Reply. Pereira A. Cryoprecipitate versus commercial fibrinogen concentrate in patients who occasionally require a therapeutic supply of fibrinogen: risk comparison in the case of an emerging transfusion-transmitted infection. Haematologica 2007; 92:846-9.

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Based on a probabilistic model to compare the pathogen safety of cryoprecipitate to a commercial fibrinogen concentrate it was concluded that in patients who occasionally need a therapeutic dose of fibrinogen, commercial fibrinogen would be marginally safer than cryoprecipitate if the new pathogen would be sensitive to inactivation; however, there would be a potential high risk of exposure if the emerging agent withstands inactivation.[1] In most of the analysed scenarios, cryoprecipitation would be safer than commercial fibrinogen, as long as a new agent is resistant to inactivation.

This report needs clarification in order to avoid possible confusion to treaters and patients. The assumed general resistance of a wide range of (non-enveloped) viruses to inactivation is incorrect. Non-enveloped viruses are resistant to solvent/detergent (S/D) treatment; pasteurisation, however, the dedicated virus inactivation step in the manufacturing process of CSL Behring's fibrinogen concentrate Haemocomplettan P, inactivates effectively a wide range of non-enveloped viruses, e.g. picornaviruses as poliovirus and other enteroviruses as well as HAV (hepatitis A virus), B19V [B19 virus (human parvovirus B19)] and caliciviruses [model virus for HEV (hepatitis E virus) as demonstrated in virus validation studies. In contrast to animal parvoviruses, B19V is sensitive to pasteurisation[2] (and unpublished data from CSL Behring). Furthermore, virus removal – not only virus inactivation – contributes significantly to the overall virus safety of the plasma-derived Haemocomplettan P (see Table 1). As demonstrated in virus validation studies, the reduction of potentially present viruses is achieved by pasteurisation (virus inactivation – manufacturing conditions for Haemocomplettan P require heat treatment in aqueous solution at 60°C for 20 hours) but also by the purification process of the desired protein fibrinogen (virus removal due to adsorption and precipitation steps). As these virus validation studies include a wide range of viruses, they provide indirect evidence that the manufacturing process might also inactivate/remove novel or unpredictable virus contamination.[3] According to current knowledge, animal parvoviruses are the most resistant viruses to physico-chemical treatment which should be used in virus validation studies of plasma-derived products (compare)[4]. Therefore, in the risk assessment for new emerging viruses an overall virus reduction capacity for Haemocomplettan P of 10^6 (6 $\log_{10}$), which was achieved for CPV (canine parvovirus) (Table 1), was considered as a minimal virus reduction factor.

The statistical analysis provided in the paper by Pereira[1] covers the risk of exposure rather than risk of infection according to statements in the paper. Nevertheless, a risk assessment regarding the safety of a commercial fibrinogen concentrate compared to cryoprecipitate was performed, as the potential risk of getting infected due to a treatment was discussed and not the potential exposure of a recipient of the treatment to a virus particle, whether infectious or non-infectious. The concluding statement, that "in contrast to what is commonly thought, our results show that cryoprecipitate would be the safer alternative unless the likelihood [*that the emerging agent will be sensitive to inactivation*] was almost certain" contradicts the concept of modelling exposure of patients rather than infection.

As the parameters used in the simulation of the magnitude of exposure to an emerging pathogen were not disclosed, the re-calculation of data is not possible. The probability of an emergence of the "AIDS-like" epidemics over a period of 15 years was assumed to be 1% for a risk assessment covering worst case conditions; however, the incidence of an epidemic should be assumed to be 100% within a defined timeframe. Therefore, in a new risk calculation the following parameters were employed:

- maximum probability of a donor being infected is 100 per 1 million donors [according to Figure 1 (year 4)]
- for cryoprecipitate: lot size 10 donations, 270 ml each/virus reduction capacity 10^1 (cryoprecipitation)

Table 1. Virus reduction capacity [$\log_{10}$] of the manufacturing process of Haemocomplettan P.

