Reconstruction of the historic time course of blood-borne virus contamination of clotting factor concentrates, 1974-1992.

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Running title Virus contamination of clotting factor concentrates

Word count: 3476
Tables / Figures: 1 table, 3 figures
References: 50

Contributions: *Study design and conceptualisation*: MM, PMcC, PS, AWT, WLI; TG, HH, JB; *laboratory testing*: PMcC, KK, KR, MXF, PR, SG; *Manuscript preparation*: PS, PMcL, HH; *Manuscript review and editing*: All.

Abstract

Background. Factor VIII and IX clotting factor concentrates manufactured from pooled plasma have been identified as potent sources of virus infection in persons with haemophilia (PWHs) in the 1970s and 1980s. To investigate the range and diversity of viruses over this period, we analysed 24 clotting factor concentrates for several blood-borne viruses.

Methods. Nucleic acid was extracted from 16 commercially produced clotting factors and 8 from non-remunerated donors, preserved in lyophilised form (exp. Dates: 1974-1992). Clotting factors was tested by commercial and in-house quantitative PCRs for blood-borne viruses hepatitis A, B, C and E viruses (HAV, HBV, HCV, HEV), HIV- types 1/ 2, parvoviruses B19V and PARV4, and human pegiviruses types 1 and 2 (HPgV-1,-2).

Results. HCV and HPgV-1 were the most frequently detected viruses (both 14/24 tested) primarily in commercial clotting factors, frequently extremely high viral loads in the late 1970s–1985 and diverse range of genotypes. Detection frequencies sharply declined following introduction of virus inactivation. HIV-1, HBV and HAV were less frequently detected (3/24, 1/24 and 1/24 respectively); none were positive for HEV. Contrastingly, B19V and PARV4 were detected throughout the study period, even after introduction of dry heat treatment, consistent with ongoing documented transmission to PWHs into the early 1990s.

Conclusions. While haemophilia treatment is now largely based on recombinant factor VIII/IX in the UK and elsewhere, the comprehensive screen of historical plasma-derived clotting factors reveals extensive exposure of PWHs to blood-borne viruses throughout 1970s-early 1990s, and the epidemiological and manufacturing parameters that influenced clotting factor contamination.

(250 words)

Keywords:

HIV-1; hepatitis A virus; hepatitis B virus; hepatitis E virus; hepatitis E virus; parvovirus; human pegivirus; hemophilia; clotting factor; Factor VIII; Factor IX

Introduction.

Haemophilia A and B are genetic diseases in which lack of factor VIII (FVIII) or factor IX (FIX) production can lead to severe bleeding disorders. Untreated haemophilia is a disabling and potentially fatal condition, and there has been substantial investment in its medical treatment, starting sequentially from the 1950s with replacement therapy by plasma transfusion, the use of cryoprecipitate that provides FVIII in a more concentrated form and the subsequent introduction of lyophilised FVIII and FIX-enriched preparations from plasma fractionation methods developed in the early 1970s (reviewed in [1]). These have now been progressively replaced by synthetically produced recombinant proteins in developed countries during the 1990s, although plasma-derived concentrates are still primarily used in low- to middle-income countries.

Throughout this period of treatment development, the virus transmission risk from pooled plasma-derived clotting factor concentrates was increasingly recognised [2]. In particular, the practice of generating products from often large pools of plasma derived from multiple donors, appeared to exacerbate the risk of transmission of hepatitis B virus (HBV) infections. Persons with haemophilia (PWHs) were additionally at high risk of developing a chronic hepatitis unrelated to HBV or hepatitis A virus (HAV) infections, termed non-A, non-B hepatitis (NANBH) and subsequently shown to result from infection with hepatitis C virus [3,4]. It was additionally recognised from the early 1980s that PWHs in the USA were at risk for developing AIDS [5], subsequently linked to the appearance of human immunodeficiency type 1 (HIV-1) antibodies from 1979 [6]. HIV-1 infection was widely documented in PWHs in other Western countries, particularly among users of US-sourced factor VIII and IX concentrates [7]. Plasma-derived clotting factors may additionally contain and transmit a range of other blood-borne viruses, including parvovirus B19V, the distantly related parvovirus, PARV4, hepatitis A virus (HAV) and human pegiviruses (HPgVs) [8-12]. The contribution of the latter viruses to blood product safety is not well defined. B19V and HAV infections occur widely in the community with respiratory and enteric routes of transmission and are typically mild or non-pathogenic resolving infections in immunocompetent individuals. HPgV type 1 (HPgV-1) is similarly widely distributed with measurable frequencies of active viraemia from persistent infections in blood donors and the wider populations without known disease associations [13]. PARV4 and HPgV-2 infections are much less common in donors and have been detected primarily in association with injecting use and concurrent HCV infection; whether they exacerbate hepatitis or cause other systemic disease is unknown [9,14,15].

