected travellers or migrants, occur in non-endemic areas where the competent Aedes vector is prevalent [90,91]. Except for a large outbreak in Madeira in 2012 with more than 2,000 cases, cases in Europe are sporadic [92,93]. In the recent years local clusters of transmission, with an imported case as primary source, were reported in Croatia and the South of France [90,91]. Surprisingly, although the burden of disease by dengue is high, only a few transmissions through transfusion have been documented, with less severe clinical manifestations compared to the mosquito transmitted infection [58,60,61].

Chikungunya

Chikungunya, originally circulating in sub-Saharan Africa, expanded in the past decade to India, Southeast Asia and the Indian Ocean. Between 2005 and 2007 Chikungunya virus emerged on Reunion Island. Although evidence was lacking, transmission through transfusion was regarded as highly plausible and a decision was made to interrupt the blood collection except for platelets on a precautionary principle; blood was imported from France [94]. In 2013 an outbreak started in the Americas, which originated on the Caribbean Island of Saint Martin, with a total of 1.6 million suspected cases in a period of 2 years [95,96].
Figure 5. WNV case in European and neighboring countries (2010-2015).
(Source: ECDC – WNV map)

Figure 6. Number of autochthonous human WNV cases on European continent in the period 2010-2015.
In Europe transmission of chikungunya is possible through the Aedes vector. In 2008, a chikungunya epidemic occurred in Ravenna in Italy (229 cases) and small clusters of local transmissions occurred in the South of France in 2010 and 2014 [92,97].

**Zika**

Zika virus is an arbovirus, first identified in the Zika Forest of Uganda (1947). Until 2007 human cases rarely occurred in Africa and South-Asia, but then it started to spread in tropical and subtropical areas where chikungunya and dengue are prevalent [98]. Recent outbreaks of Zika virus include Yap Island (2007), followed by French Polynesia (2013–2014), with further spread to New Caledonia, the Cook Islands, and Easter Island. In May 2015 the first cases appeared in Brazil [65,99-101]. It is expected that the Zikavirus continues to spread through the American continent where the Aedes vector is present. Although transmission via blood transfusion has never been demonstrated this risk cannot be ignored. During an outbreak in French Polynesia Zikavirus was detected with NAT in 42 of 1,505 blood donors (2.8%), who were asymptomatic at the time of blood donation [66]. In the recipients transfused with these viremic blood components no transmission has been found so far.

**RESIDUAL INFECTIOUS RISKS: CAUSES AND COUNTER MEASURES**

With the current multilevel safety strategies the risk of TTI in developed countries is reduced to very low levels. Nevertheless, each safety measure has its limits and residual risks for the blood-borne infections remain. Transmission through blood components still occurs [2,102-106]. Monitoring the risk of TTIs and the effectiveness of the preventive measures remains important for maintaining blood safety.

**Limitation of screening tests**

Screening tests may fail to detect an infectious agent because of various reasons:

- A negative test result may occur during the early stage of infection, the so-called diagnostic window period [102,107].
- A low-level chronic carrier state may exist in which the donor is asymptomatic and persistently testing negative in screening assays [103].
- Genetic viral diversity may cause failure to detect variants of the pathogen, for instance because of a primer mismatch in a NAT assay [108].
- Detection of viremia may be limited when the pool size of tests is too large [109-111].
- In the laboratory human, mechanical and software errors may cause false negative test results.

When a repeat donor is found to be infected, his or her previous donation (which tested negative), may have been an infectious window period donation. A lookback procedure for the potentially infectious donation is performed, by notifying the treating physician, and proposing to test the recipient(s). Identification of infected recipients enables timely
treatment and may prevent further transmission. The lookback period is determined after a risk analysis, taking into account the infectious agent, the stage of infection and the test results of archived donor samples. Several triggers for lookback procedures exist:

- Donor triggered lookback occurs after seroconversion of a repeat donor in routine screening, with previous donations possibly donated in the window period.
- Donors may be found positive in routine screening after the introduction of a new screening test. Previous, untested donations may have been infectious too.
- Infectious donors may be identified through hospital reports of a post-transfusion infection in a recipient. Other blood components of the implicated donation and other donations are potentially at risk to be infectious.
- Phylogenetic analysis, demonstrating a genetic match of the agent in donor and recipient, can help to prove the transmission.

Lookback procedures generate data which can be used to calculate the transmission risk, for example via window period donations, and thus are valuable for the surveillance of the safety of blood.

