別紙様式第 2-1

医薬品
医薬部外品　研究報告　調査報告書
化粧品

別紙 3-5

識別番号・報告回数		回	報告日 年　月　日	第一報入手日 2007 年 10 月 11 日	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称			研究報告の公表状況	Blood donor screening for parvovirus B19 in Germany and Austria. Schmidt, M. et al, Transfusion, 47, 1775-1782 (2007).	公表国 ドイツ	
販売名（企業名）						

研究報告の概要	使用上の注意記載状況・その他参考事項等
ドイツ及びオーストリアで 4 年間総計 280 万の献血検体に対して行なわれた B19 ウイルススクリーニングの結果が報告された。測定期間中，2004 年 5 月から 2006 年 1 月におけるウイルス検出頻度が最も高かった。しかし，その B19 DNA 陽性の頻度は 0.274%と低く，B19 ウイルス 1 型のみが検出された。B19 DNA が 10E5 IU/mL を超えた 50 人のドナーからは，初回献血時（T0）から 3 及び 6 ヵ月後の 2 回採血が実施された。詳細な分析の結果，ウイルス価については，T0 時点の中央値が 4.85×10E7 IU/mL から 3 ヵ月後に 4.6×10E2 IU/mL へ有意に減少し，その後 6 ヵ月時点までそのまま推移した検体と，さらに減少した検体が認められた。同時に実施された B19 ウイルス抗体分析では，3 及び 6 ヵ月後の 50 人の全ての検体から，構造蛋白 VP2 に対する中和抗体（IgG）が認められた。従って，この抗体がウイルスを中和していると考えられた。本結果から，本試験に参加中の献血業者の出荷手順を以下のように変更した。 ・10E5 IU/mL を超える高濃度の B19 DNA が検出された献血検体は廃棄とした。しかし，ドナーはその後も献血を行うことができることとした。 ・B19 DNA が 10E5 IU/mL 未満である献血検体は中和抗体を含むため安全と考えられ，輸血された。 ・特殊なリスクを有する患者（小児，妊婦及び免疫が低下した患者）に対しては依然として B19 DNA 陰性の血液製剤が推奨された。	BYL-2008-0301

報告企業の意見	今後の対応
B19 ウイルスの検出頻度は測定の時期及び方法によって，1:260 から 1:50000 まで報告に幅がある。本論文では，4 年間で計 280 万サンプルを測定しており，B19 ウイルスの検出頻度を考慮する上で，信頼性の高いデータを示したと考えられる。また，10E5 IU/ml 以下の B19 ウイルスを含有する検体では相対的に高濃度となる中和抗体が存在し，安全であることが示され，感染リスクを考慮するために重要な情報が提供されていると考えられる。 弊社のポリグロビン N の製造に使用されるミニプール血漿においては，ヒトパルボウイルス B19 に対する NAT を実施しており，10E5 IU/mL 以上が確認された場合は，そのミニプール血漿は製造工程から除去している。現在の科学水準では，ヒトパルボウイルス B19 を確実に不活化する方法は存在しないため，感染リスクを完全に排除することはできないが，伝播の可能性は非常に低いと考える。	ウイルス検出及び安全性に関する閾値に関しては今後とも情報収集に努める。

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Blood donor screening for parvovirus B19 in Germany and Austria

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BACKGROUND: Although the main transmission pathway of parvovirus B19 (B19) is typically via the respiratory route, several transfusion-transmitted infections have been reported. To increase blood safety, all blood donations to our blood donor service have been screened by a B19 minipool real-time nucleic acid testing (NAT) since April 2000. Additional customers have been screened since the summer of 2003.

STUDY DESIGN AND METHODS: In total, 2.8 million donations from Germany and Austria were screened for B19 by real-time minipool NAT. A subgroup of 50 B19 DNA–positive donors was screened for B19 immunoglobulin G (IgG) and IgM antibodies and B19 DNA over a 6-month period. Results were compared to those of 100 B19 DNA–negative donors.

RESULTS: Data accumulated over the past 6 years indicate a high incidence period from May 2004 to January 2006. In total, the incidence was 12.7 and 261.5 per 100,000 donations with high virus loads equal to or above 10^5 and below 10^5 IU per mL, respectively. Median virus concentration in the case group was 4.85×10^7 IU per mL at Time Point T0 and was reduced to 4×10^2 IU per mL at the time of the next donation (3 months later). Neutralizing antibodies (VP2) were detected in all donations if virus load was reduced to less than 10^5 IU per mL.