The discovery of widespread HIV-1 infection in PWHs in the early 1980s led to urgent measures to prevent further transmission via blood products. Virus inactivation and removal methods were adopted by manufacturers from 1984-1995 [16], including the use of solvent/detergent treatment (largely effective only against enveloped viruses) through to extreme dry heat (≥90C) and from the 1990s, viral exclusion methods such as nanofiltration and affinity purification of FVIII/FIX with monoclonal antibodies. The use of viral inactivation methods was highly effective against HIV and HCV although instances of B19V, HAV and PARV4 transmission continued to occur [9-11], reflecting their thermal stability. Donor selection was enhanced to defer donors with risk factors and symptoms of HIV infection / AIDS, followed by introduction of universal anti-HIV screening in Western countries in 1985 and the subsequent development of direct virus detection methods for p24 antigen and viral nucleic acids of HIV-1 and HIV-2 for plasma (and blood) donors.

In the current study we have assembled a large collection of unused FVIII and FIX clotting factor concentrates with expiry dates spanning the period from 1974 to 1992. Preserved in lyophilised form, they represent a “time capsule” that provides a unique record of the range blood-borne viruses circulating in the donor population in the 1970s and 1980s and potential contributory factors to transmission risk to PWHs. These include viral loads, and effects on virus detection following introduction of virus inactivation methods and donor screening and selection policies.

Materials and Methods

Clotting factor concentrates. Archived concentrates were purchased for therapeutic use and stored at +4C to -20C since manufacturer delivery, and reconstituted immediately prior to testing. Expiry dates ranged from 1974 to 1992 (Table 1). Information on manufacturer and lot number was recorded; commercially manufactured clotting factors were assumed to derive from remunerated donors; those from the UK Blood Products Ltd and the French blood service were from non-remunerated donors. FVIII and FIX ampoules were reconstituted in the indicated therapeutic volume using dH_2O.

Nucleic acid extraction. Total nucleic acid was extracted from 1400µl of resuspended clotting factor, using proportional volumes of buffers from the QIAamp Viral RNA kit (QIAGEN) and columns from High Pure Viral Nucleic Acid Large Volume Kit (Roche). Nucleic acid was eluted in 60µl of RNase free water and stored at -70°C for subsequent use.

Real-time PCR screening. HCV and HIV-1 RNA sequences were detected by calibrated real time quantitative polymerase chain reactions (RT-qPCRs; Abbott Alinity M, 700 µl test volume) and Micropathology Ltd laboratories (Roche Cobas 5800, 500 µl test volume). HBV was detected and quantified using 5 µl extracted nucleic acid [17]. HEV RNA was detected by RT-qPCR assay as previously described [18] primers modified by a 5' flap region [19] and alternative 5'-reporter and 3'-quencher dyes (MAF, TAMRA). Viral load measurements were calibrated to IU/ml using external standards from the National Institute for Biological Standards and Control.

HAV RNA as previously described [20] but without multiplexing for B19V. In-house assays for HPgV-1/-2 RNA sequences and for PARV4 and B19V DNA were used to detect and provide a relative quantitation of viral loads in the absence of external standards. cDNA template for RNA PCR was generated by random hexamer primed synthesis using 20µl of extracted nucleic acid to reconstitute lyophilised RNA to cDNA (EcoDry Premix; Takara Bio) without template dilution. Relative viral loads (RVLs) were calculated based on an assumption that Ct values of (<)45 (assay sensitivity limit) contained 1 or fewer copies in the reaction. RVLs were calculated for samples with lower Ct values (ObsCt) using the formula $2^{(45\text{-}ObsCt)}$.

HPgV-1, PARV4 and B19V sequences were amplified by newly designed RT PCR assays using conserved sense and antisense primers. Assays used either specific probe hybridisation or SYBR Green detection of the amplified product (Table S1; Suppl. Data). 1µl of cDNA template was used for both RNA and DNA viruses in a 15µl real time reaction with 7.5µl of 2x qPCRBIO SyGreen Blue Mix (PCRBIO) and 400 nM each primer. Reactions were run at 95°C/2 minutes, 40 cycles of 95°C/5 seconds and 60°C/30 seconds with fluorescence detection, followed by a melt curve generation between 70 and 90°C.

HCV genotype and HIV-1 subtype analysis. RNA from samples positive for HCV RNA was amplified in the core region as previously described [21]. A new inner antisense primer Table S1; Suppl. Data) was designed to better accommodate polymorphisms between genotypes. Amplified DNA from the 2nd round PCR was directly sequenced by Illumina (GeneWiz) next generation sequencing to generate approximately 100,000 paired end reads per sample. Genotype and subtype assignments were determined using a bespoke pipeline (Kraken2 [22]), followed by trimming to remove adapters and low-quality reads (Trimmomatic). Reads were then mapped to a collection of 140 HCV reference sequences[1] (BWA-Mem2) and statistics were collected for reads aggregated to each mapped reference (Samtools). All data analysis, scripting and plotting was conducted using Python 3.10.

[1] https://ictv.global/sg_wiki/flaviviridae/hepacivirus/table1

HIV-1 was amplified from RT-qPCR screen positive samples using nested primers in the p17*gag* (Table S1; Suppl. Data) and sequenced by the Sanger method (Source BioScience).

Results

Detection of HIV-1 and hepatitis viruses in clotting factors. We first investigated the degree of contamination of plasma-derived clotting factors used for haemophilia treatment between the early 1970s and into the 1990s for currently screened blood-borne viruses (HCV, HIV-1, HBV and HEV). Samples comprised 16 commercial preparations of factor VIII or IX and eight UK- or French-origin FVIII preparations; although dates of collection of plasma used for the clotting factors were not available, we were able to record their expiry dates, providing an approximation to the time course of plasma collection perhaps displaced by 1-2 years.