**Imported infections**

Blood donations are tested for a number of classical infectious diseases, but no testing occurs for most emerging infections abroad, because screening tests are unavailable or considered to be cost ineffective. The level of concern depends on the severity of disease (the clinical consequences in the recipient) and the prevalence of infection among donors. Often it is difficult to decide whether blood safety measures are necessary when a new agent emerges and evidence of transfusion transmission of the agent is still missing. The current trend in blood safety regarding travelling donors and imported EIDs is moving from reactive to a pro-active approach. In response to an outbreak abroad, the following counter measures can be applied:

**Geographic donor deferral**

Donors who travelled to endemic areas, and therefore are at risk of being asymptotically infected after their return, can be deferred temporarily for donation. This approach is preferred over the screening of selected donors, because of the higher costs and the remaining risk of window period donations. A geographically based deferral strategy requires identification of recent travel destinations of donors performed through specific questions. Because the epidemiological situation may vary, the definition of at-risk areas must be kept up to date constantly. This complicates the work of blood bank personnel and it may discourage donors to return to the blood bank. Therefore a universal deferral for at least 4 weeks after each travel outside Europe has been implemented in the Netherlands. Deferring donors has an impact on the donor availability and possibly on donor retention and on the blood supply. Insight in travel behavior (frequency and destination) of the donor population can be used to assess donor availability and deferral policy.
Alternatives to deferral
When too many travelling donors are expected to be deferred, the testing of returning donors instead of deferral must be considered. In addition it must be realized that potentially infectious donors may still be suitable to donate certain blood components, provided that effective inactivation or removal techniques are applied. Fortunately WNV, dengue virus, Chikungunya virus and Zika virus are enveloped viruses and therefore very sensitive to inactivation.

Occult HBV infection
Since the early 70s all donations are screened for HBsAg. The introduction of HBV DNA screening of Dutch blood donors identified several cases of occult chronically HBV infected (OBI) donors. Donations of OBI donors are negative for HBsAg, but positive for HBV DNA, with or without the presence of anti-HBs [103,112]. In OBI donors the anti-HBc is always positive. Detecting OBIs depends on the sensitivity of HBV DNA tests, the level of HBV DNA in OBI donors may be extremely low. The OBI prevalence in Europe ranges between 1:2000 and 1:15,000 [107].

Blood from OBI donations can infect the recipient [102,113,114]. OBI donors with anti-HBs levels below 100 IU/L are more likely to infect recipients [103,115]. The HBV immune status (previously HBV vaccinated or past infection), immune competence and age are important recipient determinants for the chance to become infected [103]. To definitely remove occult HBV infections from the blood supply, anti-HBc screening for all Dutch blood donations was introduced in July 2011. Blood donors testing positive for anti-HBc antibodies, with anti-HBs levels below 200 IU/L, are excluded.

Semi-immune malaria donors
Residents of endemic areas are repeatedly exposed to malaria, and develop an immune defense against malaria. They may carry a low number of malaria parasites without clinical symptoms (“semi-immune”), even years after the last exposure. Donors from endemic countries, with persistence of parasites, are an important source of transfusion transmitted malaria through cellular blood components [28,116].

“Residents of malaria endemic areas” are defined by Sanquin as persons who have lived in a malaria area for a continuous period of 6 months or more at any time in life. These persons are deferred for 3 years after returning from a malaria risk area. The deferral period can be shortened after a negative malaria test at least 4 months after leaving an endemic area. Donors with a known history of malaria infection are deferred for at least 3 years after infection. After the deferral period of 3 years a malaria antibody test should be performed and found negative.

Questionnaires and donor compliance
Donor selection before donation by means of a donor health questionnaire targeting high risk behaviour (e.g. recent high-risk sexual contact or intravenous drug use) is to identify donors who have with a history of or are exposed to a TTI. Deferring these potentially infectious donors, temporarily or permanently, reduces the risk of TTI and is an important
step in blood safety [3]. The efficacy of the questionnaire for reducing the infectious risk relies on the compliance of the donor. Reasons for noncompliance are fear of judgment, social discomfort, test-seeking behaviour, or the genuine perception that one’s blood is safe. Post-test counseling of infected new and repeat Dutch donors showed a noncompliance rate of at least 25%. The most common risk factor not to comply with the DHQ were previous diagnoses with Syphilis, having received a blood transfusion after 1980, male-to-male-sex and intravenous drug use [117].

RISK ASSESSMENTS

Despite major improvements in the blood safety process the risk of acquiring an infection through blood components is not zero. Different models have been developed for calculation of the residual risk for classical (HBV, HCV and HIV) and for emerging infectious agents.

Calculation of residual risk for tested infections
The residual risk of infectious donations entering the blood supply can be calculated using the incidence-window period model. The risk depends on the endemicity in the population (incidence in donors) and donor screening strategy (diagnostic window period) [118,119]. The residual risk (RR) of transfusion-transmitted infection per million donations can be calculated as the product of the incidence rate of infection and the window-period (WP) [RR = incidence rate * WP/365]. For HBV infection an adjustment factor for calculating the risk is needed. In case of screening for HBsAg and HBV DNA, HBV window donations may be missed, even while testing follow-up donations, because of the possibility to clear the infection in between donations, causing an underestimation of the risk [118,119]. It has been estimated that only 42 percent of donors seroconverting for HBV are identified with the HBsAg test. Therefore the observed incidence rate of HBsAg often is multiplied by 1/0.42, or 2.38.

