Systematic Evaluation of Different Nucleic Acid Amplification Assays for Cytomegalovirus Detection: Feasibility of Blood Donor Screening

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Acute primary cytomegalovirus (CMV) infections, which commonly occur asymptotically among blood donors, represent a significant risk for serious morbidity in immunocompromised patients (a major group of transfusion recipients). We implemented a routine CMV pool screening procedure for plasma for the identification of CMV DNA-positive donors, and we evaluated the sensitivities and performance of different CMV DNA amplification systems. Minipools (MPs) of samples from 18,405 individual donors (54,451 donations) were screened for CMV DNA using the RealStar CMV PCR assay (Altona Diagnostic Technologies), with a minimum detection limit of 11.14 IU/ml. DNA was extracted with a high-volume protocol (4.8 ml, Chemagic Viral 5K kit; PerkinElmer) for blood donor pool screening (MP-nucleic acid testing [NAT]) and with the Nuclisens easyMAG system (0.5 ml; bioMérieux) for individual donation (ID)-NAT. In total, six CMV DNA-positive donors (0.03%) were identified by routine CMV screening, with DNA concentrations ranging from 4.35×10^2 to 4.30×10^3 IU/ml. Five donors already showed seroconversion and detectable IgA, IgM, and/or IgG antibody titers ($\text{IgA}^+/\text{IgM}^+/\text{IgG}^-$ or $\text{IgA}^+/\text{IgM}^+/\text{IgG}^+$), and one donor showed no CMV-specific antibodies. Comparison of three commercial assays, i.e., the RealStar CMV PCR kit, the Sentosa SA CMV quantitative PCR kit (Vela Diagnostics), and the CMV R-gene PCR kit (bioMérieux), for MP-NAT and ID-NAT showed comparably good analytical sensitivities, ranging from 10.23 to 11.14 IU/ml (MP-NAT) or from 37.66 to 57.94 IU/ml (ID-NAT). The clinical relevance of transfusion-associated CMV infections requires further investigation, and the evaluated methods present powerful basic tools providing sensitive possibilities for viral testing. The application of CMV MP-NAT facilitated the identification of one donor with a window-phase donation during acute primary CMV infection.

Human cytomegalovirus (CMV) is a ubiquitous viral pathogen that causes mostly asymptomatic disease in immunocompetent individuals. In immunocompromised patients, however, CMV infection represents a significant risk for serious morbidity, e.g., due to interstitial pneumonia or hepatitis (1–3). Immunocompromised patients, such as patients undergoing hematopoietic stem cell transplantation (HSCT), solid-organ transplant recipients, infants with low birth weights, fetuses, pregnant woman, HIV patients, and patients being treated for hematological malignancies, belong to the major groups of transfusion recipients, and CMV-seronegative individuals were considered high-risk patients for transfusion-transmitted (TT)-CMV infections (4–6). The introduction of leukodepletion of blood products and provision of CMV-seronegative blood products reduced the incidence of TT-CMV infections in at-risk populations by 92%. However, TT-CMV breakthrough infections occur in 1 to 3% of high-risk patients who receive transfusions (4–6), possibly due to window-phase donations during acute primary CMV infections.

The seroprevalence rates of CMV antibodies among blood donors show geographic differences, ranging from 45.8% in Germany to 96.5% in Brazil (3, 7). Primary CMV infections in blood donors occur in all age groups, with prevalence rates between 0.2 and 1.2% (3, 6, 8, 9). The disease presentation is mostly asymptomatic, frequently with a prolonged course (10, 11). Mononucleosis-like symptoms are rare, whereas nonspecific viral disease symptoms occur often but not at significantly increased rates, compared to matched control groups (3, 5).

Determination of the prevalence of primary CMV infections among blood donors represents an important parameter for the effective prevention of TT-CMV infections (3, 12). The aim of the present study was implementation of routine CMV DNA screen-

ing according to the setup used for our standard viral nucleic acid testing (NAT) (for HIV-1, hepatitis B virus [HBV], hepatitis C virus [HCV], hepatitis A virus [HAV], and parvovirus B19 [PVB19]). The sensitivity and performance of different amplification systems were evaluated for blood donor pool screening or testing of individuals with acute or chronic infections.

