(IU) per mL.[1] It is unknown if recipients of pooled plasma products with low B19 viral titers are protected due to the neutralizing effect of B19 antibody from other units in the plasma pool, the low B19 viral titer, or a combination of both.[10] Although it has been assumed that single-unit blood components with low B19 DNA titers should, similarly, be noninfectious, this remains speculative because the mechanism of protection in the pooled plasma setting has not been established and may not apply to single-unit transfusions.

Newer information suggests that the potential for recipients to be exposed to low titers of B19 DNA from blood component transfusion is greater than previously thought. Through the use of sensitive nucleic acid test (NAT) assays, two sets of investigators have found that the prevalence of B19 DNA in donor plasma ranges from 0.5 to 0.9 percent.[11,12] Furthermore, it is now known that B19 DNA may persist in plasma at low concentration for several years in healthy individuals who could make repeat blood donations during this viremic interval.[13-16]

From 2000 through 2003, NHLBI and CDC established the Retrovirus Epidemiology Donor Study Allogeneic Donor and Recipient (RADAR) repository as a powerful tool to investigate possible transfusion-transmitted infections.[17] Our primary aim with regard to B19 infection was to use this linked donor and recipient repository to evaluate whether donations with low B19 DNA levels transmit infection. Such a transfusion transmission study would only be feasible, however, if a B19 NAT assay with appropriate performance characteristics (e.g., sensitivity, specificity, throughput) was available and if the prevalence of plasma B19 DNA in the donor population and the number of susceptible recipients were of sufficient magnitude to allow for significant conclusions to be drawn.

The primary aim of this report is to present the results of these initial investigations, which include development of a highly sensitive polymerase chain reaction (PCR) screening assay and estimation of the prevalence of plasma B19 DNA in donations represented in the RADAR repository. Our secondary aims were to evaluate the association of quantitative DNA levels with immunoglobulin M (IgM) and IgG antibody status and determine the demographic characteristics of B19 DNA–positive donors.

MATERIALS AND METHODS

Selection of repository specimens

The RADAR repository was established from 2000 through 2003 through participation of blood centers and selected hospitals at seven geographically dispersed locations throughout the United States.[17] This donor and recipient repository contains pretransfusion (or peritransfusion) specimens and follow-up specimens (collected at an interval of 6-12 months) from 3,575 enrolled recipients. The repository also contains 13,201 donation specimens given by 12,408 distinct donors that were transfused to these RADAR recipients. This portion of the repository is referred to as the linked donor-recipient repository. In addition, there is a supplementary repository of 99,906 donation specimens (contributed by 84,339 donors) from donations that were not transfused to enrolled RADAR recipients.

As previously reported, transfusion transmission studies with the RADAR-linked repository should usually only be considered if the donor prevalence of an agent is at least 0.05 percent.[17] For this study, based on some of the conservative estimates of donor B19 viremia in the literature, we determined that testing of approximately 5,000 specimens would allow us to be 95 percent confident that the prevalence of viremia in the donor population was at least 0.05 percent. Thus, 5,200 specimens (allowing for failed runs) were selected from the repository of unlinked community whole-blood and apheresis donations for B19 DNA PCR testing. A stratified sampling procedure was used to select these specimens so that they would have similar demographic, temporal, and geographic characteristics to the 13,198 community whole-blood and apheresis donation samples in the linked repository, thereby allowing for later extrapolation of the prevalence results to donations in the linked repository. The sampling scheme controlled for frequency of donations per donor, blood center where donation was given, and year and month of donation, in that order. This stratification also ensured that the distributions of other important variables, for example, age at time of donation, first-time or repeat status, and race/ethnicity were similar between the 5,200 sampled unlinked donations and the 13,198 donation samples in the linked repository. The similarity of the sampled supplementary repository subset and the linked donations was verified after the sample was selected.