Extracted RNA/DNA was assayed by standardised quantitative RT-qPCRs (Roche Cobas 5800 and Abbott Alinity) for HCV and HIV1/2 RNA sequences (Fig. 1). All (n = 12) commercial clotting factors with expiry dates between 1976-1990 were HCV RNA-positive by qPCR while those with expiry dates after 1990 and which would have been universally virally inactivated by solvent/detergent (SD) / wet heat or by dry heat were negative (n = 4) as was the FVIII preparation with an expiry date before 1976. Clotting factors with expiry dates 1974-1981 and likely used from the later 1970s and early 1980s showed systematically extremely high viral loads (10^4 – 10^5 IUs/ml) and were therefore potentially highly infectious (see Discussion). Only one clotting factor from non-remunerated donors was HCV positive (expiry date 1984), although the limited sampling possible (7/8 had expiry dates in the early 1970s or after virus inactivation from 1986) prevented a comparison of their virus contamination with that of commercial products.

HIV was much less frequently detected, with low/medium levels in three commercial clotting factors with expiry dates before 1984 (range <20 to 15,400 IUs/ml) and therefore collected before the discovery of HIV-1 and introduction of donor screening. All non-commercial clotting factors were negative although the limited sampling prevented a full comparison with commercial products. Of the three positive samples, only S49 with the higher viral load could be amplified using nested primers in the *gag* gene region for genetic characterisation. The HIV-1 variant was of subtype B and showed no polymorphic sites, consistent with contamination from a single donor (Table S2). The closest matched RNA-derived HIV-1 sequence on GenBank was HIV-1 strain SF20 amplified from a serum sample from a male homosexual in California, USA archived in 1978 (accession number KJ704794) [23] with 99% sequence identity.

All samples were screened using a recently described ultrasensitive real-time PCR for HBV DNA sequences [17]. One sample (S74; Factorate, Exp. Date: May, 1981, HCV-positive, HIV-1/2 negative; Appendix I) was reactive with a viral load of 20 IU/mL (and 29 IU/mL and 31 IU/mL on repeat replicate testing), with all others negative (assay sensitivity [LD_{95}] of 10 IU/ml). All samples were further tested for HEV RNA by in-house RT-qPCR and were negative. HAV was detected in one commercial concentrate (Kryoglobulin; expiry date 1983).

Detection of other blood-borne viruses. Four further viruses associated with acute or persistent viraemia on infection were screened by semi-quantitative PCR (Fig. 2). HPgV-1 sequences were detected in all batches of commercial clotting factor before 1989, with batches from the earlier expiry dates also showing relatively high viral loads that contrast with the lower or undetectable HCV viral loads in these four samples. However, similarly for HCV, all samples with expiry dates after 1990 were PCR-negative, potentially the result of the introduction of virus inactivation methods leading to degradation of viral RNA. Comparable results were observed from clotting factor manufactured from non-remunerated donors.

However, the samples collected in the very early 1970s from small donor pools were negative for HPgV-1 , as were all samples for HPgV-2.

PARV4 was less frequently detected (4/15) in commercial clotting factors and 0/8 in UK/French products. Contrastingly, B19V was extremely frequently detected in commercially prepared clotting factors (13/16) but less frequently in UK/French products (2/8). Viral loads showed no temporal trend, consistent with its primarily respiratory route of transmission and consequent lack of association with risk factors for HIV-1 and HCV infection. In contrast to HPgV-1 detection, B19V was frequently detected at moderate viral loads in products with expiry dates beyond the adoption of potent virus inactivation methods (see Discussion).

HCV genotypes detected in clotting factors. HCV core gene sequences were amplified by nested PCR from a selection of HCV-positive clotting factors with expiry dates ranging from 1976-1985 (Fig. 3). PCR used the original core region primers [21], and a modified assay with a more conserved inner antisense primer (see Methods). The identification of HCV genotypes is complicated by the likelihood of multiple infected donors contaminating the same batch of clotting factor, and therefore the amplicon product was analysed through Illumina sequencing and paired end reads. Each PCR product yielded approximately 100,000 reads which were assigned to different genotypes through comparison to a reference dataset of all currently assigned HCV genotypes and subtypes using our bespoke pipeline (see Methods; Fig. 3). Distributions of HCV genotypes were comparable between the original and modified primers (Table S3, Suppl. Data); with similar levels of genetic diversity recorded by each.

As expected, there was substantial genetic diversity of HCV strains in the 6 clotting factors, with genotypes 1a, 1b, 2a, 2b and 3a represented. The most commonly observed genotype was 2b, but with frequent representation of 1a and 1b and less commonly 3a. There were no evident associations between diversity and viral loads and no clear temporal trend in genotype representation with the samples available for analysis.

Discussion.