	HIV	*BVDV*	*HSV-1*	*HAV*	*CPV*
Cryoprecipitation	n.d.	n.d.	n.d.	2.4	2.8
Adsorptions / precipitation	n.d.	n.d.	n.d.		
Pasteurisation	≥ 5.7	≥ 9.1	≥ 8.1	≥ 4.31	1.72
Precipitation	3.9	2.1	1.0	1.0	1.6
Dialysis / sterile filtration / lyophilisation	n.d.	n.d.	n.d.	n.d.	n.d.
Overall Virus reduction factor	≥ 9.6	≥ 11.2	≥ 9.1	≥ 7.6	6.1

HIV: human immunodeficiency virus; BVDV: bovine diarrhea virus (specific model virus for HCV); HSV-1: herpes simplex virus type 1 (unspecific model virus); HAV hepatitis A virus; CPV: canine parvovirus (model virus for B19V); [1]Pasteurisation inactivates poliovirus by $\geq 10^{7.6}$; [2]Pasteurisation inactivates B19V (human parvovirus B19) by more than $10^{4.0}$; CPV and other animal parvoviruses are known to be resistant to physico-chemical treatment; n.d.=not determined

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- for commercial fibrinogen concentrate: lot size 10,000 donations, 800 ml each (plasma collected by plasmapheresis)/virus reduction factors $\geq 10^6$ (worst case; virus inactivation by pasteurisation limited) and ≥ 10 $\log_{10}$ (base case; effective virus inactivation by pasteurisation)
- potential virus load per ml donation from 10^3 to 10^6 infectious viruses per mL
- calculation of the virus load in a dose of product (commercial fibrinogen concentrate) according to the CPMP guideline[5] as worst case scenario: N = c x V ÷ R, where *N* is the potential number of virus particles per vial of product, *c* is the potential virus concentration in the plasma pool, *V* is the volume of plasma required to produce one vial of product, and *R* is the virus reduction factor:
  - 10,000 plasma donations (~ 800 mL) per pool for commercial fibrinogen concentrate (instead of 30,000 donations (~ 270 mL) from whole blood donations)
  - due to epidemiological situation (1 per 10,000), one donation carrying a potential virus load would be expected to enter a fractionation pool
  - 2,000 therapeutic doses per pool with one dose being prepared from 5 donations (instead of 15 from 30,000 donations)

The considered scenario is focused on the maximum risk encountered in year 4 following the epidemic outbreak. Evidently, the risk assessment for other years of a 15-year observation period would lead to lower exposure and infection risks. It should also be noted that commercial lots may be composed of more than the expected one virus positive donation, though with decreasing probability: 3 positive donations in a pool are likely to occur in 6.1% of manufactured lots, and 5 positive donations in 0.31% of lots manufactured in the peak year 4 following the outbreak.

As demonstrated in Table 2, virus exposure of patients, receiving one therapeutic dose of cryoprecipitate derived from a pool of 10 donations, is low, but all exposed patients are at high risk to be infected: considering 10,000 hypothetical patients per year (150,000 patients in 15 years according to the Brief Report)[1], in average 10 patients would be exposed with a load of infectious virus of 2.7×10^4 to 2.7×10^7 per patient. In contrast, the exposure of patients to virus particles due to therapeutic doses of commercial fibrinogen concentrate - derived from a pool of 10,000 donations resulting in 2,000 doses - is high: based on the calculation, in average 6,321 patients per year may be exposed. However, the load of infectious virus per patient is remote even under worst case conditions..

In order to assess the risk for Haemocomplettan P with regard to new emerging pathogens, the potential epidemiology of new emerging pathogens and the potential virus load in a donor during the incubation period has to be addressed, covering the following parameters: (i) likelihood of emerging viruses in the donor population, (ii) load of virus particles in a donation and (iii) load of infectious viruses in a donation. A virus can emerge either *de novo* by mutation or by crossing a species barrier to enter the human disease chain such as SARS coronavirus (SARS-CoV), menangle virus, hendra virus, or nipah virus or can re-emerge/emerge in new geographic regions as West Nile virus (WNV), Yellow Fever virus, hantaviruses or monkey pox virus. In addition, improved diagnosis may detect *new viruses* e.g., HGV/GBV-C and TTV, both with no known clinical consequences.[6] Emerging viruses in the donor population can not be excluded, but diligent surveillance of available information on new emerging viruses results in (temporal) deferral of donors based on geographic risk, in compliance with regulatory guidance (e.g., WNV, SARS-CoV); therefore, the risk of collecting a donation carrying an emerging virus can be considered to be low, most probably significantly lower than the assumed 1 case in 10,000 donors.