A 1.75-mL frozen plasma tube for each selected donation was accessed from the repository by personnel at the long-term storage facility (SeraCare BioServices, Gaithersburg, MD). Each specimen was aliquoted into three 0.5-mL subaliquots (one for B19 DNA screening and two for B19 DNA confirmation and quantitation) and one 0.25-mL aliquot (for antibody testing) with rigorous precautions to minimize the possibility of cross-sample contamination.

PCR assay development, validation, and performance characteristics

The B19 DNA assay used in this study was originally developed by Chiron Corp. (Emeryville, CA) and subsequently refined through a collaboration between Chiron and Blood Systems Research Institute (San Francisco, CA).[18] The assay format includes a magnetic-bead B19 DNA capture step followed by a real-time PCR assay that targets the VP1 region of the B19 genome. An internal control, sharing homologous primer region sequences but with a different internal probe binding sequence as the viral

target, is included in each assay tube. B19 DNA target and the internal control nucleic acid are amplified by the same primer pair but detected and distinguished by fluorophore-tagged sequence-specific probes. Five-hundred microliters of frozen plasma, thawed at room temperature, was vortexed and centrifuged briefly before the addition of lysis buffer, poly(T)-coupled magnetic beads (Seradyn, Indianapolis, IN), four viral capture primers (VSCP1, VSCP4, VSCP5, and VSCP7) with poly(A) tail, and 20 copies of internal control. The preparation was vortexed for 10 seconds and incubated in a 60°C water bath for 20 minutes, followed by incubation at room temperature for 15 minutes. The tubes were placed on a magnetic base for 10 minutes before the liquid was vacuum-aspirated. The beads were washed once with 1 mL of wash buffer (Procleix, Gen-Probe, San Diego, CA) and twice with another wash buffer (Chiron Novartis, Emeryville, CA).

All captured target DNA from the 0.5-mL input plasma and captured spiked internal control were subjected to amplification in a single PCR procedure and amplification and detection occurred in a 96-well optical plate with dual-plexed TaqMan PCR technology. TaqMan 1000 Rxn PCR core reagents were purchased from Applied Biosystems (Foster City, CA). The PCR mix was prepared by mixing 10 µL of Buffer A; 1 µL of the enzyme uracil-*N*-glycosylase (Amperase [Roche Diagnostics, Indianapolis, IN], which reduces contamination by degrading dUTP-containing amplicons from prior amplification reactions); 20 µL of $MgCl_2$; 10 µL of dATP, dCTP, dGTP, and dUTP; 0.5 µL of AmpliTaq Gold; 56 µL of sterilized water; 0.9 µL each of two amplification primers at 100 pmol per µL (VSCP8, VSCP9); and 0.25 µL of each of the two probes at 100 pmol per µL (VSCP10, VB-TAM) per sample. One-hundred microliters of the mix was added to each sample instead of the manufacturer-suggested 50 µL per sample. PCR was performed with 50 cycles of 95°C for 15 seconds and 60°C for 1 minute, after the initial Amperase (50°C for 2 min) and AmpliTaq Gold activation (95°C for 10 min). The DNA was amplified and detected with a real-time PCR system (ABI 7500, Applied Biosystems).

Features of the assay system that minimize risk of specimen-to-specimen cross-contamination of plasma or "carryover" amplicon contamination include single-tube magnetic bead target-capture and DNA purification with the Chiron/Gen-Probe–enhanced semiautomated system, single-tube amplification, and real-time monitoring of fluorescent probe binding to amplicon products with no subsequent manipulation of reaction wells; use of dUTP and UNG in each assay to destroy previous B19 amplicons before amplification; and single-use disposable reaction tubes and plates. Segregated laboratories were used for sample accessioning and preparation, preamplification target-capture, and real-time PCR.

