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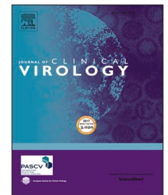

Use of polyethylene glycol precipitation and ultracentrifugation to enhance the sensitivity of hepatitis B virus DNA detection

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ABSTRACT

Background: Sensitive molecular detection of hepatitis B virus (HBV) DNA is crucial for diagnosing and managing occult hepatitis. To improve the sensitivity of HBV DNA detection, we compared the effectiveness of polyethylene glycol (PEG) precipitation and ultracentrifugation to concentrate DNA prior to extraction.

Methods: Twenty-three HBV DNA-positive samples with low viral loads were compared between the extraction of standard (0.2 mL) and larger volumes (5 mL) of plasma, through PEG precipitation of 10 mL and 20 mL of plasma, and ultracentrifugation from 35 mL of plasma. The effectiveness of the methods for HBV DNA detection was assayed by quantitative PCR. For genetic characterisation, Sanger sequencing of amplicons and targeted Illumina sequencing were used. Costs, sample capacities, and turnaround times were compared.

Results: DNA was detected in a greater number of samples using PEG and ultracentrifugation (detecting up to all 23 samples) compared to more standard extraction methods (detecting at least 18 samples). Efficiencies of recovery of HBV DNA from samples were comparable in all concentration methods. HBV and other DNA viruses, such as human herpesviruses and anelloviruses, were detected in samples and at higher read counts with PEG concentration than without. The availability, cost, relative simplicity, and throughput of PEG precipitation conferred further advantages to ultracentrifugation.

Conclusions: PEG precipitation from large volumes of plasma is a practical and economical alternative to ultracentrifugation and could be a similarly effective concentration method for low viral load samples in blood donation and clinical virology laboratories.

1. Introduction

Sensitive detection of viral nucleic acids is important for infectious diseases, as evidenced by the COVID-19 pandemic [1] and the HIV global epidemic [2]. In particular, the need for sensitive detection of hepatitis B virus (HBV) DNA cannot be overstated, where 257 million individuals worldwide have chronic infection [3]. The ability to assay low viral loads (VLs) is key for the diagnosis and monitoring of occult HBV infection (OBI). OBI is defined by undetectable hepatitis B surface antigen (HBsAg) in plasma with very low and fluctuating levels of DNA [4]. OBI is a major concern for blood safety, where ultrasensitive assays are required for the robust detection of low HBV VLs and to limit viral transmission [5].

The sensitivity of nucleic acid testing is primarily dependent on the

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volume of plasma used to extract viral nucleic acids. Ultrasensitive methods for the detection of HBV DNA have been developed with nucleic extraction from 5 mL of plasma; this method successfully measured viral DNA in samples that previously tested negative by a commercial assay [6]. The sensitivity of the detection could be improved by concentrating HBV particles from larger volumes of plasma, where high-speed ultracentrifugation was reported to pellet HBV from >10 mL of plasma [7]. However, the availability and technical difficulties of this ultracentrifugation method present major limitations to its wide-scale use in clinical virology laboratories.

Polyethylene glycol (PEG) has long been used to precipitate and concentrate HBV particles for in vitro infection studies [8]. PEG has been successfully used to enhance viral nucleic acid recovery from environmental samples [9], such as in monitoring SARS-CoV-2 circulation [10] and generating viral metagenome sequence libraries [11]. The use of PEG for viral nucleic acid concentration in human plasma has been studied less and lacks evidence in clinical and transfusion contexts. Previous research with HBV have suggested that PEG was marginally less effective than ultracentrifugation for concentrating HBV DNA from plasma [12,13]. However, VLs were not quantified in these studies, and the input plasma volumes for PEG (maximum 6 mL) and ultracentrifugation (maximum 12 mL) protocols were relatively low. Thus, a systematic comparison of PEG, ultracentrifugation, and more standard extraction protocols using large volumes of plasma would be useful for clinical virology and transfusion laboratories.

This study presents a PEG precipitation method for concentrating viral DNA from blood donor samples with low HBV VL using up to 20 mL of plasma. Quantitative PCR, Sanger sequencing, and next-generation sequencing (NGS) validated the presence of HBV DNA in extracts following the PEG method and allowed the comparison of five different protocols.

2. Methods

2.1. Samples and controls

As a convenience sample, a previously described sub-panel of 23 HBV DNA-positive plasma samples with low VL defined as 3 to 7115 IU/mL were characterised [6]. Extracted DNA from an internal control (an HBsAg-positive blood donor sample with high VL) was used for PCR assay calibrations; the internal control was calibrated to the 4th WHO International Standard for HBV DNA (National Institute for Biological Standards and Control, ref 10/266) as previously described [6]. Signed consent was obtained from all donors for the use of their samples for research to improve knowledge of the donor population. This study was approved by the Blood Supply Clinical Audit, Risk and Effectiveness Committee of NHS Blood and Transplant on 19th April 2022 and 8th January 2023.

