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Validation of a Parvovirus B19 NAT Assay for Screening of Umbilical Cord Blood for Allogenic Hematopoietic Stem Cell Donation

Philipp Steininger[a] Klaus Korn[a] Holger Hackstein[b] Erwin F. Strasser[c]

[a]Institute of Clinical and Molecular Virology, University Hospital Erlangen, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany; [b]Department of Transfusion Medicine and Haemostaseology, University Hospital Erlangen, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany; [c]Division of Transfusion Medicine, Cell Therapeutics and Haemostaseology, University Hospital, LMU Munich, Munich, Germany

Keywords

Parvovirus B19 · Nucleic acid test screening · Umbilical cord blood · Allogenic hematopoietic stem cell donation

Abstract

Introduction: Parvovirus B19 transmitted by umbilical cord blood (UCB) products may cause severe disease in allogenic hematopoietic stem cell transplant recipients. Thus, commercially available nucleic acid test (NAT) assays for highly sensitive detection of parvovirus B19 DNA validated for the specimen cord blood plasma (CBP) are required to avoid parvovirus B19 transmission by umbilical hematopoietic stem cell preparations. ***Methods:*** The multiplex cobas DPX NAT assay was validated for detection of parvovirus B19 DNA in CBP derived from citrate anticoagulated UCB units which have been processed by the Rubinstein method. In total, 363 retained CBP samples pretested negative for parvovirus B19 DNA were prepared for analyzing sensitivity, specificity, and interference of that NAT assay. The 3rd WHO International Standard for parvovirus B19 DNA was used for determining the 95% limit of detection (LOD95) by probit analysis. ***Results:*** The validation of the parvovirus B19 NAT assay for CBP demonstrated high sensitivity, specificity, intra- and inter-assay precision. Dilution series and replicate analyses showed a high linearity of the assay with a coefficient of determination above 0.99 and revealed a LOD95 of 17 International Units (IU)/mL (95% confidence interval, 14–44 IU/mL) for parvovirus B19 DNA in CBP samples. ***Conclusion:*** The validation of a commercially available parvovirus B19 NAT assay for the specimen CBP demonstrated a high assay performance fulfilling German guidelines and international regulations.

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Introduction

Umbilical cord blood (UCB) is a product of fetal origin with unique cellular properties, which is used for allogenic hematopoietic stem cell transplantation [1]. Considering the high incidence of parvovirus B19 infection in pregnant woman, which can be up to 2.4% [2], potential transmission of parvovirus B19 by UCB products is of clinical importance. Persistent virus replication in allogenic hematopoietic stem cell transplantation recipients may lead to considerable morbidity and potential life-threatening complications including failure of engraftment, pure red cell aplasia, myocarditis, and hepatitis [3–7]. Therefore, it is considered necessary to minimize the risk of parvovirus B19 transmission by nucleic acid test (NAT) screening of UCB products [8], which is mandatory by the regulation "Guideline for the preparation and use

karger@karger.com
www.karger.com/tmh

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Correspondence to:
Philipp Steininger, philipp.steininger@uk-erlangen.de

of hematopoietic stem cell preparations" (German hematopoietic stem cell preparation guideline 2019) of the German Medical Association [9].

The cobas DPX (duplex HAV and parvovirus B19 NAT) assay is intended for donor screening; however, the test is not licensed for samples of UCB. Therefore, the validation of this parvovirus B19 NAT assay is required for NAT screening of UCB products according to the requirements of the Federal Institute for Vaccines and Biomedicines (Paul-Ehrlich-Institut [PEI], Germany) and the In Vitro Diagnostic Regulation (IVDR) 2017/746 of the European Union [10]. Thus, a specimen-specific validation of the cobas DPX NAT assay in UCB-derived cord blood plasma (CBP) was performed to meet the requirements of these national and international regulations.

Material and Methods

The multiplex cobas DPX NAT assay targeting parvovirus B19 DNA and hepatitis A virus RNA was performed on an automated PCR system (cobas® 6800, Roche Diagnostics, Mannheim, Germany) with the standard instrument settings and reagents. For validation, retained CBP samples of the Department of Transfusion Medicine and Haemostaseology (University Hospital Erlangen) collected between 2019 and 2021 were used. The CBP samples were derived from UCB collected in blood bag systems (Macopharma, Tourcoing, France) with citrate-phosphate-dextrose anticoagulation and processed by the Rubinstein method as described [11]. All samples were pretested negative for parvovirus B19 DNA by the artus Parvo B19 TM PCR assay (Qiagen, Hilden, Germany). Eight CBP pools, containing 363 parvovirus B19 DNA negative CBP samples, were prepared for spiking experiments.