MATERIALS AND METHODS

Blood donors. A total of 54,451 allogeneic blood donations from 18,405 individual German blood donors were routinely screened for the presence of CMV DNA by the Uni.Blutspendedienst OWL between March and June 2013. Master pools of 96 donations were set up by combining 200- μ l EDTA-treated plasma samples; reactive pools were retested in duplicate. Repeatedly reactive pools were further tested by generation of subpools of a maximum of 10 donations (200 μ l/donor); pools were brought up to 4.8 ml with negative human plasma. Plasma specimens in positive subpools were tested individually in order to identify the individual reactive do-

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TABLE 1 DNA concentrations and antibody status for CMV-positive donors at the first positive screening result

Donor no.	Sex/age (yr) ^a	DNA level (IU/ml)	Anti-CMV IgM		Anti-CMV IgG		Anti-CMV IgA status		Time to last CMV-seronegative donation (days)
			Status	Level (U/ml)	Status	Level (U/ml)	IgA status	Avidity	
1	M/18	1.93×10^3	Positive	36.5	Positive	71.2	Positive	Low	First donation
2	F/20	4.35×10^2	Positive	52.9	Positive	54.7	Positive	Low	278
3	M/25	5.18×10^2	Negative		Negative		Negative	NT	7
4	M/26	9.22×10^2	Positive	87.5	Positive	14.7	Positive	Low	64
5	F/25	2.45×10^3	Positive	108.0	Negative		Positive	Low	61
6	F/25	4.30×10^3	Positive	>140.0	Positive	37.8	Positive	Low	24 ^b

^a M, male; F, female; NT, not tested.^b Seronegative but with an individual PCR-positive donation that had been collected outside the study period.

nors. Serological testing was performed only with samples from individual CMV DNA-positive donors. Screening for CMV DNA was performed using a RealStar CMV PCR kit (Altona Diagnostic Technologies [ADT], Hamburg, Germany). Quantification of CMV DNA in positive plasma samples was performed using four different quantification standards of the RealStar CMV PCR kit (ADT secondary standard), which were calibrated against the 1st WHO International Standard for human cytomegalovirus (HCMV) for nucleic acid amplification techniques (National Institute for Biological Standards and Control [NIBSC], Pottery Bar, Hertfordshire, United Kingdom). Samples donated at continuous intervals after the initial CMV DNA-positive donation (day 0) were available for four blood donors (one male donor and three female donors). All donors underwent predonation medical examinations and denied current diseases or any known risk factors for viral infection.

Nucleic acid extraction. (i) Pool screening. DNA extraction from 4.8 ml of plasma of master pools or subpools was performed using the Chemagic viral DNA/RNA reagent kit (Viral 5k kit; PerkinElmer Chemagen Technologie GmbH, Baesweiler, Germany) combined with the automated Chemagic Magnetic Separation Module I (PerkinElmer Chemagen Technologie GmbH). Briefly, 4.8 ml of plasma was mixed with 4.8 ml of lysis buffer, 30 μ l of protease, and 7 μ l of poly(A), followed by incubation of the samples at 55°C for 10 min. Subsequently, lysates were mixed with 15 ml of binding buffer containing 100 μ l of magnetic beads. The Magnetic Separation Module I automatically performed the nucleic acid extraction process, including binding, two washes, and elution in a final volume of elution buffer of 100 μ l.

(ii) Individual donation screening. Extraction of total DNA from 500 μ l of plasma was performed using the NucliSens easyMAG automated RNA/DNA extraction system (bioMérieux, Nürtingen, Germany). DNA was eluted in 55 μ l of elution buffer.

Real-time PCR. Amplification using the RealStar CMV PCR kit (ADT) was performed with the Rotor-Gene 3000 system (Corbett Life Sciences, Sydney, Australia), whereas amplification using the Sentosa SA CMV quantitative PCR kit (Vela Diagnostics Germany GmbH, Hamburg, Germany) was carried out on the Rotor-Gene-Q platform (Vela Diagnostics Germany GmbH). Amplification using the CMV R-gene PCR kit (bioMérieux) was implemented with the LightCycler 480 II real-time PCR system (Roche Diagnostics, Mannheim, Germany). PCR setup and conditions for the three assays were as described in the manufacturer's instructions. All assays included a heterologous amplification system (internal control) to identify possible PCR inhibition.