2.2. DNA concentration methods

Five concentration methods were compared, chosen to represent current and new methods for sensitive HBV DNA detection:

Method 1: extraction from a standard volume of 0.2 mL of plasma using the QIAamp DNA Blood Mini Kit (Qiagen) following a previously reported protocol shown to be sensitive compared to other extraction methods using similar input volumes of plasma [6].

Method 2: extraction from 5 mL of plasma using the Roche High Pure Viral Nucleic Acid Large Volume Kit [6], previously shown to yield a more sensitive detection of HBV DNA than standard extraction volumes.

Method 3: using the manufacturer-recommended plasma input volume for PEG concentration, this method involved pre-extraction concentration of HBV from 10 mL of plasma following the manufacturer's instructions for the PEG Virus Precipitation Kit (Abcam, ab102538). The pellet was resuspended in 200 µL of Virus Resuspension Solution and 800 µL of phosphate-buffered saline (PBS) and extracted the whole volume with the Roche High Pure Viral Nucleic Acid Large Volume Kit, following manufacturer instructions.

Method 4: aiming to improve sensitivity from Method 3 by increasing plasma input volume past the manufacturer's protocol, this method involved doubling the input volume of plasma (20 mL) and 5X PEG solution (5 mL) and resuspending the pellet in 400 µL of Virus Resuspension Solution and 2.1 mL of PBS, then extracting the whole volume with the same kit.

Each sample was concentrated and extracted in the same runs for Methods 2–4, including two HBV negative controls to confirm specificity.

Method 5: as a comparator to PEG for effective HBV DNA concentration, HBV was pelleted by ultracentrifugation of 35 mL of plasma at 30,000 rpm for 3 h at 15 °C using a rotor SW32 Ti of Optima XPN-100 ultracentrifuge (Beckman Coulter). Pellets were resuspended in 1 mL of Working Solution of the Roche High Pure Viral Nucleic Acid Large Volume Kit, and the whole volume was extracted following the manufacturer's instructions.

Feasibility experiments were conducted to assess the relative effectiveness of PEG concentration. Firstly, 200 µL aliquots of plasma containing 96,000 IU of HBV DNA were added, where necessary, to excess plasma that tested negative for screened blood-borne viruses to make up 200 µL, 5 mL, 10 mL, and 20 mL of plasma. These were extracted using the methods listed above. Method 5 was not evaluated in these feasibility experiments as it was an established method to concentrate DNA from large volumes effectively.

2.3. Real-time PCR

A previously described real-time PCR protocol [6] was performed with a 95 % limit of detection (LOD) of 0.45 IU/mL. 2 µL of DNA from each sample was assayed in a total 20 µL reaction volume. Extracts from each sample isolated using Method 2 to 5 were assayed in the same PCR run, including appropriate extraction and run controls. Previous measurements were included for Method 1 [6]. Ct values were converted to IU/mL via replicate 1:10 dilutions of the internal standard.

2.4. Sanger sequencing

Previously described nested PCR and Sanger sequencing protocols were followed to sequence a portion of the S gene [6], but modifying the nested PCR protocol by adding 5 µL of extracted DNA in the first round of PCR. Primers spanned regions between nucleotide positions 219 and 375 (157 bps; numbered according to the D00330.1 reference sequence) and 400 to 637 (238 bps). Consensus sequences for all samples were genotyped as previously described [6].

2.5. Next-generation sequencing

Targeted enrichment NGS was performed to investigate the relative performance of PEG concentration from 20 mL of plasma to standard extraction from 5 mL of plasma (Methods 4/3 and 2, respectively). cDNA synthesis and library preparation were performed per a previously described protocol [14], with a modification using a mixture of 2.5 µL of cDNA and 2.5 µL of original DNA extract (gDNA) as input template. Extracts for each sample were assayed in the same run with appropriate controls. Samples were sequenced using an Illumina NovaSeq 6000 (paired 150 bp) and were analysed via Castanet [14] to obtain deduplicated reads, viral genome coverage, read depth statistics, and consensus HBV genomes.

2.6. Statistical analyses

Data normality was assessed with the Shapiro–Wilk test. All non-normal data are reported as median [interquartile range]. The Friedman test with post-hoc Dunn's compared VL measurements between different

methods, the Chi-squared test compared the samples detected, and non-linear log-log regression compared expected and calculated VLs. α level was 0.05. Data analyses and visualisation were performed with GraphPad Prism (v10.4.0, LLC).