The study demonstrated highly variable frequencies and viral loads of a range of blood-borne viruses that contaminated plasma-derived blood products used to treat haemophilia until their replacement in the mid-1990s by recombinant FVII and FIX proteins. Findings of frequent detection of HCV, often at viral loads corresponding to those found in plasma of an HCV-viraemic individual, are consistent with high detection rates of HCV RNA in previous analyses of clotting factor concentrates manufactured before the introduction of virus inactivation measures in the late 1980s [24-26]. The detection of HCV RNA in clotting factors with expiry dates in the 1970s, albeit at 10-100-fold lower viral loads than those in the mid-1980s, matches clinical observations for high frequencies of NANBH in PWHs treated with pooled products [27,28].

High viral loads were detected in clotting factors throughout the period from 1976 – 1985 in the range 10^4 – 10^5 IU/ml (Fig. 1A). Levels are not dissimilar from viraemia levels in HCV-infected individuals and imply high frequencies of active HCV infection in donors for commercial clotting factors. This is consistent with a retrospective study that reported a 10% anti-HCV seroprevalence in paid plasmapheresis donors for a commercial manufacturer in the USA [29], and the previously described partitioning and concentration of most HCV virions from source plasma into cryoprecipitate used to manufacture FVIII concentrate [30]. Clotting factors would likely possess an extraordinarily high infectious load for HCV, with 20 – 50 ml volumes repeatedly transfused to a PWH during a bleeding episode. HCV can however be readily transmitted from needlestick accidents or shared needles used for injecting drug use and tattooing with blood volumes of as little as a few microlitres. It can therefore be assumed that treated PWHs would have

been repeatedly exposed to a range of HCV genotypes over the period before virus inactivation of factor VIII and IX.

We used Illumina NGS to quantify the relative frequencies of different HCV genotypes in the clotting factors used in the 1970s and 1980s in amplicons from the conserved core genome region. This confirmed the presence of a variety of HCV genotypes in each product, representing a multiplicity of infected donors contributing to each batch of clotting factor analysed. These findings are consistent with the same wide diversity of HCV genotypes infecting PWHs in England and Scotland, with genotypes 1a, 1b, 2a, 2b and 3a almost exclusively recorded [31,32] , and a high frequency of mixed infections and frequent changes in genotype in longitudinal studies consistent with multiple infection episodes [32,33].

Sporadic detection of HIV-1 in clotting factors [34] was similarly consistent with the infrequent detection and low viral loads of HIV-1 in clotting factors with expiry dates between 1983-1985 in the current study and likely use in the 1-2 preceding years. These correspond to the period between 1979-1985 in which the majority of HIV-1 seroconversions of PWHs in the UK treated with imported FVIII or FIX occurred [35]. We found a close genetic relationship between the HIV-1 strain from a commercial cryoprecipitate prep (S49; exp. date in 1984) with the SF20 strain recovered from a very early archived serum sample from a male homosexual in California in 1978 [23]. This is a member of the very early lineages that initially circulated in San Francisco prior to the subsequent AIDS pandemic and consistent with suspected origins of HIV-1 infecting PWHs around that time.

The degree of virus contamination of plasma-derived products is potentially influenced by several distinct variables. These include the frequency of infection in the donor population and the duration and level of viraemia following infection. The ability of HCV to establish persistent infection in the majority of those infected with ongoing high level viraemia is thus likely to represent the primary factor in the extremely high rates of detection in the clotting factors. Widespread distribution and a long-term persistence rate of around 20-25% in those infected with HPgV-1 as adults is similarly consistent with its high rate of detection in this and previous studies [36,37]. These findings contrast with the complete absence of clotting factors positive for the closely related HPgV-2 but are however consistent with likely extreme rarity of HPgV-2 infections even in HCV-infected people who inject drugs (PWIDs) and apparent infrequent persistence of infections [38,39].

Detection frequencies were however also relatively high for viruses such as B19V and PARV4 associated with acute resolving infections and relatively short durations of viraemia. In the case of B19V, the relatively high population incidence (with 30-50% of donors typically with serological evidence for past infection) combined with extremely high acute viraemia levels and the large pool sizes used to make clotting factors in the 1980s undoubtedly contributed to the near universal contamination of clotting factors throughout the study period. Viraemia levels in acute PARV4 infections are less clearly delineated, but its almost exclusive association with PWIDs in Western countries and low incidence of infection in the general population may have contributed to its less frequent detection (5/18 pre-1986, consistent with [40,41]) and lower viral loads compared to B19V. Its apparent disappearance in clotting factors with expiry dates after 1986 when B19V contamination continued (despite the introduction of virus inactivation methods) suggests that other measures, such as screening for HIV-1 and implementation of enhanced donor selection to exclude those with known risk factors for blood-borne virus infections, may have contributed to the observed reduction in PARV4 (and HCV).

The infrequent detection of HBV DNA in the study samples (1 from 24) using a highly sensitive PCR [17] matches previously published findings of uniform negativity of clotting factors with expiry dates before 1990 in a study using a likely less sensitive PCR assay [42]. It was commented at the time that these negative

results were inconsistent with remarkably high frequencies of past exposure to HBV in PWHs, with anti-hepatitis B core antibodies (anti-HBc) reported in >80% receiving non-virally inactivated concentrate [42,43], substantially higher than the background population anti-HBc seroprevalence in Western countries of typically <3%. However, frequent transmission of HBV from clotting factors manufactured from HBsAg-negative-screened plasma has been described [43,44], potentially the outcome of including donors with occult HBV infections with undetectable HBsAg and low levels of infectious HBV particles [45]. Detection frequencies of other human hepatitis viruses, HAV and HEV were low, with only one batch of cryoglobulin from 1983 positive by PCR for HAV RNA. The absence of detectable HEV is consistent with the absence of clear evidence of higher rate of past exposure to PWHs receiving non-virally inactivated clotting factors in retrospective sero-epidemiological surveys [46,47]. HAV transmission been reported from several centres associated with the use of solvent detergent inactivated factor VIII between 1989-1992 (reviewed in [48]), but without unequivocal evidence for increased seroprevalence in PWHs [49,50].