The validation panel consisted of 285 parvovirus B19 DNA positive, negative, and possible cross-reactive CBP samples. Assay specificity was assessed by testing 52 CBP samples. Additionally, evaluation of possible interference with the blood-borne viruses HBV, HCV, HEV, and HIV-1 as well as for other relevant vertically transmitted viruses (e.g., CMV) was performed. For this purpose, 15 validation samples were prepared by spiking CBP samples in a ratio of 3 to 1 with patient specimens tested positive for one of the following viruses: CMV, EBV, HBV, HCV, HEV, HHV-6, HIV-1, HSV-1, and HSV-2.

Linearity of the parvovirus B19 NAT assay was assessed by testing nine dilution steps of a four-fold dilution series (range: 100,000 International Units [IU]/mL – 1.5 IU/mL) of the 3rd WHO International Standard for parvovirus B19 DNA (12/208 National Institute for Biological Standards and Controls, UK). By linear regression analysis (cycle threshold [ct] values vs. logarithmic concentration values), the coefficient of determination R^2 was calculated for the mean ct values of duplicate measurements. For evaluation of the 95% limit of detection (LOD95), eight two-fold dilutions of the WHO Standard between 195 IU/mL and 1.5 IU/mL were analyzed in twelve replicates each. The LOD95 was calculated by probit analysis using SPSS version 24.0 (IBM SPSS, Chicago, IL, USA).

A special feature of UCB donations is the variable volume of the donation samples, which usually ranges between 50 mL and 150 mL [12, 13]. The variable UCB volume mixed with a constant anticoagulant volume (29 mL) in the collection bag results in different dilution factors which has to be considered for calculation of LOD95. Therefore, the experimentally determined LOD95 in CBP was multiplicated by the dilution factor of the UCB (DF_{UCB}) to calculate the maximum LOD (LOD_{max}). The maximum dilution factor is calculated assuming a minimum volume of the UCB donation (50 mL). The calculation is as follows:

$$DF_{UCB} = \left(V_{min}\ (UCB) + V_{anticoagulans}\right) \Big/ V_{min}\ (UCB)$$
$$= (50 + 29)\ mL \Big/ 50\ mL = 1.58 \quad (1)$$
$$LOD_{max} = LOD95 * DF_{UCB} = LOD95 * 1.58 \quad (2)$$

Precision was evaluated by replicate and repeat testing. Therefore, intra-assay variability (ten replicates) and inter-assay variability (ten replicates on two other days) were analyzed for a parvovirus B19 DNA positive (1.563 IU/mL) and low positive (391 IU/mL) preparation of the WHO Standard.

Results

The dilution series of the WHO Standard for parvovirus B19 DNA in CBP showed a high linearity between 100,000 IU/mL and 391 IU/mL with a coefficient of determination (R^2) above 0.99. The dilution steps of 98 IU/mL and 24 IU/mL resulted in a positive parvovirus B19 DNA detection with mean ct values of 35.63 and 36.54, respectively, while parvovirus B19 DNA was not detected at the highest dilution steps (6.1 and 1.5 IU/mL). The probit analysis revealed a LOD95 of 17 IU/mL (95% confidence interval, 14–44 IU/mL) for parvovirus B19 DNA (shown in Table 1). Calculation of the LOD_{max} by Formula (2) which takes the dilution of the CBP by the anticoagulant into account was 27 IU/mL. Replicate testing of positive CBP samples on three different days demonstrated low coefficients of variations of ct values between 0.54% and 0.84% for intra-assay variability, and between 0.75% and 0.86% for inter-assay variability (shown in Table 2). In our investigation, the specificity of the assay was 100%, since none of 52 parvovirus B19 DNA negative CBP samples and none of 15 CBP samples containing different possible interfering viruses tested positive.