3. Results

3.1. Feasibility experiments

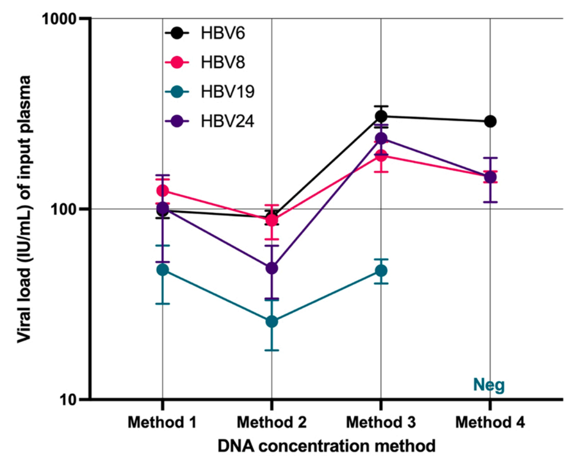
To compare the efficacy of PEG to concentrate HBV DNA from standard and large-volume extraction methods, 200 μL of plasma containing 96,000 IU of HBV DNA was separately added to 0 mL, 4.8 mL, 9.8 mL and 19.8 mL volumes of HBV-negative plasma, extracted and tested by Methods 1, 2, 3, and 4 respectively, using equal elution and test volumes of extracted DNA from each. Ct values were consistent on replicate PCR testing and comparable between methods, albeit with a modest loss in expected viral recovery when greater input plasma volumes were used (Table 1). These four methods were used to test four non-diluted samples with low VLs (27, 38, 42, and 228 IUs/mL). Taking input plasma volume into account, the estimated VLs were similar between the four methods (Fig. 1). One sample provided a negative result using Method 4; this sample was not repeated due to volume limitations, but the entire sample panel was re-concentrated for subsequent measurements.

3.2. Viral load comparison

HBV DNA was detected using real-time PCR in 19/23 samples using Method 1, 22/23 using Method 2, 23/23 using Methods 3 and 4, and 11/12 using Method 5 ($p = 0.067$). When comparing the calculated VLs of the input plasma, HBV DNA detection was proportional to the amount of input plasma (Fig. 2A). When comparing across the complete panel of 23 samples, median VLs were within 0.5 log of each other: 283 [89–704 IU/mL] with Method 1, 235 [41–1681] IU/mL with Method 2, 314 [55–3942] IU/mL with Method 3, and 1360 [60–5258] IU/mL with Method 4 (Fig. 2B). For the 12 lowest VL samples assayed with all Methods we noted comparable VL across all samples (Fig. S1).

3.3. HBV genome sequencing using large volume extraction

To investigate whether extraction from larger volumes of plasma enhanced the quantity and quality of HBV genome sequencing, DNA extracted by the five methods was amplified by two nested PCRs, and the amplicons directly sequenced. HBV DNA was successfully sequenced in a greater number of samples when input concentration volume increased: 18/23 samples using Method 1, 20/23 using Methods 2 and 3, 23/23 using Method 4, and 11/12 using Method 5 ($p = 0.234$). When detected, all samples were positive in both PCR regions except for samples with lower VL in Method 1, and the sample with the lowest VL (HBV4) which was only positive in the first PCR in two of three methods (Table 2). When detected, amplicon sequences derived from each sample were identical across the four methods, except for one or two nucleotide differences between methods for six samples; these could reflect the natural heterogeneity of the virus populations.



Method	Standard	Standard	PEG	PEG
Volume of input plasma	0.2 mL	5 mL	10 mL	20 mL
PEG added	-	-	2.5 mL	5 mL
Resuspension volume	-	-	1 mL	2.5 mL
Extraction volume	0.2 mL	5 mL	1 mL	2.5 mL
Elution volume	50 μL	50 μL	50 μL	50 μL
Effective test volume	8 μL	200 μL	400 μL	800 μL