CONCLUSION: The release of B19 DNA–positive blood products with a concentration of less than 10^5 IU per mL is thought to be safe due to the high level of neutralizing VP2 antibodies and is currently examined in a donor recipient infectivity study. In contrast, blood products with a high B19 DNA concentration ($\geq 10^5$ IU/mL), some of which did not contain neutralizing antibodies, were discarded to protect at risk individuals.

Parvovirus B19 (B19) was detected for the first time in 1975 in a blood product from a healthy donor.[1-3] During the onset of B19 infection, virus concentration can increase up to 10^{14} virions per mL.[4-6] Because B19 is a non–lipid-enveloped viral pathogen, inactivation methods like solvent/detergent treatment are ineffective for reduction of virus concentration in plasma. Most infections occur in childhood and result in a mild rash and formation of protective antibodies.[7-13] Infection normally results in seroconversion with neutralizing immunoglobulin G (IgG) antibodies affording lifelong protection from reinfection in most cases.[14] Chronic infection, however, may be associated with a poor antibody response.[15,16]

Screening for B19 DNA by minipool real-time nucleic acid amplification technology (NAT; testing in donor pools up to 96 samples per pool) was introduced into our blood donor screening protocol in 2000. NAT amplification was analyzed in a semiquantitative manner. Blood

ABBREVIATIONS: B19 = parvovirus B19; C_t = cycle threshold.

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Received for publication March 1, 2007; revision received June 26, 2007, and accepted June 26, 2007.

doi: 10.1111/j.1537-2995.2007.01443.x

TRANSFUSION 2007;47:1775-1782.

products with B19 DNA virus load equal to or higher than 10^5 IU per mL were discarded. In contrast, minipools with B19 DNA virus load below 10^5 IU per mL were not resolved, and all blood products contained were released. In any case, donors were not informed about their B19 infection and were allowed to give subsequent donations.

This study provides results for 4 years of NAT screening, including a case-controlled study for B19 antibodies performed over a 6-month period to monitor the development of structural (VP-1 and VP-2) and nonstructural (NS-1) antibodies.

MATERIALS AND METHODS

Incidence studies

Donations from six different sites were involved in the study. In Germany samples from the German Red Cross Institute Frankfurt (1,732,355 samples, Area 1) and from the German Armed Forces (99,176 samples, Area 2) were included in the study. In contrast, Austrian samples from four test areas including the Medical University of Graz (203,880 samples, Area 3), Austrian Red Cross Institute Klagenfurt (85,811 samples, Area 4), Austrian Red Cross Institute Feldkirch (51,041 samples, Area 5), and Austrian Red Cross Institute of Vienna (626,373 samples, Area 6) were included in the study (Table 1). All donations for the German Red Cross were screened by B19 real-time NAT beginning in April 2000 and in August 2003 for all other institutes. All donations were tested at the GRC Institute in Frankfurt. The screening procedure was not modified during the study period. Donations with B19 virus concentrations of at least 10^5 IU per mL were discarded, whereas minipools that contained donations with a virus load of not more than 10^5 IU per mL were not resolved. All products included in these minipools were designated as being weakly B19 DNA–positive and were released for transfusion. This procedure is in accordance with the requirements of the plasma industry, where the release level per individual donation is 10^5 IU per mL, as well as the German transfusion law, the German authorities (Paul Ehrlich Institute) and the local ethics commission, which approved of this study.

TABLE 1. B19 questionnaire and characteristics of the case and control group*

Characteristic	Group		Significance
	Case	Control	
Total number	50	50	Not done
Men/women	27/23	26/24	0.50
Age (years)	39.0 ± 10.9	44.4 ± 15.1	0.06
Chronic diseases	8/50	12/50	0.23
Tiredness	12/50	11/50	0.50
Joint pains	11/50	9/50	0.40
Neurologic symptoms	1/50	1/50	0.75
Fever, flulike symptoms	1/50	1/50	0.75
Pregnancy	12/23	12/24	0.55
Complications during pregnancy	6/12	2/12	0.10
Disease in childhood			
B19 infection	3/50	2/50	0.50
Rubella	12/50	14/50	0.41
Mumps	10/50	11/50	0.50
Chicken pox	10/50	15/50	0.18
Bordetella pertussis	1/50	3/50	0.31

* Donors of both groups were matched with regard to sex and age and were interviewed about B19-specific clinical symptoms. All women were asked about pregnancies and complications during pregnancies.