Analytical sensitivities and comparison of different amplification methods. The analytical sensitivity and precision of the three commercially available assays, i.e., the RealStar CMV PCR kit (ADT), the Sentosa SA CMV quantitative PCR kit (Vela Diagnostics Germany GmbH), and the CMV R-gene PCR kit (bioMérieux) were determined using a 2-fold dilution series of plasma inoculated with the 1st WHO International Standard for human cytomegalovirus for nucleic acid amplification techniques. DNA was extracted using the Viral 5K kit for minipool (MP)-NAT and the NucliSens easyMAG system for individual donation (ID)-NAT. Analysis was performed for each assay with 6 dilution steps and 24 repli-

cates. The 95% limit of detection (LOD) was calculated by probit analysis using the SPSS software (IBM SPSS Statistics, version 21; IBM GmbH, Ehningen, Germany).

Serological testing. Plasma from CMV DNA-positive donors was screened for the presence of CMV-specific IgM and IgG antibodies with a Liaison XL instrument, using Liaison CMV IgG II and CMV IgM II immunoassays (Diasorin Germany GmbH, Dietzenbach, Germany). The presence of CMV-specific IgA antibodies and IgG avidity was determined using the corresponding anti-CMV enzyme-linked immunosorbent assays (ELISAs) (IgA and CMV IgG avidity; Euroimmun AG, Lübeck, Germany). The CMV-specific antigens in samples from donors were further characterized using the recomLine CMV IgG/IgM assay (Mikrogen GmbH, Neuried, Germany). Samples were analyzed according to the manufacturer's instructions.

RESULTS

Screening of blood donors. A total of 6 of 18,405 individual blood donors were CMV DNA positive (0.03%) (Table 1). The serostatus and antibody avidity revealed that all donors had acute primary CMV infections; the donors denied any current diseases during predonation medical examinations. One donor had a NAT-only-positive donation, and a second donor had a positive IgM titer but the IgG result remained negative. Confirmation of CMV infections was performed by determination of the serostatus in subsequent samples, showing complete seroconversion with IgM-negative and IgG-positive status for all donors. CMV DNA-positive donations were immediately rejected for transfusion.

Progression of asymptomatic CMV infections. The progression of CMV DNA and CMV-specific IgA, IgM, and IgG antibodies in plasma is shown in Fig. 1. The day of the first detection of CMV DNA by PCR screening was defined as day 0. CMV viremia in samples from donor 1 lasted over 130 days, with alternating positive and negative results and a maximum value of 2.98×10^3 IU/ml. IgA, IgM, and IgG antibodies were detected in parallel at the first sampling point. The maximum IgM titer was observed at that sampling point, followed by a rapid decrease until day 15 and a subsequent linear decrease toward the reference range. However, IgM titers above the reference range were still detectable after 130 days. The IgG titer was still increasing after the first detection of CMV DNA, to a maximum value of 71.2 U/ml on day 3, followed by consistent titers at that level until day 130. IgA antibodies were clearly detectable at the first and second sampling points, followed by alternating positive and negative values around the reference range. Low-affinity antibodies were observed until day 88, followed by borderline results for the next sampling time points.

For donor 2, maximum viremia was also observed on day 0, followed by a continuous decrease until day 95. Similarly, CMV-

FIG 1 Progression of CMV DNA and anti-CMV IgA, IgM, and IgG antibodies. Displayed are the courses of CMV DNA concentrations (○) and immune responses, i.e., CMV IgA (▽), CMV IgM (□), and CMV IgG (◆) levels. The day of detection of CMV DNA by PCR screening was defined as day 0. Gray horizontal lines, cutoff values for the different serological assays.