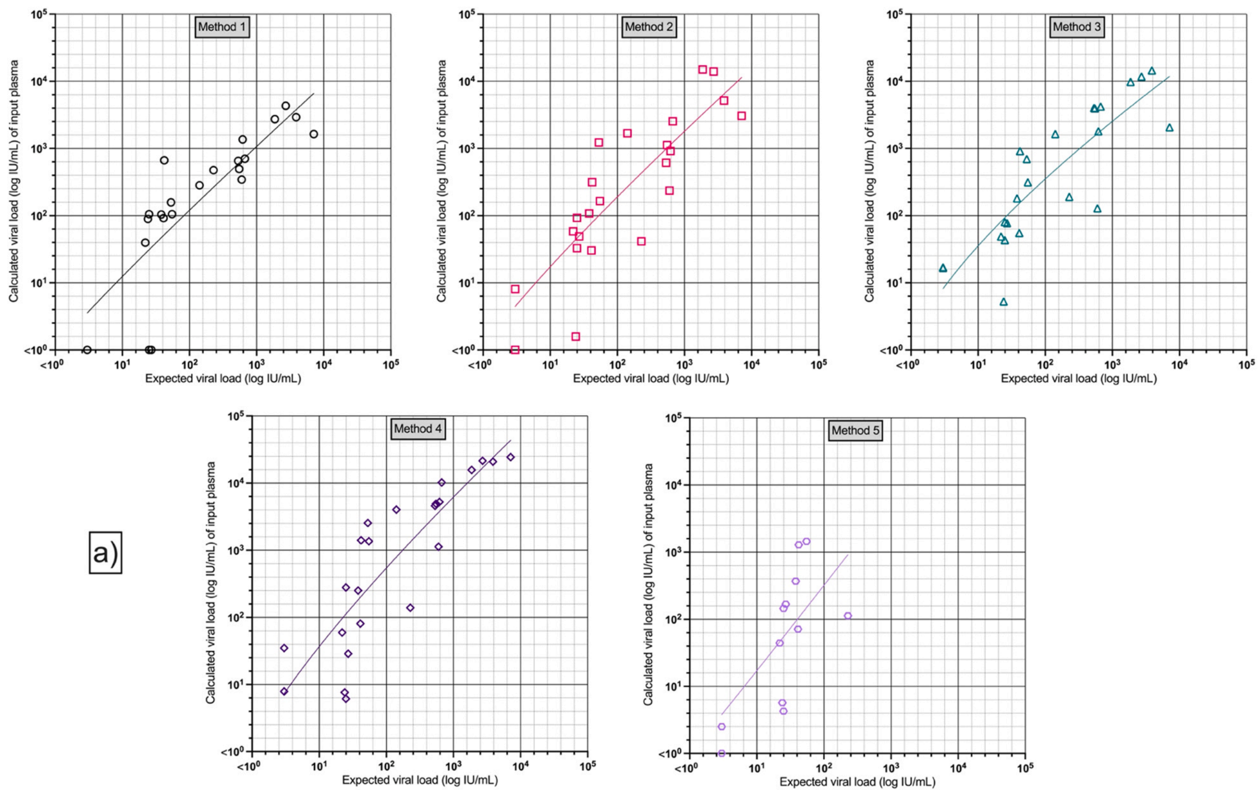
Fig. 1. Comparison of HBV viral loads obtained from four concentration methods for four samples: standard extraction using 0.2 mL of plasma (Method 1) and 5 mL of plasma (Method 2), polyethylene glycol (PEG) precipitation using 10 mL (Method 3) and 20 mL (Method 4) of plasma. The error bars indicate the mean and standard error of the mean from three PCR replicate measurements. The volume of plasma sample used, PEG, resuspension volumes, extraction volumes, elution volumes and effective test volumes ([volume of input plasma]/[elution volume]*[PCR template volume]) are displayed below the plot. Neg, sample not detected by this method, which could be explained by potential difficulties in large pellet resuspension with the doubling of input volumes than recommended in the manufacturer's protocol.

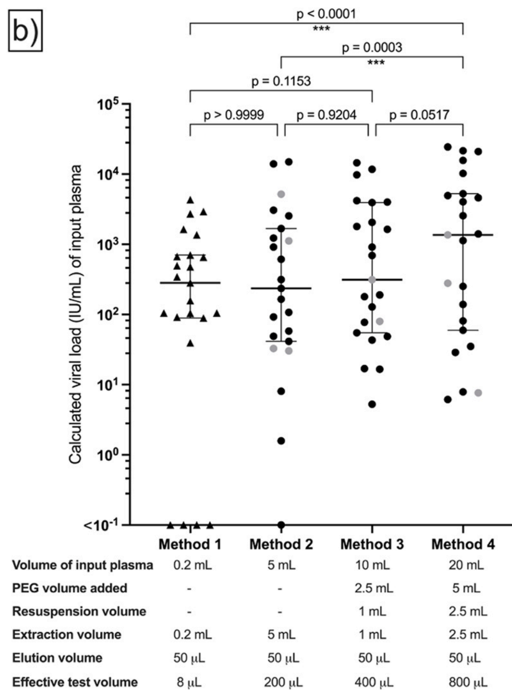
3.4. HBV NGS coverage

Six representative panel samples with a range of HBV VLs from 24 to 3881 IU/mL were extracted by Methods 2 and 4 (or Method 3 for HBV22 because of sample volume limitations) and assayed through a multi-pathogen targeted capture NGS protocol (Table 3). HBV sequences were obtained from five of the six samples using Method 3/4, but only two of the six using Method 2. Consensus sequences from HBV12, HBV22, and HBV25 were consistent with the sequences derived by nested PCR, with 0, 1, and 3 nucleotide differences in the 395 nucleotide amplicon, respectively. The three samples detected by Method 4 produced 8–95 deduplicated HBV reads. For the two samples that were detected by both methods, nearly two-fold and ten-fold HBV reads with greater genome coverage were obtained using PEG compared to Method 2 (Table 3). Anellovirus sequences were detected in three samples, Epstein-Barr virus (EBV) DNA detected in a fourth sample with Method