Donor substudy (case-control study)

A group of 50 B19 DNA–positive blood donors with a virus concentration of at least 10^5 IU per mL at the index donation (Time Point T0, high-virus-load group) was analyzed in a prospective study involving two subsequent blood draws (with the first occurring approximately 12 weeks after the index donation), for B19 DNA concentration as well as B19 antibodies. The 50 donors were randomly selected from all B19 DNA–positive donors ($\geq 10^5$ IU/mL) residing in Area 1.

In addition, 100 B19 DNA NAT-negative donors were screened for B19 antibodies as a control group. Both the case and the control groups were comparable with regard to age and sex (Table 1). All donors positive for the presence of B19 DNA ($\geq 10^5$ IU/mL) at the index donation (case group) and 50 randomly selected members of the control group were interviewed by standard questionnaire within 4 weeks after the donation about clinical symptoms of a B19 infection (Table 1).

B19 screening techniques

Routine testing. An aliquot of 100 µL plasma of each blood donation was pooled overnight into minipools containing up to 96 samples per pool. The complete pool of up to 9.6 mL was centrifuged at 58,000 × *g* for 60 minutes at 4°C. Supernatants were discarded and pellets were subjected to nucleic acid extraction with a viral RNA kit (QIAamp, Qiagen, Hilden, Germany). Five-microliter aliquots of the total eluted volume of 75 µL were subjected to polymerase chain reaction (PCR) amplification for B19 DNA. Two positive controls and at least three quantitative standards (10^6, 10^5, and 10^4 IU/mL) were included in each PCR procedure.[17-19]

Resolving of B19 DNA–positive minipools. All samples achieving a positive B19 DNA minipool NAT result with a virus concentration of less than 10^5 IU per mL were released as weakly positive B19 DNA donations without resolving the minipool. In contrast, all

minipools that yielded a B19 DNA concentration higher than 10^5 IU per mL were resolved by creating subpools from archive plates. Next the identified B19 DNA–positive samples were discarded and all negative or weakly positive B19 DNA samples included in the minipool were released for transfusion.

NAT. Real-time quantitative amplification of B19 DNA was performed with a CE labeled B19 PCR kit (DRK Baden-Württemberg-Hessen, Frankfurt, Germany) according to the manufacturers' instructions with a thermocycler (ABI PRISM 7000, 7700, 7300, or 7900HT; Applied Biosystems, Foster City, CA). Five microliters of extract was analyzed in a total volume of 25 µL. The assay contains reagents and enzymes for the specific amplification of the VP1-capsid protein gene of B19. Thermal cycling was as follows: 50°C for 2 minutes, 95°C for 15 minutes, 10 cycles of 95°C for 10 seconds and 62°C for 30 seconds, 40 cycles of 93°C for 10 seconds, and 56°C for 40 seconds.

Data analysis was performed with the computer software (sequence detection software, Version 1.6.3, Applied Biosystems). A positive real-time PCR result is reflected by an increase in the fluorescence intensity of a reporter dye. After PCR, the number of PCR cycles necessary to reach a defined fluorescence threshold in each sample was defined as the cycle threshold (C_t). The C_t value is related to the amount of PCR product and therefore to the original amount of target present in the PCR procedure. Low C_t values indicate a high initial target amount and high C_t values indicate the opposite.

Sensitivity and specificity of the DRK B19 PCR kit. Sensitivity was analyzed in accordance with the directive of European Commission 98/79/EC. Probit analysis was done on at least 24 replicates of each dilution from a dilution series containing at least six steps. The calculation was performed on nonlog converted data. Specificity was tested with 200 negative plasma samples. Additionally, the amplification efficiency of different B19 genotypes (Genotype 1, Genotype 2 [Subtype A6], and Genotype 3 [Subtype V9]) was evaluated. Genotype 3 was obtained from a Ghanaian blood donor service.[20-22]

Precautions to prevent B19 DNA cross-contamination. All steps of NAT (pooling, enrichment by centrifugation and extraction, master mix preparation, and amplification) were performed in separate rooms. All rooms were equipped with ultraviolet light and were decontaminated once per week. The daily decontamination procedure included decontamination of all workbenches, pipettes, and centrifuges with a disinfectant (Bacillol Plus, Bode, Hamburg, Germany) and sodium hypochlorite (Roth, Karlsruhe, Germany). All PCR procedures were monitored by the addition of at least six negative controls. PCR procedures were only valid if all negative controls gave a negative test result. All personnel performing nucleic acid extraction and resolution of highly B19 DNA–positive pools have been thoroughly trained to be competent in performing these procedures without cross-contamination.

