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# Virus safety of human blood products – diagnostics and prevention

## Virussicherheit humaner Blutprodukte – Diagnostik und Prävention

**Lutz G. Gürtler***

Friedrich Loeffler Institut für Medizinische Mikrobiologie der Universität Greifswald, Greifswald, Germany

### Abstract

Relevant blood transfusion-transmitted viruses are still HBV, HCV and HIV. As newly emerged infectious agent, the vCJD prion is relevant as well and will cause difficulties within the next decade, since there is no diagnostic tool applicable to blood donation testing. Still, the most effective measure for the virus safety of blood is donor selection followed by antibody and nucleic acid testing, followed by derichment and inactivation performed to the extent possible.

For selected recipients, relevant viruses are CMV and Parvovirus B19. Diagnostic testing for both viruses is no challenge. The prevalence of HTLV-I is very low in Germany but higher in many other countries. Therefore epidemiological surveillance is sufficient to control the spreading of this retrovirus in Germany.

As the West Nile fever virus and the SARS coronavirus demonstrate, old viruses may newly enter the donor population and the efficient way to prevent these viruses from spreading is still the usual quarantine for donors who have visited contaminated areas.

The history of blood transfusion shows that by taking appropriate measures safety will be increased, but also that new infectious agents will enter the donor population in future years.

**Keywords:** blood safety; blood transfusion; vCJD.

### Zusammenfassung

Für die Bluttransfusion weiterhin relevante Viren sind HBV, HCV und HIV. Das vCJD Prion ist als neuer Infektionserreger in jüngster Zeit relevant geworden und wird, da kein für die Blutspende geeigneter diagnostischer Test vorhanden ist, sicherlich noch die nächste Dekade relevant bleiben. Die Sicherheit der Blutspende wird immer noch wesentlich von der Selektion der Spender beeinflusst, zusätzlich von Antikörper- und Nukleinsäure-Tests, gefolgt von Abreicherung und Inaktivierung, soweit dies möglich ist.

Für ausgewählte Patienten sind CMV und Parvovirus B19 relevant, deren diagnostische Erkennung keine Probleme bereitet. HTLV-I hat in Ländern ausserhalb Deutschlands wesentlich höhere Prävalenzen, sodaß derzeit epidemiologische Überwachungsstudien ausreichen, um die Übertragung dieses Virus einzuschränken.

Wie das West Nil Fieber-Virus und das SARS-Coronavirus zeigen, können alte Viren jederzeit in die Spenderpopulation eingebracht werden. Zur Prävention der Übertragung beider Viren durch Spender, die aus Ländern mit erhöhtem Risiko kommen, ist die herkömmliche Quarantäne immer noch gut und ausreichend.

Die Geschichte der Bluttransfusion hat gezeigt, dass durch geeignete Massnahmen die Sicherheit verbessert werden kann und dass vorauszusehen ist, dass neue Infektionserreger in den kommenden Jahren immer wieder in die Spenderpopulation eingeschleppt werden.

**Schlüsselwörter:** Blutsicherheit; Bluttransfusion; vCJD; Virus.

### Blood and blood products

By definition, blood for therapeutic application in humans is either whole blood, which is transfused only exceptionally, or, usually, erythrocyte concentrate, which is leukocyte depleted. Around 10% of the erythrocyte concentrate is still plasma and thus a further differentiation whether a virus is cell-bound or present freely in plasma will not be made in this article.

The majority of other transfused blood products transfused encompass platelet concentrates and, less frequently, granulocyte concentrates. More than 20% of the platelet concentrate is plasma and thus most viruses transmitted by blood are also transmitted by this derivative. The concentration of some viruses such as cytomegalovirus, whose concentration is higher in gran-

*Korrespondenz: Prof. Dr. Lutz G. Gürtler, Friedrich Loeffler Institut für Medizinische Mikrobiologie, Universität Greifswald, Martin Luther Str. 6, 17487 Greifswald, Germany
Tel.: +49 (0)3834-865560
Fax: +49 (0)3834-865561
E-mail: guertler@uni-greifswald.de

ulocytes than in lymphocytes or in plasma, is unimportant for the transfusion safety in this respect.

Plasma, in the form of fresh frozen plasma, is kept in quarantine to reduce the risk of virus transmission and to increase safety. In plasma components, the viral burden is reduced by the fractionation process and a highest possible level of virus safety is reached by the mandatory inactivation process.