Preliminary assay development work used a series of dilutions of the CBER parvovirus B19 DNA standard to determine where to set the assay cutoff as well as to estimate the resultant assay analytic sensitivity. Figure 1 shows box and whisker plots of testing results for 30 replicates at each of four dilutions (30, 15, 7.5, and 3.75 IU/mL). Based on these studies, the assay cutoff was established as follows: a specimen was classified as reactive if a signal was detected at not more than 40 cycles (cycle threshold [C_T] ≤ 40), indeterminate if C_T was more than 40 but not more than 45, and negative if there was no signal detected or if a C_T was more than 45. An apparent negative result was interpreted as invalid if the C_T of the internal control was more than 45.

Because the chosen assay cutoff was designed to maximize assay sensitivity, an algorithm was developed for final test interpretation so as to avoid classifying nonspecific reactivity on a single assay run as a confirmed-positive result (see Fig. 2). All initially positive, initially indeterminate, and invalid specimens were retested in duplicate with two separate 0.5-mL subaliquots on plates that included quantitative run standards. This testing

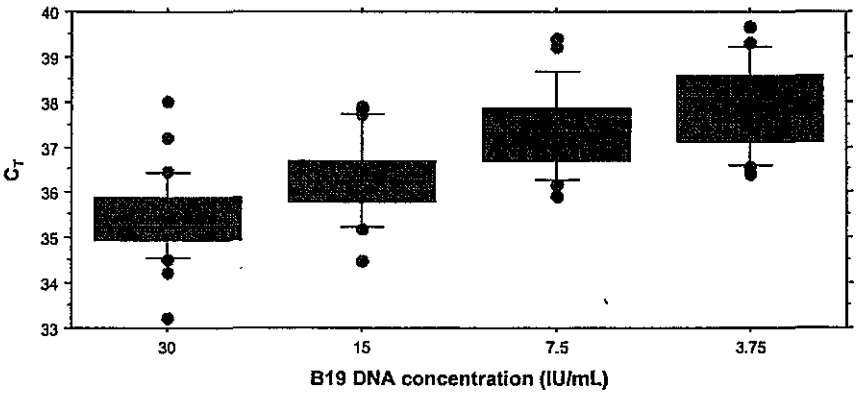

Fig. 1. Assay validation and selection of assay cutoff based on replicate testing of dilutions of the CBER parvovirus B19 DNA standard. The input per amplification was 0.5 mL. Twofold dilutions of CBER-validated B19 DNA standard were run with 30 replicates at each concentration. The standard was diluted with pooled plasma negative for B19 DNA and B19 antibody. The y-axis represents PCR C_T. The x-axis represents B19 DNA concentration per milliliter of plasma. The top and bottom whiskers on the box plot represent the 90th and 10th percentiles, respectively. The top of the box represents the 75th percentile and the bottom of the box represents the 25th percentile. The line inside the box represents the median. Negative plasma aliquots were also tested (data not shown). Of 204 B19-negative plasma aliquots, 203 yielded negative assay results (C_T > 40). One negative control sample amplified at 36.30 C_T.

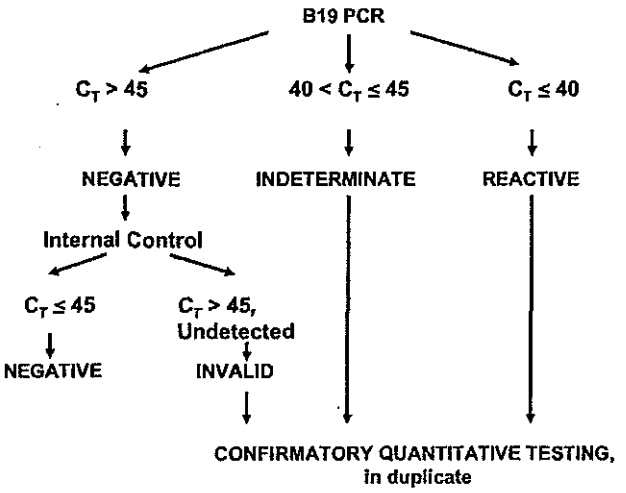

Fig. 2. B19 DNA testing algorithm.

served both as confirmation and as quantitation. The final interpretation of the qualitative PCR assay was based on the results of the three individual assays (i.e., the initial screening assay and the duplicate repeat assays). Specimens were classified as B19 DNA–positive if at least two of three tests showed reactivity at a C_T of not more than 40 cycles and indeterminate if at least two tests showed reactivity at a C_T of not more than 45, with one or both of these C_T values more than 40, and negative if both of the duplicate repeat assays were negative.