Screening for B19 antibodies. Samples were screened with two assays for IgM and IgG antibody detection. A parvovirus IgG and IgM assay (recomLine, Mikrogen, Neuried, Germany) was used to analyze antibodies against VP-2, VP-N, VP-1S, VP-2r, VP-C, and NS-1 epitopes. Band intensities were compared with a control band and were scored as –, +/–, 1+, 2+, 3+, or 4+. Additionally all samples were screened with the microtiter plate–based B19 enzyme immunoassay (EIA; Biotrin, Dublin, Ireland) for IgG and IgM antibodies. All antibody assays were performed according to the manufacturers' instructions.

Antibody adsorption. Eight samples with a B19 DNA concentration of more than 10^5 IU per mL and eight samples with a B19 DNA load below 10^5 IU per mL were analyzed for B19 IgG antibodies by use of the recomLine assay. In these samples, virus load was determined by real-time NAT before and after treatment with a protein G column (MAb Trap kit, Amersham, Uppsala, Sweden). One-hundred microliters of each sample was filtered through a protein G column and washed with 5 mL of binding buffer. The flowthrough of the binding step was centrifuged at 58,000 × *g* for 1 hour at 4°C followed by a standard minipool extraction protocol.

B19 sequence analysis. Sequence analysis was performed as described in detail by Hokynar and colleagues.[23] Overlapping amplicons of 1000 bp that spanned the entire protein coding region of the genome were used. Primers (NSofwd and NSirev, NSsfwd and NSorev, p6 and p3, p9 and rtsrev, and rt1 and VP2orev) were used for sequencing plus and minus strands. Amplification products were sequenced directly with a cycle sequencing ready reaction kit (BigDye Terminator, Applied Biosystems, Darmstadt, Germany) and a DNA sequencer (ABI PRISM 310, Applied Biosystems).

Statistical analysis

The sensitivity, standard deviation (SD), and coefficient of variation (CV) of the real-time PCR test were calculated with computer software (Excel 2000, Microsoft Corp., Redmond, WA). For the Probit analysis, another computer program (SPSS 12.0, SPSS, Chicago, IL) was used. Comparison between the case and control groups was calculated with Fisher's exact test or the t test. Statistical significance was assumed if p values were less than 0.05.

RESULTS

Incidence of B19 in different areas

B19 incidence between 2003 and 2006 was demonstrated for six different areas (Fig. 1). There was a high incidence

period of B19 from May 2004 to January 2006 in all screened regions. The highest incidence was found in Areas 4 and 5, although the incidence of B19 DNA–positive donors with a high virus load (B19 DNA concentration $\geq 10^5$ IU/mL) as well as with low B19 DNA virus loads (B19 DNA concentration $<10^5$ IU/mL) did not differ significantly between the areas (Table 2).

Sequence analysis of the 50 B19 NAT–positive blood donors included in the substudy identified only Genotype 1 strains. Sequence analyses of all B19 DNA–positive samples are currently being processed to better understand the genotype distribution in our donor population.