### Viruses transmitted by blood

Viruses may be divided in enveloped and non-enveloped species or, depending on their size, in large and small viruses. Very small viruses such as picornavirus and parvovirus pass membranes that are used to remove viruses by filtration more easily. Enveloped viruses are more effectively inactivated by solvent detergent and mostly by heat, thus this kind of differentiation is used in this article [1].

### Enveloped viruses

- Herpes virus: cytomegalovirus (CMV), human herpes virus 6 and 7 (HHV6, HHV7), Epstein Barr virus (EBV), human herpes virus 8 (HHV8).
- Retrovirus: human immunodeficiency virus 1 and 2 (HIV), human T-cell leukaemia virus (HTLV-I).
- Flavivirus: tick-borne encephalitis virus, Dengue fever virus, West Nile fever virus, Japanese encephalitis virus, GB virus C (formerly called hepatitis G virus-which does not cause hepatitis or any known disease).
- Hepacivirus, which is a remote member of flavivirus: hepatitis C virus (HCV).
- Hepadnavirus: hepatitis B virus (HBV).
- Coronavirus: transmission by blood is only possible when the virus is not cleared within one month after the appearance of diarrhoeal symptoms.

### Non-enveloped virus

- Parvovirus B19.
- Picornavirus: most easily transmitted are enteroviruses, among them Coxsackie virus, Echovirus and hepatitis A virus (HAV).
- Calicivirus: hepatitis E virus (HEV).
- Papovavirus with the members papillomavirus and polyomavirus BK and JC.

### Infectious protein – not a virus

- Prion agent-Creutzfeldt Jakob disease (CJD) and variant Creutzfeldt Jakob disease (vCJD).

### Transfusion-relevant viruses

Most of the viruses cited are without any relevance for transfusion medicine, since they are either transmitted in childhood and induce long-lasting immunity, such as coronavirus or polyomavirus, or since they are highly prevalent in the population, such as Epstein Barr virus with a prevalence of $>90\%$, or since, when present, they are quickly eliminated by cross-reacting antibodies, as are most of the enteroviruses.

Only a few viruses remain important for transfusion:

- Transfusion-relevant:
  Hepatitis B virus (HBV)
  Hepatitis C virus (HCV)
  Human immunodeficiency virus (HIV)
- Partially transfusion-relevant for selected patients:
  Cytomegalovirus (CMV) in children and immunosuppressed patients

  Parvovirus B19
- Regionally transfusion-relevant:
  Human T-cell leukaemia virus (HTLV-I)
- Seasonally transfusion-relevant:
  West Nile fever virus
  SARS Coronavirus

**Hepatitis virus** HBV and HCV viruses cause hepatitis and, in chronic disease, hepatocellular carcinoma. The incubation period until clinical onset of hepatitis may last several months [2]. The chronic carrier rate amounts to 10–20% in HBV-infected individuals and to 60–70% in HCV-infected individuals. The virus might be present in blood permanently in high, low or undulating titres so that periodically no virus is detectable since it is incorporated in immune complexes or cell-bound. Some 5% of the German population have been exposed to HBV, and approximately 1% is infected with HCV. HBV replication is hindered by lamivudine and adefovir, HCV replication by ribavirin in combination with pegylated interferon alpha [3, 4].

The main route of HBV transmission is sexual contact, followed by blood contamination through intravenous (IV) drug use, tattooing and close exposure to a chronic carrier. HCV is transmitted mainly by IV drug consumption, further by sexual contact.

**Human immunodeficiency virus** HIV always causes chronic disease with immunodeficiency and opportunistic infections or opens the way for lymphoma and carcinoma. AIDS develops approximately 10 years after infection. In blood, the virus is free in plasma and cell-bound in T-helper lymphocytes and macrophages. There is no period in the life of an HIV-infected patient when the virus is absent from blood [5]. The prevalence of HIV in the German population differs: it may range from less than 0.01% in rural areas to as much as 1% in large cities. Presently, more than 20 drugs are available to reduce HIV replication, however, no combination will lead to the elimination of the virus.

The main route of HIV transmission is sexual contact, thus the virus will not be eliminated from the population without a vaccine; the second frequent route of transmission is intravenous drug consumption [6].

**Cytomegalovirus** Cytomegalovirus (CMV) is a virus that infects many human cells. In blood, lymphocytes, macrophages and granulocytes will be infected. As is typical for all herpes viruses and as known from HIV, CMV always induces chronic infection. CMV leads to severe disease only when a status of immunodeficiency evolves. CMV replication is hindered by nucleotide analogues such as ganciclovir, by foscarnet and cidofovir. The prevalence in the German population depends on the age, i.e. increases with age; in the average, it reaches 50% [7].