For determining DNA concentration, quantitative run standards were placed on each plate in duplicate. For confirmation and quantitation of initially reactive specimens, standards from 1000 to 31.25 IU per mL were tested in twofold dilutions. For repeat testing of indeterminate and invalid specimens, standards from 125 to 31.25 IU per mL were tested in twofold dilutions to prevent any cross-contamination of the specimens from high-titer standards. Quantitative results were determined by comparing the C_T of the specimen to the C_T of the known standards on the same test run. The assigned quantitative value for each specimen was the average of the two duplicate quantitative assays (including zero for a negative test result). Specimens with low C_T (C_T < 30) were diluted 1:10 and 1:100 and then run in triplicate at each dilution, and the quantitative result was the mean of the three test results at the most appropriate dilution.

Analysis of additional replicates of the CBER standard (30-60 replicates performed on twofold serial dilutions with concentrations of 30, 15, 7.5, 3.75, 1.88, and 0.94 IU/mL) established that the 50 percent limit of detection (LOD) of the assay was 1.6 IU per mL (95% confidence interval [CI], 1.2-2.1 IU/mL), and the 95 percent LOD was 16.5 IU per mL (95% CI, 10.6-33.9 IU/mL). To allow for the possibility that quantitation might not be precise at the lower limits of detection, we categorized all specimens with quantitative values of 0 to less than 20 IU per mL as having a value of less than 20 IU per mL.

PCR testing of study specimens

Initial B19 DNA testing was performed in singlicate with one 0.5-mL plasma aliquot. Testing was performed in 96-well microtiter plates. Each plate contained two known positive, two blinded negative, and two blinded positive controls as well as up to 90 study specimens. All positive controls were prepared by the testing laboratory (BSRI) from the CBER parvovirus B19 DNA standard and were diluted to contain 100 IU per mL B19 DNA. The known controls were introduced into each test batch by the testing laboratory whereas the blinded controls were introduced into each specimen batch by the repository facility. Runs were considered valid if at least one of the two known positive and one of the two known negative controls gave a valid, expected result. Figure 3 shows the high consistency of assay performance on the known and blinded positive control specimens for 56 screening test runs based on C_T; the C_T for the known controls (Fig. 3A) was 33.36 ± 2.96 and the C_T for the blinded controls at the same concentration was 34.09 ± 2.71 (Fig. 3B). All positive controls reacted with the exception of 5 of 112 known positive controls with invalid results and 1 of 112 blinded positive controls with a false-negative result. In addition, 110 of 112 negative controls were negative, 1 gave an invalid result, and 1 gave an indeterminate result.

All initially positive, indeterminate, and invalid specimens were rerun in duplicate with two separate subaliquots on plates that included quantitative run standards. Because of limitations of specimen volume, this testing served both as confirmation and as quantitation (see above).

B19 antibody testing

All confirmed B19 DNA–positive and indeterminate donations were tested for the presence of B19 IgG and IgM antibodies against a recombinant VP2 protein with FDA-licensed test kits (Biotrin, Dublin, Ireland). Testing was performed in singlicate with the 0.25-mL subaliquot. If results fell into the equivocal zone, the assay was repeated in singlicate and the repeat result was taken as the overall final result for the specimen.