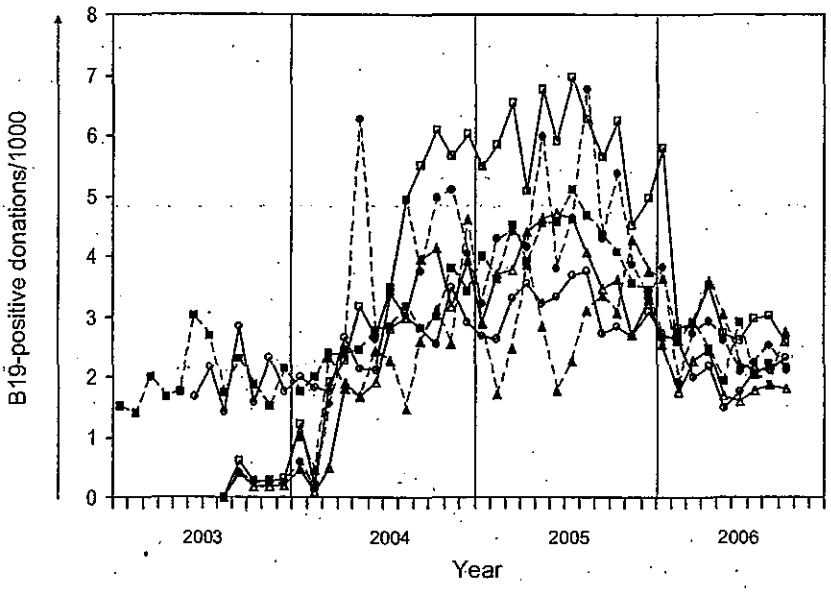

Fig. 1. Incidence of B19 virus infections between 2003 and 2006. Donations were screened for B19 by real-time minipool NAT. Incidence was increased between May 2004 and January 2006, especially in Areas 4 and 5. (■) Area 1 = GRC Institute Frankfurt; (▲) Area 2 = German Armed Forces; (△) Area 3 = Medical University of Graz; (□) Area 4 = Austrian Red Cross Institute Klagenfurt; (●) Area 5 = Austrian Red Cross Institute Feldkirch; and (○) Area 6 = Austrian Red Cross Institute Vienna.

Monitoring of B19 DNA–positive blood donors

In the substudy, two additional donations were taken from 50 B19 DNA–positive multiple-time donors (high-virus-load group) randomly selected from all B19 DNA–positive samples in Test Area 1 to determine B19 DNA concentration and the course of antibody development to B19.

All donors included in the substudy were B19 DNA–positive with a virus load of more than 10^5 IU per mL at the index donation (Time Point T0). The virus load was significantly reduced within 12 weeks from a median of 4.85×10^7 IU per mL (T0; SD) to 4.6×10^2 IU per mL (SD; T1; Fig. 2) and either remained at this level or declined further at Time Point T2. Additional follow-up in a subset of these donors beyond Time Point T2 revealed that B19 DNA concentration was stable around the NAT detection level for up to 1 year (range, 100 and 1500 IU/mL; data not shown). All samples from donors of the case group were below the release level of 10^5 IU per mL at Time Point T1.

B19 antibody levels were investigated with an enzyme-linked immunosorbent assay and a line probe assay. Both commercially available B19 antibody assays gave comparable results for B19 IgM (Table 3) and IgG (Table 4) antibodies. At each time point, IgM antibodies were detected more frequently ($p < 0.05$) in the case group compared with the control group, and antibody titers generally showed an increase from Time Point T0 to Time Point T1 followed by a decrease at Time Point T2. IgM antibodies against the nonstructural protein (NS-1) were not detected at any time point. In contrast, neutralizing IgG antibodies against VP-2 were detected in all samples of the high-virus-load group

TABLE 2. Incidence of B19 virus infections in different areas per 100,000 donations*

	B19 DNA virus load (IU/mL)													
	Area 1		Area 2		Area 3		Area 4		Area 5		Area 6		All	
Year	$>10^5$	$<10^5$	$>10^5$	$<10^5$	$>10^5$	$<10^5$	$>10^5$	$<10^5$	$>10^5$	$<10^5$	$>10^5$	$<10^5$	$>10^5$	$<10^5$
2003	9.3	185.2	1.0	200.3	0.0	19.6	0.0	29.9	0.0	23.9	ND†	ND	6.3	172.0
2004	17.9	254.3	13.6	239.4	9.3	217.0	25.1	340.3	50.0	279.1	11.5	212.9	16.3	247.7
2005	25.0	395.1	12.6	300.6	36.0	345.8	3.9	580.9	6.2	434.7	2.7	295.7	19.4	362.8
2006	5.7	237.1	3.7	209.9	6.0	170.8	0.0	317.1	0.0	255.4	3.6	268.9	4.5	227.6
All	15.0	269.8	9.1	245.0	15.7	221.3	9.3	366.1	17.6	289.7	6.1	259.1	12.7	261.5

* Donations were tested from six different areas in Germany and Austria. Incidence was demonstrated in two groups: 1) donations with high B19 DNA virus load over 10^5 IU/mL and 2) donations with low B19 DNA virus load below 10^5 IU per mL. Incidence increases were observed in all areas in 2004 and 2005 for both groups.
† ND = not done.