Table 1
Comparison of Ct values and estimated viral loads obtained in PCR assay replicates between four DNA concentration methods for equal volumes (0.2 mL) of diluted internal control in negative plasma. The same amount of HBV DNA (96,000 IU) was extracted with Method 1 and added to Methods 2, 3, and 4 to extract a total volume of 5 mL, 10 mL, and 20 mL. R, replicate number; VL, viral load; SEM, standard error of the mean.

Method	Testing volume	Expected VL (IU/mL)	PCR assay 1				PCR assay 2			
			R1 (Ct value)	R2 (Ct value)	R3 (Ct value)	Observed mean VL ± SEM (IU/mL)	R1 (Ct value)	R2 (Ct value)	R3 (Ct value)	Observed mean VL (IU/mL) ± SEM
1	0.2 mL	480,000	27.15	27.21	27.26	442,630 ± 10,894	26.92	27.11	27.01	478,012 ± 20,977
2	5 mL	19,200	27.23	27.76	27.81	13,287 ± 2017	27.12	28.12	27.99	11,279 ± 3096
3	10 mL	9600	27.98	28.09	28.23	4444 ± 248	29.15	28.97	29.15	1769 ± 88
4	20 mL	4800	28.44	28.38	28.40	1743 ± 25	28.77	28.78	28.74	1153 ± 11





	Method 1	Method 2	Method 3	Method 4
Volume of input plasma	0.2 mL	5 mL	10 mL	20 mL
PEG volume added	-	-	2.5 mL	5 mL
Resuspension volume	-	-	1 mL	2.5 mL
Extraction volume	0.2 mL	5 mL	1 mL	2.5 mL
Elution volume	50 µL	50 µL	50 µL	50 µL
Effective test volume	8 µL	200 µL	400 µL	800 µL

Fig. 2. Comparison of viral loads obtained from the five concentration methods: standard extraction without concentration using 0.2 mL (Method 1) and 5 mL of plasma (Method 2), polyethylene glycol (PEG) precipitation using 10 mL (Method 3) and 20 mL (Method 4) of plasma, and ultracentrifugation using 35 mL of plasma (Method 5): A) Comparison of calculated viral loads (normalised to input plasma volume) for each methods, with log-log non-linear regression lines shown and axes drawn to the same scales for each plot. B) Comparison between Methods 1 to 4 (samples were concentrated, extracted, and tested concurrently for Methods 2 to 4) showing calculated viral loads (normalised to input plasma volume) for the set of 23 samples, where the lines indicate the median [interquartile range] for each method. The volume of PEG, resuspension volumes, extraction volumes, elution volumes and effective test volumes ([volume of input plasma]/[elution volume]* [PCR template volume]) are displayed below the plot. *P*-values were obtained from the Friedman test with Dunn's multiple comparisons test. Lighter-coloured dots indicate measurements from 9.5 µL extracts instead of 2 µL due to limited extract, as measurements were initially performed with 9.5 µL extracts. IU/mL, international units per millilitre.

Table 2
A comparison of the sequence completeness (shown by percentage %) and sequence identity of the genome region of length 395 nucleotides derived from amplifying two overlapping genome regions by nested PCR. Sequences assembled from amplicons in the four methods were identical to each other, except for samples with coloured cells. Grey indicates one dissimilar nucleotide from the shaded methods compared to the unshaded methods. Orange indicates two dissimilar nucleotides compared to the other methods. Blue indicates two identical sequences with two dissimilar nucleotides compared to the other methods. '-' indicates where ultracentrifugation was not performed on samples with higher viral loads. 'NS' indicates where sequences were not able to be obtained. * indicates where only one of two amplicons was able to be sequenced. mL, millilitre.