Additionally, to determine IgG and IgM prevalence in B19 DNA–negative donations, we first randomly selected a subset of 520 donation specimens from the 5200 donations that had been selected for PCR testing (see above). This sampling occurred before obtaining the PCR results on the 5200 donations. IgG antibody testing was performed on 505 of the 520 donations, 501 of which were subsequently found to be B19 DNA–negative. For IgM antibody, due to kit availability issues, a random subset of

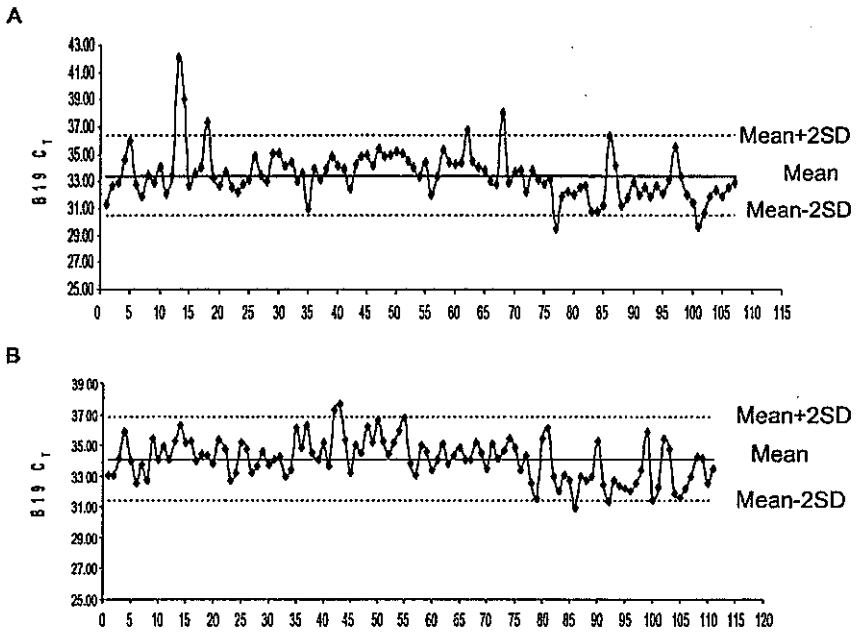

Fig. 3. (A) Control chart: 100 IU per mL known controls. Two known positive controls were included per run. The data plotted were controls for 56 plates, which includes 107 valid results and excludes 5 invalid results (C_T mean ± 2SD = 33.36 ± 2.96). (B) Control chart: 100 IU per mL blinded controls. Two blinded positive controls were included per run along with two blinded negative controls (data not shown). The data plotted were positive controls for 56 plates, which includes 111 valid results and one false-negative result (C_T mean ± 2SD = 34.09 ± 2.71).

194 of 366 of these specimens identified as IgG-positive were tested.

Demographics

The following information was available for each donation in the RADAR repository: donor identification number, age at time of donation (categorized as ≤25, 26-35, 36-45, 46-55, 56-65, ≥66), sex, race/ethnicity (Asian, black non-Hispanic, Hispanic, white non-Hispanic, other non-Hispanic), first-time or repeat donor status, education level (<high school, high school degree, some college education, college degree, graduate or professional degree), history of transfusion, the center at which the donation was collected, and date of donation (categorized by calendar year of donation or by season, i.e., occurring in the winter, spring, summer, and fall).

Statistical analysis

We calculated the proportion of donations that were confirmed positive by PCR with associated 95 percent CI as well as the prevalence of IgM and IgG (and their 95% CI) in DNA-positive and DNA-negative donations. We evaluated whether the prevalence of IgM in DNA-positive donations varied as a function of B19 DNA level (categorized as <20, 20 to $<10^2$, 10^2 to $<10^3$, and 10^3 to $<10^4$ IU/mL) by conducting a Fisher's exact test (SAS/STAT 9.1, 2004, SAS Institute, Inc.). We determined the 50 and 95 percent LOD of our PCR assay with associated 95 percent CI by probit analysis with PC SAS Version 8.2.