As an analysis of the relationship between clotting factor contamination and infection of PWHs treated in the 1970s – 1980s, the study has limitations, including a lack of numerical power to analyse the separate contributions of a large number of possible variables influencing blood product infectivity (*eg*. virus epidemiology in the donor population, degree of persistence in donors and PWHs, resistance to virus inactivation and donor selection). Secondly, while expiry dates of the individual clotting factors were recorded, these do not have a fixed temporal relationship with manufacture or donation time, preventing precise matching of factor VIII/IX contamination with infection of PWHs. Nevertheless, the study does record the extraordinary diversity and frequent high viral loads of a wide range of blood-borne viruses that PWHs were exposed to from their therapy over a prolonged period.

The combined analyses of epidemiologically and physically distinct viruses provides a valuable framework to compare effects of interventions, such as virus inactivation and more effective donor screening on viral loads and likely infectivity. Future investigations will use agnostic metagenomic next generation sequencing (NGS) methods to expand the analysis of the range and genetic diversity of viruses in the clotting factors and further and further characterise virus exposure in this patient group who have been historically sadly affected by this issue.

Funding. National Institutes for Health Research (NIHR) grant code NIHR203338.

Conflict of interest: MM has received honoraria for lecturing, grant reviewing and advisory committee participation from NovoNordisk, Takeda, Grifols and Sanofi. The other authors declare no conflict of interest with publishing this manuscript.

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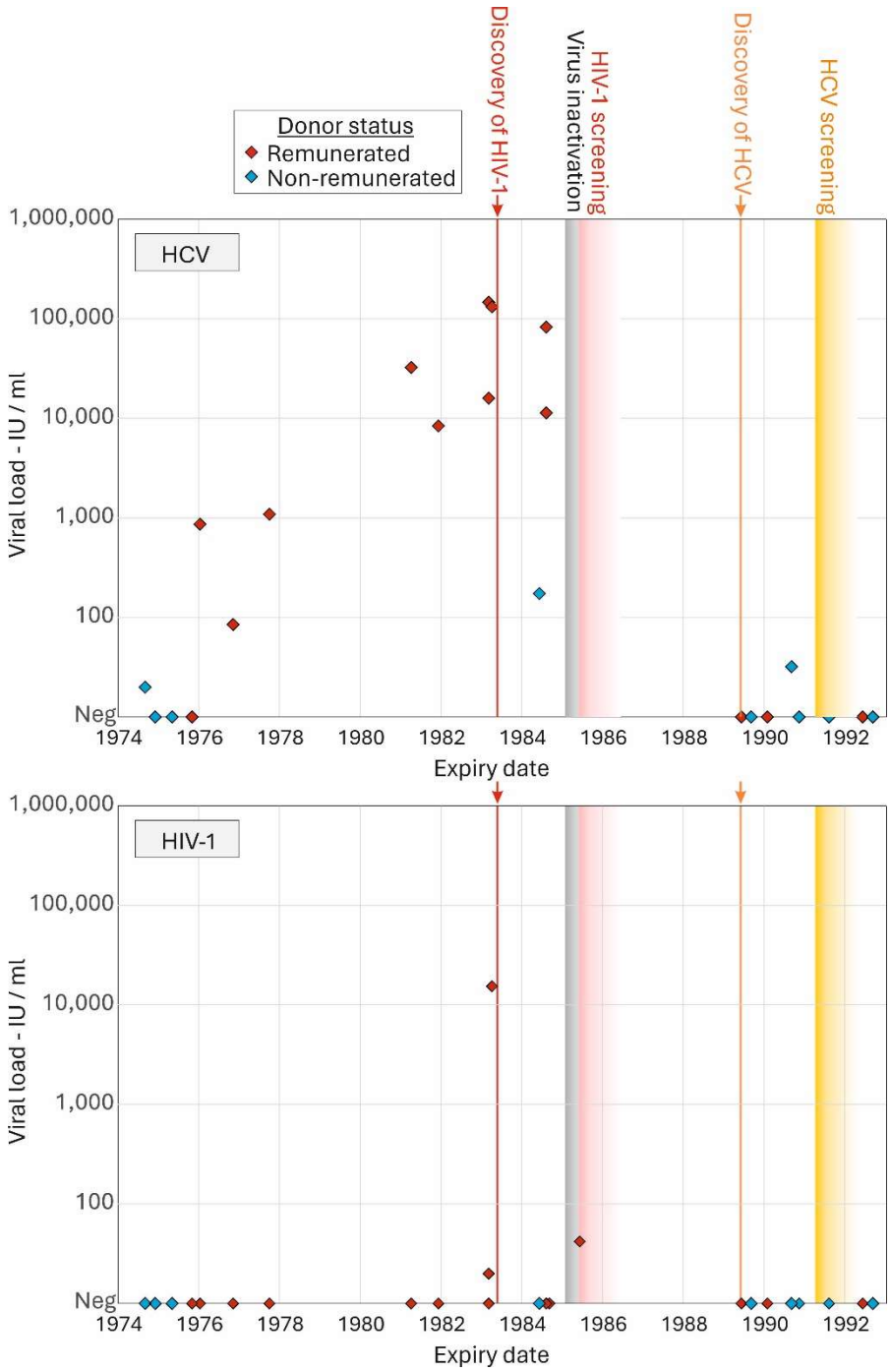
Table 1. Clotting factor information and testing results