The main route of transmission is contact with body fluids, partially by blood transfusion.

**Parvovirus B19** Parvovirus B19 replicates in proerythroblastic cells and can cause severe to lethal anaemia. This non-enveloped virus induces an extended immune response and lifelong immunity. No therapeutic drug is available. The general antibody prevalence in the German population is 70%, the virus is usually acquired in childhood by droplets and close contact to saliva. Subjects who lack the P-antigen in the erythrocyte are not susceptible to parvovirus infection [8].

**Human T-cell leukaemia virus** Like all retroviruses, HTLV-I induces a lifelong chronic infection. After decades, HTLV may induce T-cell leukaemia, spastic paraparesis or myelopathy. HTLV is mainly prevalent in tropical areas, but also in some countries of South and South East Europe. The prevalence in Germany is still so low that screening is done only for epidemiological studies. The main route of HTLV-transmission is sexual contact. HTLV-I is only found cell-bound in T-lymphocytes and not as free virus in plasma. According to current knowledge, HTLV-II induces no disease. There is no drug available to suppress virus production [9].

As long as the prevalence in Germany is as low as it is today, HTLV antibody screening will not be necessary; when the prevalence increases, testing will be mandatory as is already the case in neighbouring countries like for example England, France and Italy.

**Prions** Until today, no case of vCJD disease has been detected in Germany. Two reports from 2004 of vCJD agent transmission by blood are available from England [10–14]. Most susceptible to the accumulation of the prion protein in nerve cells and the subsequent destruction of neuronal cells are subjects with the homozygous codon 129M (M stands for methionine) of the prion gene on chromosome 20, which is found in 40–50% of the German population [10]. The transmission of the scrapie prion protein is possible by the oral route and by application of contaminated material during medical intervention, like dura mater implantation, and by blood transfusion [11, 12].

Autoclaving for 15 min. at 131°C and 3 atm is insufficient to inactivate the infectivity of the scrapie prion protein. Thus there is presently no procedure to inactivate infectivity neither in blood nor in blood products [13, 14].

**West Nile fever virus** West Nile virus (WNV) is transmitted to man by mosquito bite and may therefore be seasonally transmitted in the Northern hemisphere during summertime. It causes flu-like symptoms and finally encephalitis and may be lethal in 1–10% of the cases. Viraemia normally lasts for only 2 to 3 weeks, but occasionally, the virus may circulate in small amounts for 6 to 8 weeks [15]. WNV induces lifelong immunity. A new epidemic spread of WNV occurred in New York in 1999 and WNV has spread since over the North American continent and reached Mexico and some Caribbean countries in 2004.

Blood donors returning from North America from June to November have to pause for one month as a measure of prevention. An antibody test and RNA detection tests (PCR, TMA) are available, but presently not used in Germany since there is no need for them.

WNV has been spreading in Europe for centuries due to the close contact to birds that migrate to Africa in winter. Normally, only animals are affected, humans are affected in rare cases only. Since WNV is closely related to Japanese encephalitis virus and other flaviviruses of this group, these flaviviruses might induce immunity against WNV in Europe.

**SARS coronavirus** This virus is a member of a new coronavirus group which was first identified in 2003 [16]. It is spread mainly by droplets and direct contact to feces, saliva and aerosol. Severe symptoms are diarrhoea and pneumonia, death usually occurs only in the elderly. Viraemia is high for 2 weeks and cleared after 3 weeks, but in some cases it may persist for 6 weeks. The country of origin of SARS coronavirus is still South China.

There is presently no commercial antibody screening test or PCR available, but in several virological laboratories in-house tests work with high quality.