We compared the distribution of demographic characteristics between donation groups (i.e., DNA-positive and DNA-negative donations) with chi-square statistics or, for small cell sizes, either the Fisher's exact test (SAS/STAT 9.1, SAS Institute, Inc.) or the Fisher-Freeman-Halton test (StatXact Version 6, 2004, Cytel Software Corp., Cambridge, MA). This latter test is a generalization of the Fisher's exact test for 2 by 2, to an r-by-c contingency table.

RESULTS

Of the 5200 specimens originally selected for DNA testing, screening results were obtained for 5020. Results were not obtained for 180 specimens that were part of two runs which failed due to equipment problems. There were 113 initially reactive specimens (2.25%), 26 initially indeterminate specimens (0.52%), 56 initially invalid specimens (1.12%), and 4825 negative specimens. After retesting, 43 of the initially reactive specimens confirmed as positive, 2 were reclassified as indeterminate, and 68 were reclassified as negative. For the indeterminate specimens, 1 was reclassified as positive, 22 remained indeterminate, and 3 became negative. All initially invalid specimens retested as negative.

Summarizing the screening and retesting results, we found that 44 specimens (0.88%) were DNA-positive, 5 (0.10%) were indeterminate, and 4971 (99.02%) were negative. In 35 (80%) of the confirmed-positive specimens, all three tested replicates reacted in the PCR assay. DNA prevalence was 0.88 percent with a 95 percent CI of 0.64 to 1.2 percent.

Figure 4 shows that the percentage of confirmed-positive specimens was inversely related to the C_T value obtained on the initial screening test run. Specimens that initially reacted at a C_T value of less than 37 were confirmed as positive 86 percent of the time, whereas specimens with a C_T value of between 37 and 40 were confirmed 16 percent of the time.

Table 1 presents the quantitative DNA levels grouped into four categories as well as the antibody status of the 44 B19 DNA confirmed-positive donations. The median DNA level for all of our confirmed-positive donors was 22.75 IU

per mL. Twenty-one of 44 specimens had DNA levels of less than 20 IU per mL (conservatively determined to be the lower limit of quantitation of the assay), and if we consider only those donors who had DNA levels of more than 20 IU per mL, then the median DNA level for these 23 donors was 105 IU per mL (interquartile range, 42-481 IU/mL), with the highest value being 1869 IU per mL. Specimens with reactivity on two of the three replicates had lower DNA levels than specimens reactive on all three replicates (data not shown).

All B19 DNA confirmed-positive donations had detectable B19 IgG antibody, whereas in the control group of 501 PCR-negative donors, IgG was present in 73 percent (95% CI, 68%-77%). IgM antibody was detected in 10 B19 DNA confirmed-positive donations and was assigned an equivocal status in 2 additional cases. IgM seropositivity was associated with increasing DNA concentration ($p = 0.0013$). The median DNA level for the 10 IgM-positive donations was 297 IU per mL, and all three donors with B19 DNA titers of more than 10^3 IU per mL were IgM-positive. IgM was not detected in any of 194 DNA-negative, IgG-positive donors (95% CI, 0.00%-1.88%).

Donors who were not more than 45 years old were more likely to be viremic than donors older than 45 years:

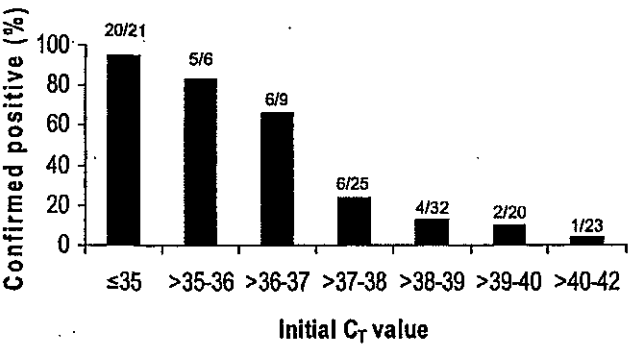

Fig. 4. Confirmation of B19 DNA reactivity relative to the C_T obtained on initial PCR screening. Confirmation of all 136 initially reactive specimens. There were no initially reactive specimens with C_T between 42 and 45 cycles. All 4884 specimens with a C_T value of more than 45 on the initial test run were classified as negative.