Study no	Clotting Factor	Batch no.	Expiry Date	Volume	HCV[1] (IU/mL)	HIV-1 (IU/mL)	HBV (IU/ml)	HEV (IU/ml)	HPgV-1 Ct	PARV4 Ct	B19V Ct	HAV Ct
S6	BPL FACTOR IX	3C30912	01/10/1974	100	20	<10	<10	<50	45.0	45.0	45.0	45.0
S60	FACTOR IX	C319-6	01/01/1975	100	<10	<10	<10	<50	45.0	45.0	45.0	45.0
S5	BPL FACTOR VIII	646	01/06/1975	100	<10	<10	<10	<50	45.0	45.0	45.0	45.0
S41	PROTHROMPLEX	05D474	29/11/1975	10	<10	<10	<10	<50	33.7	45.0	36.0	45.0
S42	PROTHROMPLEX	51274	08/02/1976	10	867	<10	<10	<50	34.1	37.0	33.3	45.0
S30	FRACTION R	05A0575	03/12/1976	10	85	<10	<10	<50	32.8	45.0	18.6	45.0
S51	FEIBA	05A0576	28/10/1977	20	1091	<10	<10	<50	33.7	45.0	35.6	45.0
S74	FACTORATE	T33403	01/05/1981	20	32500	<10	25	<50	32.3	45.0	35.3	45.0
S72	FACTORATE	T51309	01/01/1982	20	8427	<10	<10	<50	32.8	45.0	45.0	45.0
S70	FACTORATE	B322303	01/04/1983	20	31400	20	<10	<50	31.9	33.1	35.1	45.0
S49	KRYOBULIN	09M02381	01/04/1983	20	147048	10911	<10	<50	29.2	45.0	22.1	30.1
S88	FACTORATE	V32303	01/04/1983	20	28400	<10	<10	<50	31.5	32.5	35.5	45.0
S25	BPL FACTOR VIII	HLB3084	01/07/1984	15	174	<10	<10	<50	34.6	45.0	45.0	45.0
S90	PROFILATE	A31930	01/09/1984	20	82800	<10	<10	<50	33.4	35.3	32.6	45.0
S66	FACTORATE	X24302H	01/09/1984	20	11399	<10	<10	<50	31.6	45.0	45.0	45.0
S68	FACTORATE	X45507H	01/07/1985	20	17000	42	<10	<50	28.2	45.0	36.6	45.0
S7	OCTA-VI	880808-0	01/07/1989	20	<10	<10	<10	<50	45.0	45.0	30.0	45.0
S83	BPL FACTOR VII	7D221H	27/09/1989	20	<10	<10	<10	<50	36.8	45.0	38.3	45.0
S59	FACTOR 8Y	FHC0140	21/02/1990	10	<10	<10	<10	<50	45.0	45.0	37.1	45.0
S1	BPL FACTOR IX	FJA0060	26/09/1990	20	32	<10	<10	<50	45.0	45.0	45.0	45.0
S77	BPL ANTITHROMBIN III	AT2281	04/12/1990	10	<10	<10	<10	<50	45.0	45.0	36.4	45.0
S47	FRENCH VWF	87800070	01/09/1991	20	<10	<10	<10	<50	45.0	45.0	35.5	45.0
S57	OCTAPLAS	1281496	01/07/1992	200	<10	<10	<10	<50	45.0	45.0	45.0	45.0
S58	FRENCH ANTITHROMBIN	11000310	01/10/1992	10	<10	<10	<10	<50	45.0	45.0	45.0	45.0