### Diagnostics of virus contamination of blood

A virus consists of proteins which, when liberated in the organism, induces an immune response. This will occur around 4 weeks after virus entry for Parvovirus B19, 6 weeks for HIV and 8 to 12 weeks (16 weeks in exceptional cases) for HBV and HCV. A further viral constituent is nucleic acid (DNA for CMV, HBV and parvovirus and RNA for HCV and HIV), which may be detected by widely used methods such as PCR (polymerase chain reaction), TMA (transcription mediated amplification) or similar methods generally abbreviated as NAT (nucleic acid testing). The big advantage of NAT is its ability to detect the

**Table 1** Variability of the viral load in blood and 1 human infectious dose (HID) suitable for transmitting a virus.

| Virus | Genome | 1 HID for blood | Average concentration in blood per ml | Stability at 20°C |
|---|---|---|---|---|
| HBV | DNA | 30 | $10^{6-9}$ | 3–6 months |
| HCV | RNA | 1.000 | $10^{6-7}$ | 4 weeks |
| HIV | RNA | 500 | $10^{4-5}$ | 1 day |
| CMV | DNA | 1.000 | $10^{2-5}$ | 1 week |
| HTLV | RNA | 1.000 | Cell-bound | A few days |
| Prion | none | 1 IU | 1 to 120 | Years |

presence of virus directly or after concentration, for example by ultracentrifugation, in the specimen. More important is the fact that the diagnostic window period formed by antibody testing is tremendously shortened. Thus, by using NAT as single donation testing, HBV may be detected 33 days, HCV 22 days and HIV 11 days after virus entry. For viral load amounts see Table 1.

**HBV** Mandatory screening is done by the determination of HBsAg, additional voluntary screening by HBV-NAT by the majority of the transfusion services in Germany. In low dose virus carriers, HBsAg and HBV liberation by liver cells might be below the detection limit and thus HBV is transmitted. To prevent this rare event (1:100.000 to 1:500.000), anti HBc screening is performed in several countries and 50% of those escaping the detection by HBsAg and HBV-NAT are eliminated from the donor pool by positivity in anti-HBc testing.

Normally, the eight genotypes of HBV are sufficiently detected by HBsAg and NAT. Nevertheless, there are a few Asian escape variants that cause negative results in sensitive HBsAg tests. Anti-HBc positivity and simultaneously anti-HBs positivity with titers $>100$ IU/l indicate resolved HBV infection, with no risk of transmitting HBV by blood and blood product.

**HCV** Tests available are anti-HCV as a second generation ELISA and HCV-NAT. Both tests are mandatory for HCV screening. Since the introduction of HCV-NAT in Germany in 1998, no transfusion-associated HCV transmission has been reported, indicating that these two tests are a valuable combination for the safety of blood transfusion.

HCV consists of six genotypes. Antibodies against all genotypes are detected with the currently available ELISA. The false positivity rate is usually below 0.4%. All ELISA-reactive specimens have to be checked for specificity in an immunoblot such as RIPA or LIA. Since HCV-NAT is performed in the highly conserved non-coding region of the genome, a failure to amplify this virus has not been observed.

After infection with HCV, antibodies will be detectable lifelong. HCV presence in blood might undulate so that antibody positive and NAT negative is a frequent constellation. This donor might still transmit HCV and therefore has to be permanently excluded from the donor pool.

**HIV** HIV is composed of different viruses such as HIV-1 with the subgroups M, N and O and HIV-2 with the subgroups A to G. In Germany, the most frequent virus is HIV-1 M:B (i.e. subtype B of group M), which was introduced in Germany around 1978. One blood donor infected with group O virus has been identified, the number of HIV-2 infected patients in Germany is around 100, thus the main diagnostic challenge is the detection of HIV-1 group M.

Antibody screening assays in Germany detect all types and groups of HIV. The false positive rate of the ELISA is about 0.2%, therefore, reactive ELISA results have to be confirmed in an immunoblot (Figure 1). Strips with conventionally separated and recombinantly produced antigens are available (Figure 1B). Despite several efforts, interpretation is not standardized. Safe interpretation is performed, when positivity is stated only when the 2 glycoproteins gp120 and gp 41, and another protein from the gag or pol gene are present. Similarly, for HIV-2, both glycoproteins gp105 and gp36 have to be present, and in addition, a further protein from the gag or pol region. Cross-reactivity between HIV-1 and HIV-2 is variable: the most conserved protein is the integrase (p32) followed by the capsid protein (p24), followed by the transmembrane protein (gp41-gp36).

During infection with several agents, immune complexes will be formed. When they are present in serum, they may complex with HIV bands on the strip and lead to mostly faint staining of bands or intensive staining of single bands. The interpretation of such a pattern is indeterminate. The formation of immune complexes is facilitated within acute and chronic infection, during pregnancy, after blood transfusion and transplantation and during attacks of severe allergy. A further reason may be the presence of autoantibody. An indeterminate blot result has to be resolved, either by re-checking in a newly drawn specimen after two to four weeks or by NAT with specific primers for the detection of HIV-1 group M and O and HIV-2, or by using specific immunoblot strips for HIV-1 group M or group O or HIV-2. A part of these assays is not commercially available.