1.24, 1.30, and 1.41 percent of donors not more than 25, 26 to 35, and 36 to 45 years, respectively, were viremic compared to 0.23, 0.37, and 0.00 percent of donors 46 to 55, 56 to 65, and more than 65 years old ($p = 0.0008$). Further, 1.85 percent of first-time donors were viremic compared to 0.66 percent of repeat donors ($p = 0.007$). Although DNA prevalence estimates appeared to be higher for spring (1.14%) and summer (1.18%) donations than for fall (0.44%) and winter (0.67%) donations, these differences did not achieve significance ($p = 0.09$). There was no significant association with geographic region (based on center where the donation was given), calendar year of donation, sex, race/ethnicity, education level, or transfusion history.

DISCUSSION

In this study we applied a highly sensitive B19 DNA assay to 5020 individual donations to determine the prevalence of plasma B19 DNA in donors from seven different geographic areas of the United States using specimens collected over a 4-year interval. We believe this to be the largest such study performed on individual donations rather than on large pools of donations, as is routine in the plasma manufacturing sector.

We found that the prevalence of B19 DNA in plasma was 0.88 percent with a 95 percent CI of 0.64 to 1.2 percent. Our data can be compared with several recent European studies that used somewhat less sensitive B19 DNA assays. Thomas and coworkers[11] tested 16,859 Belgian blood donors in pools of 60 donations with an assay with 95 percent LOD of 96.6 IU per mL and found a B19 DNA prevalence of 0.16 percent. Candotti and colleagues[12] tested 1,000 UK whole-blood and platelet donors in minipools of 10 donation specimens and found a prevalence of 0.9 percent with a nested PCR with a 95 percent LOD of 25 IU per mL; this prevalence was very similar to that found in our study. Plentz and colleagues[8] found a 0.7 percent prevalence with an assay with a 50 percent LOD of 60 to 80 copies per mL when retrospectively testing 1,806 blood products transfused in a hematology ward. In contrast, studies reported in the plasma manufacturing sector have reported much lower prevalence, ranging from 0.008 to 0.04 percent; it is notable that these

TABLE 1. Quantitative PCR and antibody results on confirmed-positive specimens*

Viral load (IU/mL)	Number of confirmed-positive specimens	IgM-positive and IgG-positive	IgM-equivocal and IgG-positive	IgG-positive only
<20	21	1 (5)	1 (5)	19 (90)
20 to $<10^2$	11	2 (18)	0 (0)	9 (82)
10^2 to $<10^3$	9	4 (44)	1 (11)	4 (44)
10^3 to $<10^4$	3	3 (100)	0 (0)	0 (0)
Total	44	10 (23)	2 (4)	32 (73)

* Data are reported as number (%).

studies used NAT assays that were designed to lack sensitivity so as to only detect units from donors in the stage of acute viremia with DNA concentrations of more than 10^5 or 10^6 IU per mL.[19-21]

The generally accepted understanding of the natural history of B19 infection in immunocompetent individuals such as blood donors states that viremia occurs approximately 1 week after infection and persists in high titer for approximately 5 days. With the development of IgM antibody at approximately 12 days after infection (followed within days by IgG antibody), viremia levels drop precipitously and viremia usually disappears within weeks.[22,23] IgM antibody becomes undetectable after several months (although this precise duration is unknown) but IgG persists long term and is thought to convey immunity to reinfection. As a variation of this usual natural history, plasma viremia may persist for more than 6 months to several years in some cases, and recent data suggest that B19 may persist in other tissue sites (e.g., skin, synovia) for a much longer period of time in a significant percentage of individuals.[13-16,24]