Sample	Viral Load (IU/mL)	Genotype	Method 1	Method 2	Method 3	Method 4	Method 5
11	7115	A	100%	100%	100%	100%	-
22	3881	D	100%	100%	100%	100%	-
3	2715	A	100%	100%	100%	100%	-
9	1871	E	100%	99.7%	100%	100%	-
12	670	C	100%	100%	100%	100%	-
17	619	D	100%	100%	100%	100%	-
14	599	A	100%	100%	100%	100%	-
25	550	D	95.9%	100%	100%	100%	-
1	533	D	100%	100%	100%	100%	-
8	228	D	100%	100%	NS	100%	100%
15	141	E	100%	99.7%	99.7%	100%	-
5	55	B	100%	100%	100%	100%	100%
23	53	A	100%	100%	100%	100%	-
6	42	E	100%	100%	100%	100%	100%
21	41	C	60.5%*	100%	100%	100%	100%
24	38	A	39.5%*	98.5%	99.7%	100%	100%
19	27	C	60.5%*	100%	100%	100%	100%
7	25	A	NS	NS	NS	100%	100%
16	25	A	NS	99.7%	99.5%	100%	100%
26	24	A	NS	100%	100%	100%	100%
10	22	D	60.5%*	100%	100%	100%	100%
2	3	D	NS	NS	NS	92.2%	NS
4	3	A	NS	NS	39.5%*	39.5%*	100%

4, while none were detected in DNA extracted by Method 2. With Method 3 compared to Method 2, greater anellovirus read counts were observed for two samples and greater human herpes virus (HHV)-8 reads for one sample. In summary, the PEG methods greatly enhanced sequence coverage across HBV and other viral genomes (Fig. 3).

3.5. Comparison of practicality, costs, and time

Lastly, we compared key factors contributing to the practicality and cost-effectiveness of the different methods (Table 4). The standard extraction method required smaller volumes of plasma and allowed the testing of more samples than PEG or ultracentrifugation. Ultracentrifugation required equipment and technical expertise that may be lacking and costly to implement in standard clinical diagnostic and transfusion laboratories. Hands-on times were similar in the four extraction protocols, but calculated per sample, they would be longer for PEG and longest for ultracentrifugation because of smaller concentration batch sizes.

4. Discussion

By comparing a panel of HBV-positive samples with low VL, this study has shown that PEG precipitation from up to 20 mL of plasma is an effective method for concentrating viral DNA and enhancing analyte sensitivity, comparable to ultracentrifugation. This method could enable the detection and genomic investigations of low amounts of viral DNA when large volumes of plasma are available, such as from blood donors. The unique properties of the non-toxic and chemically inert PEG solution contribute to its efficacy in detecting and characterising viruses in samples with low VL. By inducing interactions that preferentially

Table 3
Comparison of deduplicated read counts (dedup rds) and genome coverage proportion (COV) for six selected samples where extracted DNA from Method 2 and Method 4 (or Method 3 for HBV22) were assayed in parallel through a multi-pathogen target capture NGS method. Grey boxes indicate samples run in the method with half-cDNA and half-gDNA, whilst the other three samples were run on a standard cDNA protocol. Anellovirus coverage was not computed as reads may have originated from multiple co-infecting strains or genotypes. HBV, hepatitis B virus; AV, anellovirus; HHV, human herpesvirus; EBV, Epstein-Barr virus.

Sample	Viral load (IU/mL)	Method	HBV		AV	EBV		HHV8	
			Dedup Rds	COV	Dedup Rds	Dedup Rds	COV	Dedup Rds	COV
HBV22	3881	Method 2	254	0.92	-	0	-	-	-
		Method 3	459	0.96	-	3	0.02	-	-
HBV12	670	Method 2	0	-	0	-	-	-	-
		Method 4	95	0.91	11	-	-	-	-
HBV25	550	Method 2	35	0.90	3	-	-	-	-
		Method 4	343	0.99	32	-	-	-	-
HBV1	533	Method 2	0	-	0	-	-	-	-
		Method 4	12	0.41	3	-	-	-	-
HBV7	25	Method 2	0	-	0	-	-	5	0.05
		Method 4	8	0.17	2	-	-	37	0.23
HBV26	24	Method 2	-	-	31	-	-	-	-
		Method 4	-	-	107	-	-	-	-

crystallise biological macromolecules such as DNA and virions in the interpolymer spaces between PEG molecules [15], PEG reduces the solubility of viral particles that are concentrated and precipitated from the solvent supernatant. PEG has also been used to enhance HBV e-antigen detection [16], where treating plasma with PEG may also increase the detection sensitivity for novel biomarkers for OBI [17].