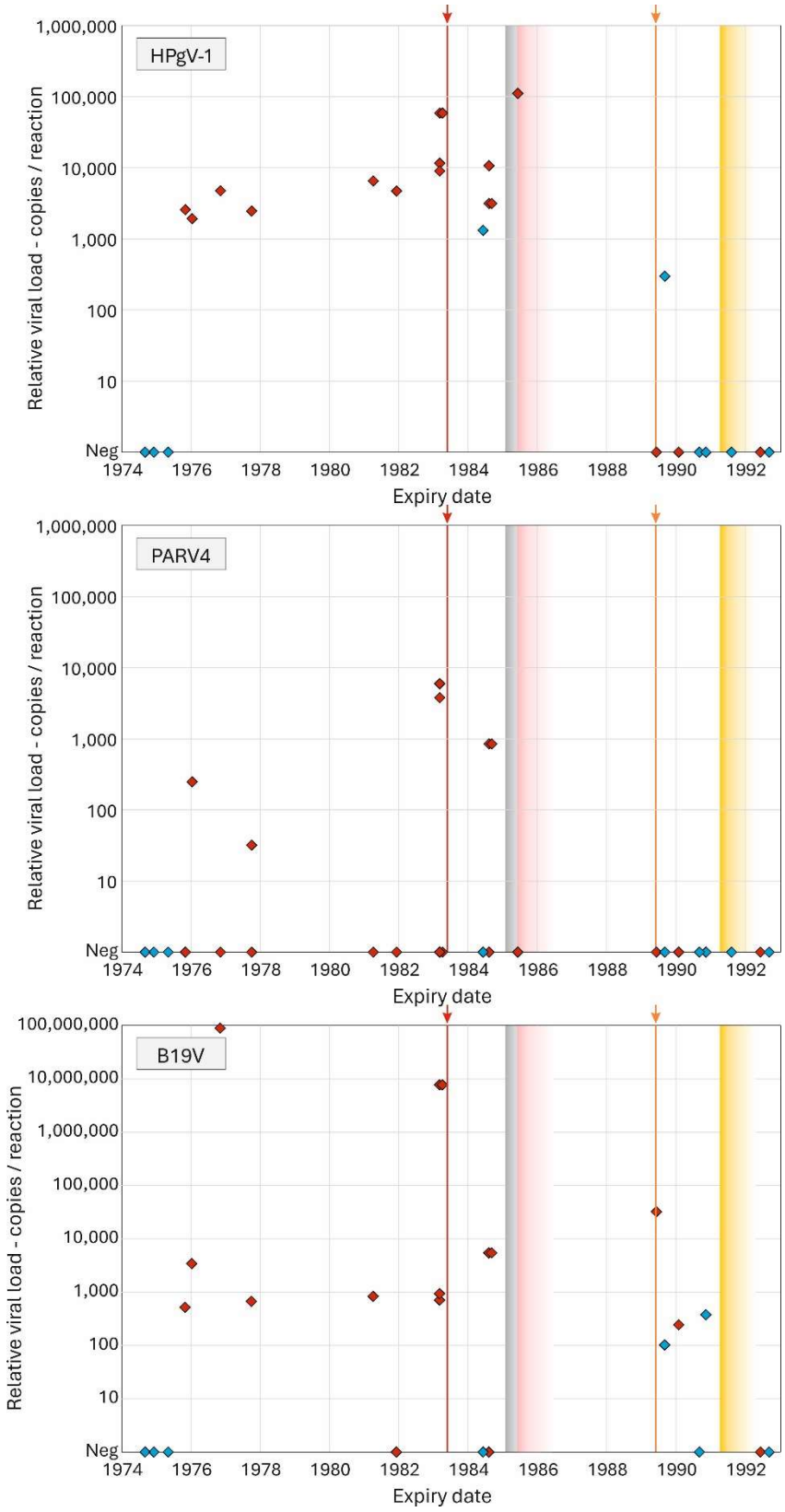
[1]Positive sample shaded in yellow

Figure 1. Viral loads of HCV and HIV-1 detected in clotting factors



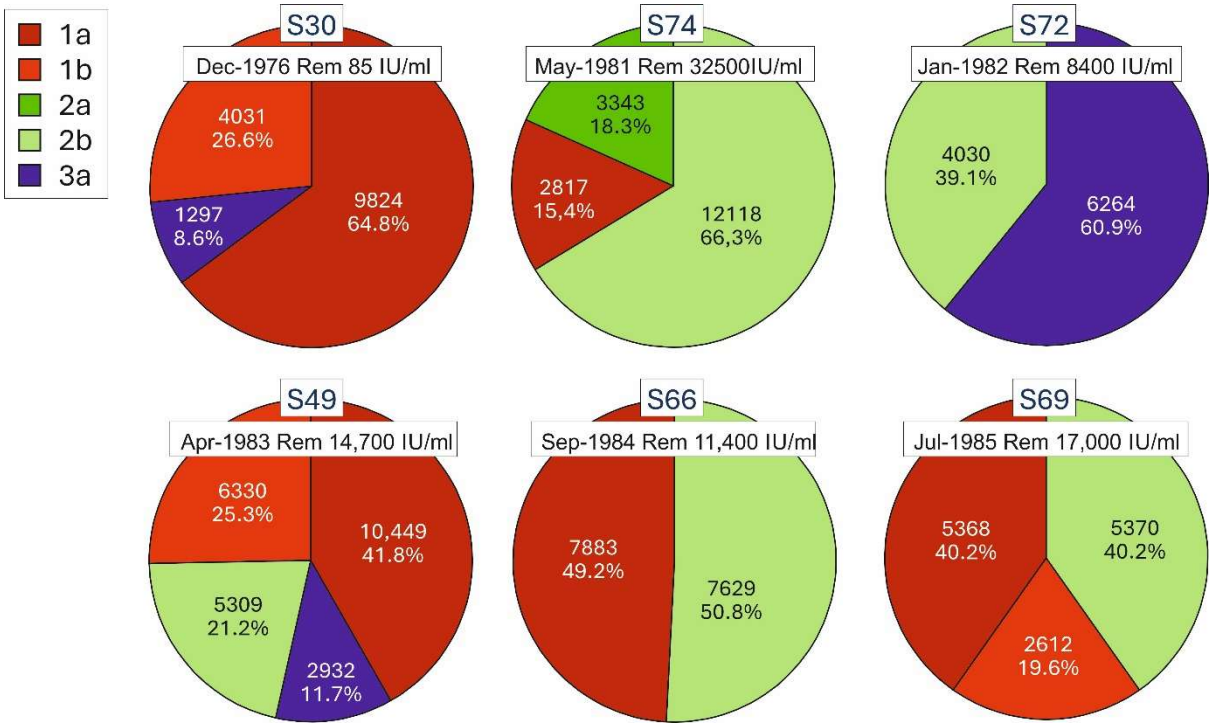
Plot of viral loads for the commercial (red) and UK/French origin (blue) factor VIII and IX clotting factors plotted against manufacturers' expiry date (raw data provided in Table 1). The approximate timing of the introduction of measures to reduce viral contamination (HIV, HCV screening, and virus inactivation) are indicated by vertical bars). Remuneration status of the donors is indicated by red and blue symbols.