The estimated residual risk in the Netherlands for HBV, HCV and HIV transmission by window period donations, before the introduction of PCR screening, has been calculated to be respectively 5.2; 0.6 and 0.3 transmissions per million donations [120]. The introduction of testing for HBV DNA, HCV RNA and HIV RNA reduced these risks theoretically to 2.8; 0.1 and 0.2 transmissions per million donations [120].

Calculation of residual risk for emerging infections
In the USA, the risk for collecting donations from WNV viremic donors has been estimated by Biggerstaff and Petersen using sero-epidemiologic data from the WNV outbreak was [121,122]. Historical data on course of WNV infection, timing and duration of viremia, number of infected persons during the outbreak and duration of outbreak were used in the calculation. It was estimated that for each WNV meningoencephalitis case there are 140 WNV
infected cases in the population [121]. Assuming that all transfused blood components of asymptomatic WNV viremic blood donors transmit infection to recipients, the mean risk was estimated to be 1.8 per 10,000 donations (95% CI, 1.4-2.2/10,000) during the WNV outbreak in Queens, New York [121].

The European Up-Front Risk Assessment Tool (EUFRAT) is designed to analyse EID outbreaks and to estimate the associated risk for blood recipients. This web-based tool, developed in 2011 for the ECDC, is a ready-to-use application providing stepwise estimates of the risk of receiving contaminated blood in outbreak situations in Europe [123]. For a reliable estimate this needs several input parameters of the specific agent, the course of infection and disease and blood bank information concerning donation patterns and applied blood safety measures. If travel characteristics are known, this tool can also be used to estimate the risk from returning travelling donors.

SCOPE OF THIS THESIS

Blood transfusion in developed countries is extremely safe. A myriad of regulations and safety measures is in place to guarantee this level of safety. The boundary between risks that should be covered by safety measures, and residual risks that are acceptable, is unclear. This study focuses on the yield of safety measures that cover small infectious risks. Possibly the yield of certain safety measures is so limited that the residual risks involved are acceptable. Safety measures that were studied include:

1) When a repeat donor is found to be infected, his or her previous donation may have been an infectious window period donation.
   • What is the risk of window period donations as assessed by the analysis of the lookback procedures among recipients of pre-seroconversion donations? (chapter 2)

2) The screening for HBV DNA resulted in the detection of “HBV DNA positive only” donations. The HBV strains in these donations were sequenced and categorized.
   • What are the categories and characteristics of HBsAg negative and HBV DNA positive healthy blood donors? (chapter 3)

3) The introduction of screening for HBV DNA resulted in the detection of donors with various forms of HBsAg-negative HBV infection. To assess the consequences for recipients an extensive lookback operation was performed.
   • What is the transmission rate of (previous) donations from donors with an “HBV DNA-only” test result? (chapter 4)

4) Transfusion transmitted diseases are more common in new donors as compared to repeat donors. Acute infections occur in donors as a result of incompliance and test-seeking behaviour. Pre-donation testing of new donors may reduce the risk of in-
fectious window period donations. This study describes the incidence of acute HBV, HCV and HIV infection in new donors versus repeat Dutch donors during the period 2009-2013.

• What is the potential value of predonation screening of candidate donors, considering the proportion of incident transfusion-transmissible infections in candidate donors, in first-time donors, and in repeat donors? (chapter 5)

5) Temporarily deferring travelling donors will decrease the risk of infected donations, but the yield of this measure is unclear. Estimation of the risk of infected donors can be performed using the incidence of infection in the travel destination and Dutch tourist travel data.

• What is the yield of donor deferral after travelling abroad, considering the infection risk for donors returning from various affected areas? (chapter 6)

6) Due to an increase of travel and migration of donors, more donors are at risk for infection during their stay abroad. Donors returning from risk areas will be temporarily deferred. Deferring too many donors will jeopardize the blood supply. A balance needs to be found to optimize blood safety and blood supply. Study of the travel behavior of the donor population will give insight in the risk for certain import infections.

• What is the travel behavior of Dutch blood donors, and what are the consequences of different deferral strategies on donor availability? (chapter 7)

7) A tool to estimate the transfusion transmission risk from travelling donors for blood safety in the Netherlands is available, namely the European Upfront Risk Assessment Tool (EUFRAT).

• What is the transfusion transmission risk from Dutch blood donors who travelled to Suriname and the Dutch Caribbean? Are the model estimates comparable with the actual dengue infection risk as measured among Dutch travellers? (chapter 8)
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