specific IgA, IgM, and IgG antibodies were present on day 0. The IgM and IgA titers continuously decreased toward borderline values after 30 days, with negative values after 98 days; IgG titers continuously increased to 97.5 U/ml. Low-avidity antibodies were observed within the first 38 days, changing to high avidity after 98 days. In samples from donor 5, CMV DNA was undetectable subsequent to the initial positive screening, followed by intermittently positive and negative results. IgA and IgM antibodies were already detectable on day 0, followed by decreasing values, whereas IgG antibodies were first detected after 34 days. Only low-affinity antibodies were observed until day 34. Samples from donor 6 showed DNA-positive results in a previous donation that had been collected outside the study period. That donation showed no anti-CMV IgA, IgM, or IgG. The viremia of that donor reached the maximum value of 3.30×10^3 IU/ml on day 0 and continuously decreased until its disappearance on day 46. IgA was detectable on day 0, in parallel with the maximum IgM titer (>140 U/ml), followed by borderline results for the next 17 days and disappearance after 28 days. IgM titers continuously decreased toward negative values after 63 days, which was linked to a parallel constant increase in IgG titers. Low-affinity antibodies were observed on day 7, followed by borderline results between day 46 and 63.

All donors showed antibodies (IgM and IgG) against nonstructural or tegument proteins in the seropositive samples (see Table S1 in the supplemental material). Antibodies (IgG) against membrane glycoprotein B (gB) were detected only in IgG-positive samples (see Table S1 in the supplemental material). Donor 3 was not tested, since no anti-CMV IgA, IgM, or IgG antibodies were detected.

Comparison of NAT assays. The analytical sensitivities and precisions of different assays, which potentially can be implemented in our routine viral nucleic acid screening, are shown in Table 2 (MP-NAT) and Table 3 (ID-NAT). For blood donor pool screening, all assays showed almost equally high analytical sensitivities, with 95% detection limits of 10.23 IU/ml (95% confidence interval [CI], 7.87 to 15.81 IU/ml) for the Sentosa SA CMV quantitative PCR kit, 11.08 IU/ml (95% CI, 7.11 to 47.94 IU/ml) for the CMV R-gene PCR kit, and 11.14 IU/ml (95% CI, 8.49 to 17.65 IU/ml) for the RealStar CMV PCR kit. The Sentosa SA CMV quantitative PCR kit had the greatest sensitivity, i.e., 37.66 IU/ml (95% CI, 25.31 to 144.39 IU/ml), for individual donor screening using the Nuclisens easyMAG system for DNA extraction, followed by the RealStar CMV PCR kit with 49.30 IU/ml (95% CI, 39.60 to 68.63 IU/ml) and the R-gene PCR kit with 57.94 IU/ml (95% CI, 46.04 to 82.42 IU/ml). With LODs being determined with reference to the Sentosa SA CMV quantitative PCR kit as the most sensitive assay, deviations in the LODs ranged from 0.85 to 0.91 IU/ml (for MP-NAT, with extraction with the Chemagen Viral 5k kit) or from 11.61 to 20.28 IU/ml (for ID-NAT, with extraction with the Nuclisens easyMAG system) for the two other assays. The CIs for all assays overlapped over a wide range, and the mean values for the CMV R-gene PCR kit and the RealStar CMV PCR kit were located within the confidence interval for the Sentosa SA CMV quantitative PCR kit; therefore, there is no evidence that the assays had statistically significant differences. Wider fluctuations observed using the Nuclisens easyMAG extraction method might be due to the smaller input volume.

The reproducibility of all assays was demonstrated by analyzing the intra-assay and interassay variations for the cycle threshold