Discussion

The implementation of the cobas DPX NAT assay for screening of parvovirus B19 DNA in UCB requires validation for the fulfillment of national guidelines and international regulations [9, 14, 15]. The recent In Vitro Diagnostic Regulation (IVDR) 2017/746 requires

Table 1. Limit of detection determination for parvovirus B19 DNA in CBP

Parvovirus B19 DNA (IU/mL)	Number of replicates	ct value (mean±SD)	Fraction positive
195	12	35.2±0.28	1.0
98	12	35.9±0.24	1.0
49	12	36.7±0.36	1.0
24	12	37.5±0.37	1.0
12	12	38.3±0.61	0.67
6.1	12	38.6±0.44	0.50
3.0	12	38.7	0.08
1.5	12	-	0
LOD95 (IU/mL)		17 (95% CI, 14–44)	

CI, confidence interval; ct, cycle threshold; IU, international units; LOD95, 95% limit of detection; SD, standard deviation.

Table 2. Results of intra-assay and inter-assay validation

	Negative sample	Low positive sample	Positive sample
Day 1	Intra-assay (n = 10)		
Ct value (mean±SD)	Negative	34.1±0.22	32.6±0.27
CV (%)	–	0.65	0.82
Day 2	Intra-assay (n = 10)		
Ct value (mean±SD)	Negative	34.4±0.19	32.6±0.20
CV (%)	–	0.54	0.60
Day 3	Intra-assay (n = 10)		
Ct value (mean±SD)	Negative	34.4±0.27	32.9±0.28
CV (%)	–	0.79	0.84
	Inter-assay		
Ct value (mean±SD)	Negative	34.3±0.26	32.7±0.28
CV (%)	–	0.75	0.86

ct, cycle threshold; CV, coefficient of variation; SD, standard deviation.

a specimen-specific validation of analytical sensitivity including the intended matrix [14], while the German hematopoietic stem cell preparation guideline 2019 mandates parvovirus B19 DNA analysis in CBP for testing of allogenic UCB donations [9]. Since testing of UCB samples is not part of manufacturer's claims for the cobas DPX NAT assay [16], validation of this assay for CBP as a matrix is required according to the regulations of the Federal Institute for Vaccines and Biomedicines for modified CE-licensed NAT assays [10]. The cobas DPX NAT assay on the cobas 6800 PCR system allows high-throughput screening with a high degree of automation. In addition, assays for all other viruses relevant in screening of blood products are available on that platform which allows an efficient workflow in donor testing.

Validation of the cobas DPX NAT assay, including the determination of LOD95 and assay precision, was performed with the WHO Standard for parvovirus B19 DNA. Compared to blood donations with standardized volumes, UCB collection volumes are highly variable (up to a factor of 3). The collection of low UCB volumes results in dilution by the anticoagulant, which reduces the sensitivity of parvovirus B19 DNA detection in the CBP. Thus, the experimentally determined LOD95 must be corrected by the dilution factor for estimating the LOD95 for the lowest acceptable amount of blood in the original UCB product. According to the German hematopoietic stem cell preparation guideline 2019, the sensitivity for the detection of parvovirus B19 DNA has to be at least 500 IU/mL for single-donation testing. The validation of the cobas DPX NAT assay in CBP revealed a LOD95 of 17 IU/mL and a LOD_{max} of 27 IU/mL after correction for the dilution factor. This is in accordance with the manufacturer's information on the LOD95 of

17.4 IU/mL for plasma collected in EDTA anticoagulant [16]. These LODs are about 20-fold below the minimum sensitivity for the detection of parvovirus B19 DNA required by hematopoietic stem cell preparation guideline 2019. Therefore, this method also offers the possibility of pool testing strategies.

In conclusion, the cobas DPX NAT assay is suitable for single-donation testing of parvovirus B19 DNA in citrate anticoagulated CBP specimens and meets the national regulations. Due to the high sensitivity of this assay, further validation for pool testing to reduce the cost of testing is feasible.

Statement of Ethics

Samples used in this work were obtained as part of routine patient care and diagnostics and were processed in an anonymized fashion. Therefore, use of these samples for this validation purpose did not require ethical approval in accordance with local/national guidelines.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Conceptualization, methodology, and experiments, and original draft preparation: Philipp Steininger and Erwin F. Strasser; analysis and interpretation of data: all authors; resources: Klaus Korn and Holger Hackstein; all authors reviewed and edited the manuscript.

Data Availability Statement

All data generated or analyzed in this validation are available on request from the corresponding author.

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