Whilst the effectiveness of PEG precipitation in concentrating viral nucleic acids from environmental samples has been reported [18], as has its greater recovery efficiency for pelagic viral DNA to ultracentrifugation [19], our study findings add to the limited literature on protocols using PEG precipitation to concentrate viral nucleic acids from human plasma. A previous study showed that concentrating plasma using PEG (with 6 mL input volume and detecting 39.2 % of samples) was less efficient than ultracentrifugation (with 12 mL input volume and detecting 54.4 % of samples; $p = 0.079$) for the detection of HBV DNA in samples that were non-discriminated reactive from a commercial PCR assay [12]. A further study showed that ultracentrifugation (no volume of serum stated) was marginally superior to PEG precipitation (using 100 µL of serum) to concentrate HBV DNA; preparation by ultracentrifugation detected two further DNA-positive samples than PEG [13]. Using much greater volumes of input plasma in both methods and assessing metrics not previously considered, such as VL comparisons and sequencing metrics, our study provides a new perspective, suggesting that both methods are similar in their concentration of nucleic acids in low VL plasma. Our findings highlight the potential applicability of PEG in the transfusion context for clinical virology stakeholders to consider.

Since the volumes used for ultracentrifugation are larger (35 mL) than PEG (20 mL maximum), ultracentrifugation may still be beneficial since it can concentrate almost double an effective test volume of 1.4 mL compared to 0.8 mL. However, the comparative costs and practicality of these methods may not justify the marginal superiority of ultracentrifugation in concentrating nucleic acids.

Despite larger volumetric concentration factors, both PEG and ultracentrifugation methods present limitations. Practical barriers exist for obtaining and processing large volumes of samples in routine clinical practice outside the blood donation setting. Indeed, limited plasma volume restricted the repetition of experiments in this study, such as for samples that produced false-negatives. The detection failure of one sample using ultracentrifugation could be explained by the difficulty of resuspending small and often translucent pellets. Potential lack of reproducibility would affect the detection of very low VL, where full pellet resuspension is important. Further, large pellets produced by the 20 mL modified PEG protocol present difficulties in resuspension, which may have mediated the non-detection of one sample in the feasibility experiment.

In conclusion, PEG precipitation presents a feasible alternative to ultracentrifugation for concentrating viral DNA at low copy numbers from large volumes of plasma. Compared to standard extraction methods, PEG precipitation or ultracentrifugation could be utilised to ascertain the infectious status of blood donations more sensitively, such as those with OBI. Further studies could investigate the performance of PEG precipitation on more standard and available volumes of plasma.

CRediT authorship contribution statement

Michael X. Fu: Writing – original draft, Visualization, Project administration, Methodology, Investigation, Formal analysis, Conceptualization. **Osmany Larralde:** Writing – review & editing,