Limited data are available on the virus load of potentially emerging viruses in the donor population. For WNV, the virus load in an asymptomatic donor is in the order of 1 to 5×10^3 genome copies/ml of plasma[7] with an average of less than 100 infectious units per ml blood[8]. For SARS-CoV, a relevant titre in plasma can be

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Table 2. Expected peak exposure to infectious viruses in Year 4 after epidemic outbreak with unknown virus.[1]

	Cryoprecipitate		*Commercial Fibrinogen Concentrate (e.g. Haemocomplettan P)*							
Load infectious virus per ml donation	10^3	10^6	10^3		10^4		10^5		10^6	
Virus reduction factor	10^1	10^1	10^6	10^{10}	10^6	10^{10}	10^6	10^{10}	10^6	10^{10}
Lot size (# of donors)	10	10	10.000	10.000	10.000	10.000	10.000	10.000	10.000	10.000
Volume of donation [ml]	270	270	800	800	800	800	800	800	800	800
Probability of lot contamination [$pL=1-(1-p)^n$]	0,001	0,001	0,63	0,63	0,63	0,63	0,63	0,63	0,63	0,63
Total patients exposed (10,000 treatments)[2]	10	10	6321	6321	6321	6321	6321	6321	6321	6321
Virus load per exposure (CPMP)	2.7×10^4	2.7×10^7	0,0004	4×10^{-8}	0,004	4×10^{-7}	0,04	4×10^{-6}	0,4	4×10^{-5}

[1] *Maximum of epidemic (according to Figure 1)*
[2] *Number of patients exposed to donations collected at maximum of epidemic (10,000 treatments per year)*

excluded as even in a clinical case maximally 10^4 genome copies/mL and in pre-clinical situation a virus titre in the order of 1,000 genomic copies/ml or less could be detected.[9,10] As shown for WNV and the animal parvovirus MVM (minute virus of mice),[11] the ratio of virus particles/genome copies to infectious virus can reach 10,000 to 1 for different viruses and virus populations; these published data are in line with unpublished data from CSL Behring for a wide range of viruses. Therefore, the assumed maximum virus load of around 10^6 infectious virus particles per ml plasma in the risk assessment (Table 2) corresponds to a virus load of at least 10^7 NAT detectable units (virus particles/genomic copies). The assumed maximum load of infectious virus can only be expected in a seronegative donor or an individuum with clinical symptoms. The latter will not be accepted as a donor and seroconversion occurs generally within a short period of time after infection, thus the probability of collecting high-titre plasma from asymptomatic donors in the incubation period of emerging viruses is low.

The potential risk for patients to be infected by emerging viruses was calculated for either cryoprecipitate or commercial fibrinogen concentrate (Haemocomplettan P), taken into consideration worst case scenarios as a prevalence rate for an emerging virus in the donor population of 100 per 1 million, a very high load of infectious virus in a donation and a minimal virus reduction capacity for Haemocomplettan P as demonstrated for animal parvoviruses. Based on these parameters, the conclusion in the paper by Pereira,[1] that cryoprecipitate would be the safer alternative (at least in the case that emerging viruses would not be inactivated) is incorrect as the manufacturing process of Haemocomplettan P has an inherent capacity to remove and inactivate a very wide range of viruses including enveloped and non-enveloped viruses.

Experience from the 1980s, when HIV or HCV were unknown and donors were not deferred or donations interdicted due to unavailability of screening assays, demonstrate that Haemocomplettan P is safe for these viruses as well as for HBV and HAV. No proven case of transmission of these viruses was ever reported. This fact proves that Haemocomplettan P was already virus safe in the case of these "new emerging" viruses.

In conclusion, the treatment of patients with fibrinogen concentrates as Haemocomplettan P is very safe with regard to virus transmission including the transmission of new emerging viruses due to the virus reduction capacity inherent in the manufacturing process of Haemocomplettan P. Based on the risk assessment provided in this letter, Haemocomplettan P is safer than cryoprecipitate under the discussed conditions.

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