Figure 2. Relative viral loads of other blood-borne viruses detected in clotting factors



Plot of viral loads of HPgV-1, PARV4 and B19V in clotting factors. Symbols and reduction measures as described in Fig. 1.

Fig. 3. Distribution of HCV genotypes and subtypes in clotting factors



Read totals and proportions of totals of HCV reads mapped to reference sequences of currently classified genotypes and subtypes (n = 140).

SUPPLEMENTARY DATA

Table S1. Sequences of primers and probes used for amplification of HPgV-1, B19V and PARV 4 sequences in real time PCR.

Name	Pos[1]	Sequence
HCV Core region; reference sequence AF011751		
Core_OS	288	ACT GCC TGA TAG GGT GCT TGC GAG
Core_OAS	751	ATG TAY CCC ATG AGR TCG GC
Core_IS	321	AGG TCT CGT AGA CCG TGC AHC ATG
Core IAS	724	CAY GTR AGG GTA TCG ATG AC
Core_New IAS	637	GAC ARG AGC CAH CCY GCC CA
HIV-1 p17*gag* region; reference sequence K03455		
gag OS	796	GCG AGA GCG TCA GTA TTA AGC GG
gag_OAS	1319	TCT GAT AAT GCT GAA AAC ATG GG
gag_IS	836	GGG AAA AAA TTC GGT TAA GGC C
gag_IAS	1270	CTT CTA CTA CTT TTA CCC ATG C
Human pegivirus type 1; reference sequence U44402		
HPgV-1_S	100	CGG CCA AAA GGT GGT GGA TG
HPgV-1_AS1	244	CAA CAC CTG TGG ACC GTG C
Human pegivirus type 2; reference sequence KT427414		
HPgV-2_S2	374	GGC CGA CTA TAA TAC CTC CTC
HPgV-2_AS2	503	CGC AAG GAA TGC GCA CAG C
PARV4; reference sequence AY622943		
PARV4_S1	2992	TGA ACC AGA CCT TGA GCG GCC
PARV4_AS2	3131	CGT ACC GTT CAT CAT GAT GYT TTG C
Parvovirus B19; reference sequence AY386330		
B19V_NS1-F	2083	AATGCAGATGCCCTCCAC
B19V_NS1-R	2275	ATGATTCTCCTGAACTGGTCC

[1]Position of the 5' base in the indicated reference sequence

Table S2. Sequence of HIV-1 strain detected in S49[1]

>S49
GGGGGAAAGAAAAAATATANATTAAAACATATAGTATGGGCAAGCAGGGAGCTAGAACGATTCGCAGTTAATCCTGGCCTATTAGAAACATCAGAAGGCT
GTAGACAAATACTGGGACAGCTACAACCAGCCCTTCAGACAGGATCAGAAGAACTTAGATCATTATTTAATACAGTAGCAACCCTCTATTGTGTGCATCA
AAAGATAGATGTAAAAGACACCAAGGAAGCTTTAGAGAAGATAGAGGAAGAGCAAAACAAAAGTAAGAAAAAAGCACAGCAAGCAGCAGCTGACACAGGA
AACAGCAGCCAGGTCAGCCAAAATTACCCTATAGTGCAGAACATCCAGGGGCAAATGGTACATCAGGCCATATCACCTAGAACTTTAAAT

[1]KRYOBULIN; batch 09M02381; exp. date: 04/1983

Table S3. Genotype and subtype detection frequencies in six clotting factors using original (co) and modified PCR with new antisense inner primer (cn)

	co					cn				
Sample	**1a**	**1b**	**2a**	**2b**	**3a**	**1a**	**1b**	**2a**	**2b**	**3a**
S30	9,824	4,031			1,297	10,544	3,993			
S49	10,449	6,330		5,309	2,932	7,486	6,272		6,496	1,199
S66	7,883			7,629		6,850			6,300	
S68	5,368	2,612		5,370		4,195	2,563		5,161	
S72				4,030	6,264			4,373	6,972	3,076
S74	2,817		3,343	12,118		2,960		3,883	12,733	
Total	36,341	12,973	3,343	34,456	10,493	32,035	12,828	8,256	37,662	4,275