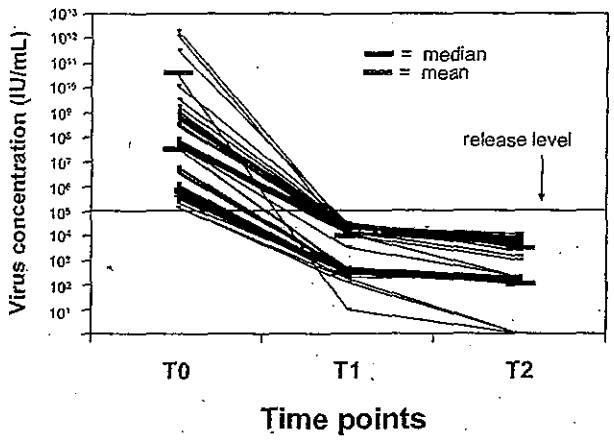

Fig. 2. Virus load during the 6-month study period. All donors of the case group were B19 DNA–positive at the index donation with a value of more than 10^5 IU per mL (highest concentration was 2.1×10^{12} IU/mL). The black bar represents the median virus concentration and the gray bar indicates the mean virus concentration of all donations for each time point. Virus load was significantly reduced from Time Point T0 to Time Point T1. The SDs were 3.5×10^{11}, 1.0×10^4, and 3.5×10^3 for T0, T1, and T2, respectively.

(case group) at Time Point T1 and T2 without any exception. Both antibody assays and titers were significantly higher in the case group compared to the control group. Likewise, antibodies against nonstructural antigens of NS-1 increased up to 92.3 percent from Time Point T0 to Time Point T2.

In one experiment, plasma from donors with B19 DNA concentrations of more than 10^5 IU per mL and with B19 DNA concentrations of less than 10^5 IU per mL was filtered through protein G columns. The viral load was determined before and after IgG absorption. Reduction of the B19 virus concentration was significantly higher in samples with low virus load and high IgG antibodies titers as shown in Table 5. In two of eight samples (viral load, $<10^5$ IU/mL), no virus was detectable after column filtration. In the other six samples, low virus concentrations were detected (mean C_t value, 30.6; virus concentration, <100 IU/mL).

All donors included in the case-control substudy were matched by age and sex and were interviewed with a standard B19 questionnaire about clinical symptoms (Table 1). Typical clinical symptoms for B19 infections such as tiredness, joint pain, or complications between pregnancies did not significantly differ between groups.

TABLE 3. IgM antibodies in the case group and the control group*

	Biotrin EIA	Mikrogen immunoblot					
Group	VP2 (%)	VP-2p (%)	VP-N (%)	VP-1S (%)	VP-2r (%)	VP-C (%)	NS-1 (%)
Case							
T0†	42.9	42.9	42.9	57.1	42.9	35.7	0.0
T1	71.4	71.4	85.7	85.7	50.0	35.7	0.0
T2	23.1	46.2	69.2	69.2	38.5	30.8	0.0
Control							
T0	1.8	9.2	4.6	6.1	1.5	1.5	0.0

* Fifty B19 DNA–positive donors were screened for IgM antibodies by two different assays to detect antibodies against structural (neutralizing) and nonstructural antigens at three time points, and the data were compared to results from 100 B19 DNA–negative blood donors (control group).
† T0 = index donation.

TABLE 4. IgG antibodies in the case and control groups*

	Biotrin EIA	Mikrogen immunoblot					
Group	VP2 (%)	VP-2p (%)	VP-N (%)	VP-1S (%)	VP-2r (%)	VP-C (%)	NS-1 (%)
Case							
T0†	35.7	35.7	28.6	28.6	28.6	21.4	0.0
T1	100	100	100	100	100	85.7	57.1
T2	100	100	100	100	100	76.9	92.3
Control							
T0	74.8	73.3	71.0	68.7	46.6	16.0	14.5

* Fifty B19 DNA–positive donors were screened for IgG antibodies by two different assays to detect antibodies against structural (neutralizing) and nonstructural antigens at three time points, and the data were compared to results from 100 B19 DNA–negative blood donors (control group).
† T0 = index donation.