Since, as described in HCV infection, free virus might be absent in plasma depending on the immune status,

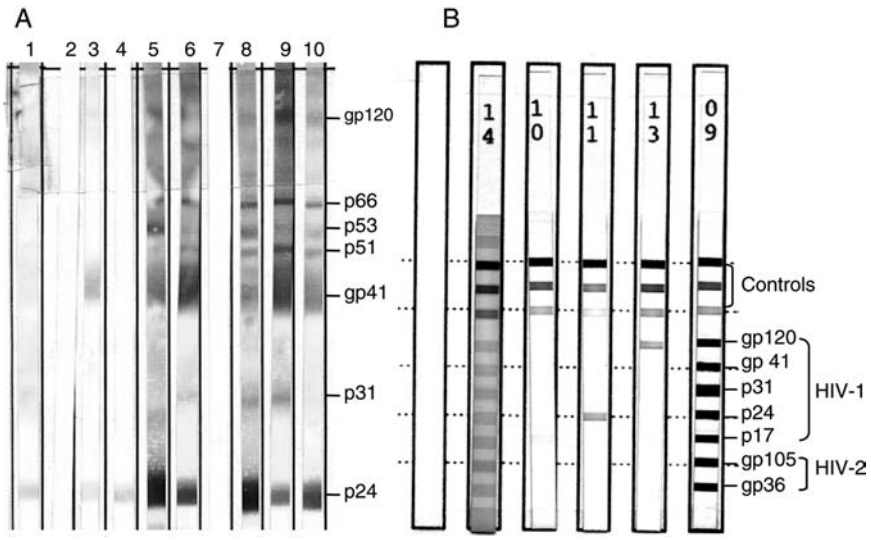


**Figure 1** Typical pattern of the band coloration in immunoblot strips. (A) Left side: Blots strips were loaded with purified virus, strips 1 to 3 are indeterminates, with the coloration of multiple faintly stained bands in strip 1, with no bands in strip 2, with staining of gp120 and gp41 and p24 in strip 3 and only p24 in strip 4. The following strips 5 to 6 and 8 to 10 are true positive, while strip 7 has no visible bands. (B) The right side presents an example of recombinant HIV proteins lined on a strip: strip 09 on the right side is the control, with all bands visible including the two lower ones which represent HIV-2 gp105 and gp36, strip 13 is indeterminate with single coloration of gp120, strip 11 as well with single coloration of p24 and strip 10 shows only the control bands. Strip 14 shows unspecific coloration of all bands due to the presence of immunocomplexes.

HIV-NAT is not a confirmatory assay for antibody screening. Commercially available HIV-NAT for blood donor screening only detects HIV-1 group M and N with sufficient sensitivity, clearly not HIV-2 RNA. Thus there is room for future improvement of HIV diagnostics as well.

HIV-NAT screening of blood donations in Germany has been mandatory since May 2004 and will contribute to reduce the risk of HIV transmsission by blood or blood product to less than 1 in 10 million donations.

**CMV** Antibodies to CMV are detectable by ELISA around four to seven weeks after virus entry. As mentioned earlier, CMV always induces a chronic infection, but frequently without clinical symptoms. The presence of virus in blood may be detected by the pp65 or pp68 antigen assay, mostly by fluorescent screening of infected cells in blood smears, or by NAT. Both methods allow the quantification of the viral burden of a patient, but when the tests are positive, CMV will definitely be transmitted by blood or blood product.

There is no general screening for CMV. The presence of antibody as well as of virus indicates infectivity. Patients who should not receive CMV-positive blood are children, immunosuppressed patients and pregnant women, even though they themselves might be carriers of CMV.

### Parvovirus B19

This virus is of importance only for immunosuppressed individuals and children not previously exposed and for pregnant women. Antibodies are detected by ELISA. The presence of antibody indicates immunity and virus clearing, with the exception of some low-dose carriers (approximately 5–10%) with viral loads in plasma around and below 5.000 copies/ml. This low dose is still sufficient for parvovirus B19 transmission. Usually parvovirus B19 antibodies will neutralize the virus.

Parvovirus B19-NAT is performed routinely in plasma donations for plasma component preparation, since inactivation of this virus is cumbersome. If parvovirus B19-free blood or blood product has to be transfused, both antibody screening and parvovirus B19-NAT in single donor assay have to be performed.