The antibody findings in our study can be used to assess the stage of viremia that we detected in our B19 DNA–positive donors. We did not detect any B19 DNA–positive donors who lacked both IgM and IgG antibody nor did we detect any donors with a DNA concentration at or above 10^5 or 10^6 IU per mL, which would be characteristic of the several-day interval after infection. Given the low rate of detection of high-titer DNA in previous studies, it is not surprising that with the testing of 5020 donations in this study, we did not detect any such donations. We also did not detect any donors in the short window period where IgM antibody is present but IgG is absent. We detected 10 donors who were positive for the presence of IgM and IgG (23% of the 44 B19 DNA–positive donors) and an additional 2 who were IgM-equivocal. These donors were probably in a relatively early stage of infection, that is, within the first several months of acquiring infection. Consistent with the known natural history of B19 infection, the IgM-positive, DNA-positive donors had higher DNA levels than the IgM-negative, DNA-positive donors. We detected 32 DNA-positive donors who were IgG-positive only: 28 of these had DNA levels of less than 10^2 IU per mL; the median DNA level in these donors (as for all 44 DNA-positive donors) was lower than that previously reported by other investigators. We believe either that these DNA-positive, IgG-positive donors were at the tail end of resolving their B19 viremia or that some of these donors may have had very-low-titer B19 DNA that persisted for longer than predicted by the standard natural history model. Future longitudinal studies will be needed to distinguish these possibilities. The 73 percent prevalence of B19 IgG seropositivity and the lack of IgM antibody in our B19 DNA–negative control donors were consistent with reports in other donor cohorts.[11,12,25]

B19 infections are known to occur with a spring and summer preponderance and to vary in annual frequency in cycles that span several years.[25] In our study, the B19 DNA prevalence was higher in spring and summer donations, but did not achieve significance. This may be due to limitations in sample size or alternatively to our use of a highly sensitive NAT assay that may have allowed us to detect B19 DNA–positive donors for a relatively long period of time after acquisition of infection, thereby masking the expected temporal findings. The significant association with younger age (<age 46) may correlate with an increased likelihood of possible B19 exposure in young adults from contact with young children or with a lower degree of susceptibility among previously infected older adults. First-time donors showed higher prevalence of viremia than repeat donors, which may in part reflect the younger age distribution of first-time donors or may be due to other unexplained factors as has been seen with other infectious agents (e.g., human immunodeficiency virus, hepatitis C virus) in the donor population.[26]

The real-time B19 TaqMan PCR assay used in this study gave reproducible results on known standards, had a low failed run rate, gave a low rate of invalid specimens due to internal control failure, and showed no evidence of sample cross-contamination. Furthermore, the assay had a high analytic sensitivity at the chosen cutoff (50% LOD, 1.6 IU/mL; 95% LOD, 16.5 IU/mL). Although our choice of a relatively high C_T cutoff introduced nonspecificity on initial testing (presumably due to nonspecific probe binding), our confirmatory algorithm minimized false-positive results by requiring a reactive result on a second aliquot subjected to the full extraction, amplification, and detection procedure before designating the donation as confirmed positive for the presence of B19 DNA.

At present, interventions for preventing B19 transfusion from blood components have not been implemented in the vast majority of developed countries, due in part to the prevailing view that blood components with low levels of B19 DNA will not transmit B19 infection. Recently several authors have cited the need for studies to verify this hypothesis.[11,12] The results of the study reported in this article have established that there is sufficient statistical power to carry out such a B19 transfusion transmission study with the real-time B19 TaqMan PCR assay to test specimens in the RADAR repository. This conclusion is based on the demonstrated donor B19 DNA prevalence of 0.88 percent combined with a sufficient number of B19-susceptible recipients in the RADAR repository; that is, we observed that 22 percent of tested RADAR recipients were B19 IgG–negative on their pretransfusion specimen (data not shown), which was similar to the 27 percent prevalence of IgG seronegativity in tested donors. We are now actively engaged in per-

forming a linked B19 transfusion transmission study using the RADAR repository.

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