TABLE 2 Analytical sensitivity of pool screening (MP-NAT)^a

Parameter ^b	RealStar CMV PCR kit	Sentosa SA CMV PCR kit	CMV R-gene PCR kit
No. positive/total no. (% positive) for sample concn of:			
25 IU/ml	24/24 (100)	24/24 (100)	24/24 (100)
12.5 IU/ml	23/24 (97.1)	23/24 (98.2)	22/24 (97.3)
6.25 IU/ml	18/24 (77.7)	21/24 (79.8)	21/24 (75.1)
3.13 IU/ml	18/24 (57.8)	14/24 (57.9)	16/24 (52.0)
1.56 IU/ml	10/24 (46.5)	10/24 (45.2)	7/24 (39.6)
0.78 IU/ml	8/24 (41.0)	9/24 (39.0)	6/24 (33.7)
95% detection limit (95% CI) (IU/ml)	11.14 (8.49–17.65)	10.23 (7.87–15.81)	11.08 (7.11–47.94)
Intra-assay variability (mean $C_T \pm$ SD) (VC%) ^c			
CMV	30.65 $\pm$ 0.52 (1.71)	32.54 $\pm$ 0.73 (2.25)	34.50 $\pm$ 0.53 (1.52)
IC	22.7 $\pm$ 0.18 (0.79)	29.64 $\pm$ 0.85 (2.88)	28.43 $\pm$ 0.35 (1.22)
Interassay variability (mean $C_T \pm$ SD) (VC%) ^c			
CMV	30.99 $\pm$ 1.19 (3.84)	32.27 $\pm$ 0.97 (3.01)	34.83 $\pm$ 1.49 (4.29)
IC	22.15 $\pm$ 0.98 (4.42)	30.53 $\pm$ 1.05 (3.44)	27.90 $\pm$ 0.69 (2.47)

^a Extraction was performed with the Chemagen Viral 5K kit (4.8 ml of plasma).^b C_T , cycle threshold; SD, standard deviation; VC%, variation coefficient; IC, internal control.^c Intra-assay and interassay variabilities were calculated for the concentration of 25 IU/ml as an example.

(C_T) values. The intra-assay variability was calculated from eight replicates, and the interassay variability was determined from three independent PCR runs with eight replicates per run. The standard deviations for the CMV target and the internal control were C_T values of <1.0 , and the intra-assay and interassay variations were less than 5% for all evaluated assays, independent of the extraction method used (Tables 2 and 3).

DISCUSSION

It has been shown that the concentrations of plasma CMV DNA correlate with the progression of CMV disease (13, 14). Therefore, the molecular detection of CMV DNA is essential for the diagnosis of acute CMV and the assessment of disease progression through monitoring of active CMV infections (virus clearance versus persistence). Early studies using different PCR assays, mainly in-

house tests, revealed considerable differences in their sensitivities and specificities, confirming the need for implementation of validated CMV assays (15). Therefore, the performance of different commercially available amplification systems was evaluated. These methods could be used for screening of individuals with acute or chronic infections as well as for blood donor pool screening, since the prevention of TT-CMV infections is the subject of recurrent debate (16). The risk of TT-CMV infections in at-risk populations was reduced by 92% after the introduction of leukodepletion in 2002 and the provision of CMV-seronegative blood products (4). However, a residual risk of TT-CMV breakthrough infections persists, on the order of 1 to 3% among high-risk patients who receive transfusions (4, 5). This remaining risk is most likely based on residual white blood cells (WBCs) despite leukodepletion. Additionally, non-cell-associated infectious viri-

TABLE 3 Analytical sensitivity of single-donation testing (ID-NAT)^a

Parameter ^b	RealStar CMV PCR kit	Sentosa SA CMV PCR kit	CMV R-gene PCR kit
No. positive/total no. (% positive) for sample concn of:			
100 IU/ml	24/24 (100)	24/24 (100)	24/24 (100)
50 IU/ml	22/24 (95.4)	24/24 (99.6)	20/24 (90.4)
25 IU/ml	17/24 (64.5)	17/24 (73.1)	19/24 (59.1)
12.5 IU/ml	12/24 (38.9)	7/24 (34.4)	8/24 (38.0)
6.25 IU/ml	6/24 (27.1)	9/24 (18.2)	7/24 (28.3)
3.13 IU/ml	3/24 (22.0)	0/24 (12.2)	4/24 (23.9)
95% detection limit (95% CI) (IU/ml)	49.30 (39.60–68.63)	37.66 (25.31–144.39)	57.94 (46.04–82.42)
Intra-assay variability (mean $C_T \pm$ SD) (VC%) ^c			
CMV	31.08 $\pm$ 0.60 (1.93)	32.83 $\pm$ 0.49 (1.50)	36.05 $\pm$ 0.61 (1.69)
IC	20.96 $\pm$ 0.30 (1.45)	32.24 $\pm$ 0.65 (2.02)	27.84 $\pm$ 0.09 (0.32)
Interassay variability (mean $C_T \pm$ SD) (VC%) ^c			
CMV	31.36 $\pm$ 0.95 (3.04)	32.66 $\pm$ 0.65 (1.98)	35.68 $\pm$ 0.92 (2.56)
IC	21.13 $\pm$ 0.54 (2.58)	31.51 $\pm$ 0.85 (2.70)	27.65 $\pm$ 0.35 (1.26)