**HTLV** Antibodies to HTLV are detectable six to eight weeks after infection and persist lifelong. Since the virus continues to exist mainly in T-lymphocytes, HTLV-NAT is performed sensitively after enrichment of lymphocytes from blood, as RNA for cytoplasma and as proviral DNA from the cell nucleus.

Antibody screening is generally sufficient to exclude infected donations. ELISA-reactive specimens have to be confirmed in the western blot, with interpretation roughly narrowing that described for HIV.

**vCJD** Until today, no sensitive method has become available for the detection of prion in blood. With the ELISA established in Zurich, 50 MIU/ml (MIU is mouse infectious units) may be detected, while one unit is sufficient for transmitting infection. High titers of prion are only reached in the symptomatic phase of the disease, when a donor would be excluded as well by the specific clinical

signs. Low titer of prion in blood is sufficient for the transmission of this infectious agent. Since the scrapie prion protein acts as a chaperone and converts the natural prion to the protease-resistant form, and thus the converted prion is also recognized by the immune system as self, there are and will be no antibodies produced against the scrapie prion protein. Prion protein is detected by immunohistology in tissues of the brain, tonsils, lymph-node and spleen, a procedure unsuitable for blood donor screening.

The prion as infectious element harbours no nucleic acid and therefore, no NAT can be established to detect the prion. Thus donor selection and exclusion is presently the only effective measure to prevent prion transmission by blood. Leukocyte depletion will only remove half of the prion burden of blood.

**Prevention of drawing blood from infected donor**

**Primary prevention: donor selection and testing, possibly vaccination** Historically, there have been many successful measures to reduce the transmission of infectious agent by blood, such as specific antibody testing, NAT and most effectively, donor selection and exclusion of people at higher risk for acquiring infection than the general population. All procedures act together in such a way that in Germany, the risk of transmitting the relevant viruses is very low-generally less than 1 in 500.000. This low transmission risk justifies the associated financial burden. Outside Europe, even HCV screening has not been implemented in all countries.

The final goal for blood transfusion is zero risk. For HCV and HIV, we are coming closer to this threshold, for HBV, further efforts are needed. One is the introduction of anti-HBc testing, which is discussed, and another is the implementation of HBV vaccination, most rationally combined with the HAV vaccination for all blood and plasma donors. The cost of vaccination is similar to that of annual screening, but the long-term benefit for transfusion recipients and the general population is much higher. Finally, whether HAV/HBV vaccination will be performed is also a political decision, which is pending.

Safety and efficacy trials for HCV-vaccination are presently in phase 3 and results should be awaited for some more years. For HIV, no vaccine produced until now has been effective, thus an HIV vaccine will not be an alternative for decades. CMV will be a problem for the immunosuppressed. Since parvovirus B19 is a non-enveloped virus which induces lifelong immunity, its transmission might be prevented by a vaccine, should this vaccine become effective and available.

**Secondary prevention: leukocyte depletion, inactivation and banning transfusion recipients as donors**
**Leukocyte depletion** Passing blood through filters that absorb leukocytes will remove around 80% of the white cells and thus reduce the risk of infection for CMV and vCJD by approximately 50% [17] and for HTLV by 80%. For the other transfusion-relevant viruses cited, no significant risk reduction is achieved.

Despite this incomplete elimination of virus, the big advantage of leukocyte depletion is the prevention of alloimmunization against several antigens, for example HLA antigens, and therefore, this method will not be withdrawn.

**Inactivation of infectious agents in blood product**
Several components have been tested for decades [18]. Recently, derivatives of psoralene have again been propagated for clinical trials and application, with on the one hand the demonstration of virus inactivation and on the other hand the formation of neoantigens, which may cause side effects. The procedure is limited due to the fact that not all virus is inactivated, like polio virus as an example for enterovirus. Nevertheless, the procedure shows that also in cellular products the inactivation of infectious agent is possible to the benefit of the recipient. To draw a final conclusion, the results of further application studies need to be evaluated.

**Exclusion of transfusion recipients as donors** This measure was introduced in France more than 10 years ago as one method to reduce the risk of HIV transmission. As a way to reduce the risk of vCJD transmission, this procedure was also implemented in England, Switzerland and Canada in 2004. As far as known some 4 to 10% of all donations will be missed when this measure is introduced in Germany. When safety is the highest priority, this procedure might work to reduce the risk of transmitting vCJD or other, not yet identified infectious agent transmitted by blood.

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