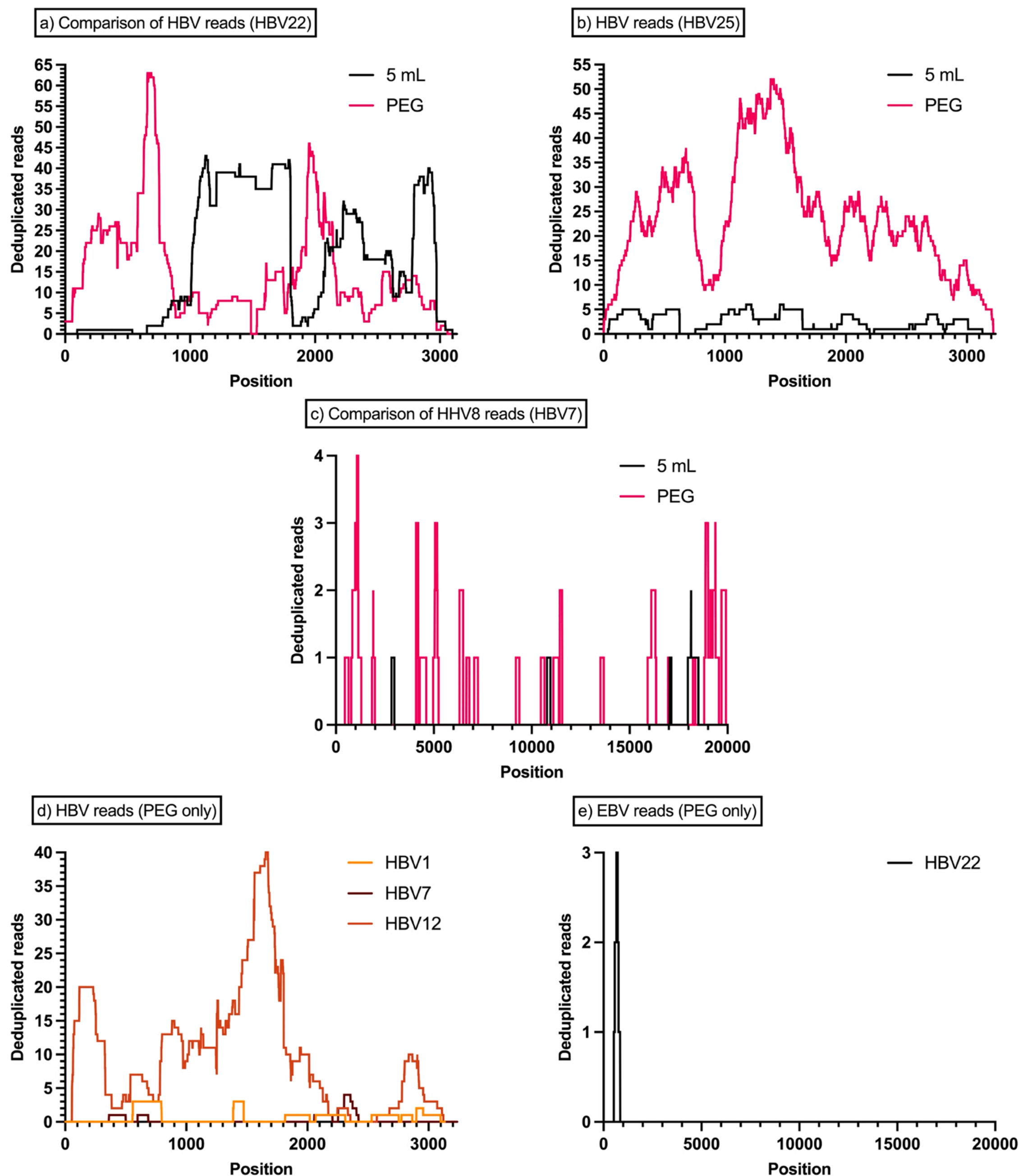


Fig. 3. Read depth plots across whole viral genomes for the samples run through the next-generation sequencing protocol. Plots a) to c) show the comparison of genome coverage for HBV and HHV8 detected in samples by both 5 mL (Method 2) and PEG methods (Method 3 or 4), whilst d) and e) show genome coverage for HBV and EBV detected in samples using only the PEG method. Anellovirus coverage is not shown due to viral heterogeneity. HBV, hepatitis B virus; PEG, polyethylene glycol; HHV, human herpesvirus; EBV, Epstein-Barr virus.

Methodology, Investigation. **Richard Mayne:** Writing – review & editing, Methodology, Investigation. **Kai Kean:** Methodology, Investigation. **Kaitlin Reid:** Methodology, Investigation. **Monique Andersson:** Writing – review & editing, Supervision. **Tanya Golubchik:** Writing – review & editing, Methodology, Funding acquisition. **Jane A. McKeating:** Writing – review & editing, Conceptualization. **Lisa Jarvis:** Writing – review & editing, Methodology. **William L. Irving:** Writing – review & editing, Methodology, Formal analysis. **Peter Simmonds:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization. **Heli Harvala:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Table 4
Comparison of different factors between standard extraction, extraction preceded by polyethylene glycol (PEG) precipitation, and extraction preceded by ultracentrifugation methods. NA, not applicable; £, Great British Pounds.

Factor	Method		
	Standard	PEG	Ultracentrifugation
Principle of concentration	Binding nucleic acids to silica fiber filters	Solution-based chemistry	Sedimentation coefficient
Practicality			
Volume of plasma required	0.2 mL or 5 mL	10 mL or 20 mL	35 mL
Number of clinical samples per run	90 (0.2 mL) or 21 (5 mL)[a]	10[b]	6
Technical expertise required	Standard	More than standard (pellet resuspension)	Even more than standard (meticulous sample preparation and pellet resuspension)
Instrument availability	Standard	Standard	Only in certain laboratories
Costs			
Instrument/rotor (approximate upfront cost)	Microcentrifuge for 0.2 mL (£2500); Benchtop megafuge for 5 mL (£7500)	Benchtop megafuge (£7500)	Ultracentrifuge (£90,000 [centrifuge £65,000 plus rotor £25,000])
Tubes needed before extraction	NA	50 mL regular tube (£0.20 each)	Ultra-clear tube (£11.50 each)
PEG Precipitation Kit, cost per sample	NA	£6 for 10 mL £15 for 20 mL	NA
Extraction cost	£3 per sample (for 0.2 mL) £10 per sample (for 5 mL)	£7.50 per sample	£7.50 per sample
Approximate times			
Pre-extraction total hands-on time	NA	2 h	2 h
Pre-extraction total waiting time	NA (for 0.2 mL) Overnight (for 5 mL)	Overnight	5 h
Total extraction time	3–5 h	2 h	2 h

[a] Total 24 for each run, with an additional two negative controls and one low-positive control.
[b] Total 8 for each run, with additional negative control and low-positive control.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jcv.2025.105802.

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