^a Extraction was performed with the Nuclisens easyMAG system (0.5 ml of plasma).^b C_T , cycle threshold; SD, standard deviation; VC%, variation coefficient; IC, internal control.^c Intra-assay and interassay variabilities were calculated for the concentration of 100 IU/ml as an example.

ons related to the detection of plasma CMV DNA, as observed in primary CMV infections or reactivation of latent infections (6, 17, 18), might be a cause of breakthrough infections. Currently, three different strategies in addition to leukodepletion, with different assets and drawbacks, have been proposed to avoid TT-CMV infection, i.e., (i) provision of seronegative blood products (19), (ii) transfusion of blood products from long-term-seropositive donors (16), or (iii) transfusion of CMV DNA-negative blood products (20).

The transfusion of CMV-seronegative blood products is a reasonable approach to avoid the transfusion of blood products from donors with latent infections or late-phase primary infections, but it could involve the risk of transfusion of blood products from donors with primary CMV infections (window-period donations), since IgG antibodies after primary CMV infection do not occur until 6 to 8 weeks after infection (11). A published report of CMV breakthrough cases after transfusion of CMV-seronegative blood products (21) suggested a role for window-period donations, but further studies are required to assess the incidence of window-period donations (12, 22). Indeed, the infectivity of these window-period donations needs to be elucidated, and even seronegative donors in the window period may present a low risk regarding TT-CMV infection (23). To our knowledge, the characterization of window-period donations is limited to one study that detected four individual donors (median CMV DNA concentrations, 40 IU/ml in plasma and 400 IU/ml in whole-blood samples) (6). We identified one window-period donation in this study, with a plasma CMV DNA concentration of 5.18×10^2 IU/ml.

The transfusion of blood products from long-term-seropositive donors is connected to the residual risk of reactivation of latent infections. To date, however, CMV DNA has not been observed in plasma samples from CMV-seropositive donors (positive for anti-CMV IgA, IgM, and IgG [24] or anti-CMV IgG [18]). Recently, Ziemann et al. proposed a novel strategy that included the provision of WBC-reduced blood products from donors with anti-CMV seropositivity for >1 year (6). Transfusion of CMV DNA-negative blood products contains major requirements for blood donation screening. The most rapid and effective solution would be the implementation of CMV screening within the current routine screening procedures, which most often include minipool screening of up to 96 plasma samples. Currently available MP screening procedures are restricted to screening of donor plasma for non-cell-associated viral particles, since the availability of methods for whole-blood screening is limited to single-donation testing, with major cost implications. In this study, we demonstrate the technical feasibility of CMV blood donor screening on a routine basis, with compatibility with the virus NAT screening used in our blood transfusion service. Indeed, CMV DNA in plasma from newly seropositive donors has been detected with different frequencies, ranging from 1.6% (24) to 44% (18), most likely as a result of significant differences in assay sensitivities or long intervals between the last seronegative sample and the subsequent seropositive sample (18). The annual seroconversion rate among German blood donors was determined to be 1% (3, 5, 10). The prevalence of primary CMV infections among blood donors was determined to be between 0.2 and 1.2% (3, 8, 9). Taking these findings into account, the detection of CMV DNA in only 0.03% of 54,451 donations in this study is at least 1 order of magnitude lower than the expected rate, which is most likely due to the large

pool size of 96 samples. However, we initially selected the current pooling strategy to demonstrate the feasibility of CMV NAT blood donor screening. We have shown that the sensitivity of the methods compared in this study allowed both MP screening to identify CMV DNA-positive donors and screening of individuals for the presence of CMV DNA. However, the pool size needs to be adjusted in the future, since concerns remain regarding the required sensitivity. Unfortunately, the absence of detailed knowledge of important factors regarding transfusion-associated CMV infections, including the infectious dose, the infectivity of cell-associated versus free CMV DNA, the influence of neutralizing antibodies (5), the role of anti-CMV antibodies in the recipient, and the frequency of clinically apparent transfusion-transmitted CMV infections, limits determination of the minimum required sensitivity. Furthermore, it remains to be determined whether plasma CMV DNA is infectious. The presence of CMV DNA in the blood cannot be equated inevitably with the presence of infectious virus (16). Several studies analyzed the presence of viable CMV in the peripheral blood of donors but, with the exception of a single study by Diosi et al. (25), viral cultures often failed to detect viable CMV (26), most likely due to the reduced detection limit of shell vial assays (27). To date, the correlation of CMV DNA in the blood with the presence of infectious virus remains unresolved.

Regarding the sensitivity of 11.14 IU/ml determined for MP-NAT and the observed CMV DNA concentrations in affected blood donors, we have decided to reduce the initial pool size to 24 samples in further studies. This pool size will provide enhanced sensitivity of 268 IU/ml per single donation in our setting, covering at least the majority of viremic phases observed during the demonstrated progression of CMV viremia in this and other studies (10). Viral loads observed in primary CMV infections ranged from 40 to 500 IU/ml (window period [6; this study]) to over 1,000 IU/ml (6), 1,600 copies/ml (24), 4,500 genomic equivalents/ml (conversion factor of approximately 1:1 for units per milliliter) (10), and 5,000 IU/ml (this study). It remains to be seen whether this approach would detect all relevant viremic phases that could result in TT-CMV infections.

The natural course of CMV infections was described for 13 blood donors, revealing maximum DNA levels in plasma ranging from <100 to 4,500 genomic equivalents/ml and a medium time to DNA clearance of 137 days (10). Interestingly, comparable courses of DNA levels in plasma and WBCs were observed, with slightly longer CMV DNA detection and considerably higher concentrations in plasma than in WBCs. We observed a similar progression, although the alternating positive and negative detection of CMV DNA in samples from donor 1 and donor 5 might be due to the analysis of plasma samples instead of whole-blood samples. Since two of the six positive samples found in this study demonstrated this pattern, the following question arose: how many of the negative samples were actually CMV positive but were not detected? This could be the result of lack of sensitivity for plasma samples or could be caused by the natural course of CMV infection. There is an ongoing debate regarding which specimen type (whole blood or plasma) should be used for CMV detection. The frequency of detection of CMV in whole blood depends strongly on the extraction method used and the sensitivity of the downstream amplification assay. Measuring CMV viral loads in whole-blood specimens can enhance test sensitivity, but it was demonstrated that virus levels in plasma correlated reasonably well with levels in whole blood, suggesting that both were indicative of ac-

tive infection (28). It remains unclear whether extremely low levels of CMV DNA that were detectable only in whole-blood samples correlated with a relevant viremic phase or infectious dose that could result in TT-CMV infections, since blood products were further treated by leukodepletion before transfusion.

Finally, the definition of day 0 for the first detection of CMV DNA by blood donor screening limits exact conclusions regarding the duration of viremia, the occurrence and presence of CMV-specific antibodies, and calculation of the diagnostic window. Earlier occurrence of CMV viremia is most likely, which is supported by the presence of CMV-specific IgA, IgM, and IgG antibodies on day 0. Furthermore, the accumulation of asymptomatic CMV infections observed in this study represents the preselection of apparently healthy, immunocompetent individuals voluntarily donating blood and lacking detectable physical symptoms.

Identification of blood donors with acute (window-period) CMV infections could be ensured only by molecular genetic screening using a sensitive PCR method. The currently used strategy involving the transfusion of CMV-seronegative blood products will invariably miss such individuals. In conclusion, we demonstrated sensitive, reliable, and uncomplicated implementation of a semiautomated screening method during routine analysis of blood donors for the presence of CMV DNA. The occurrence of TT-CMV infections requires further investigation, and the evaluated methods present powerful basic tools providing sensitive possibilities for viral testing.

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