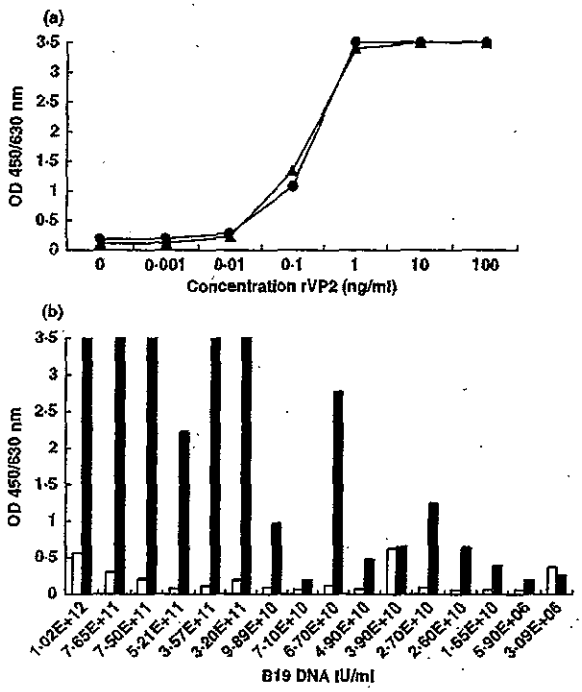

Fig. 1 Determination of B19 antigen enzyme immunoassay (EIA) assay sensitivity. (a) Two independent batches of recombinant capsid VP2 (rVP2), V056 (circles) and V057 (triangles) were decimally diluted to determine assay sensitivity. (b) Comparison of specimen diluents used in the detection of B19 viral capsids. Specimens were diluted in either Tris-buffered saline Tween-20 (TBST) (clear boxes) or a low pH proprietary reagent (filled boxes). Error bars represent the standard deviation from the mean.

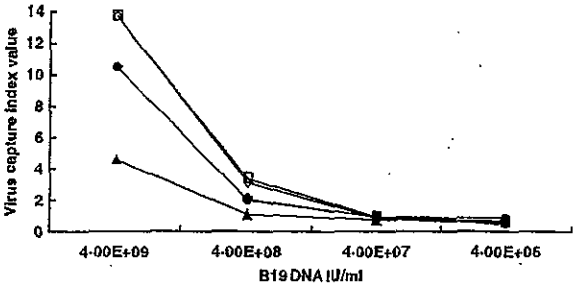

Fig. 2 Determination of antigen assay sensitivity using titrations of polymerase chain reaction (PCR)-quantified viraemic specimens. Viraemic plasma Bt72 (diamonds), Bt73 (squares), Bt80 (triangles) and genotype 2 Bt81 (circles) were decimally diluted in B19 negative serum to determine assay cut-off.

Results

Assay optimization and validation

Figure 1a shows identical standard curves [absorbance$_{450/630\,nm}$ vs. B19 recombinant VP2 capsid concentration (ng/ml)] generated from two independent batches of recombinant VP2 capsids in the B19 antigen EIA. These standard curves show that the minimal detectable level of B19 VP2 capsid detectable was 0·01 ng/ml, which theoretically equates to $1·9 \times 10^6$ viral particles per ml.

However, detection of B19 viraemic plasma in the same assay format required the implementation of an alternative specimen diluent (Fig. 1b). Here, dilution of viraemic specimens (n = 16) in a low pH, proprietary diluent, compared to using Tris-buffered saline Tween-20 (TBST), facilitated a considerable increase in virus capture in the majority of specimens (0- to 30-fold). Only one specimen ($3·9 \times 10^{10}$ IU/ml B19 DNA) that was negative for B19 IgM did not display a significant signal increase post-treatment, but did remain positive. Interestingly, the two specimens with the highest absorbance values in the assay without low pH pretreatment were IgM negative.

Non-viraemic plasma remained unreactive when subjected to the same pretreatment (data not shown). Assay specificity was determined by screening non-viraemic plasma (n = 20), all of which were unreactive in the antigen EIA based on the cut-off calibrator sample (data not shown).

The assay sensitivity (limit of detection) was estimated using dilutions of viraemic specimens and was shown to be approximately between 4×10^7 and 4×10^8 copies per ml B19 DNA (Fig. 2). However, the cut-off calibrator used in the EIA contained 10^9 copies per ml B19 DNA as determined by qPCR, which equates to 2×10^7 copies B19 DNA per microwell. To further define the limit of detection, plasma specimens (n = 17), containing a range of B19 DNA concentrations and B19 IgM/G reactivity, were subsequently screened in the antigen EIA. Table 1 shows that 53% (9/17) of specimens, all of which contained greater than $1·4 \times 10^{11}$ copies per ml B19 DNA, were also detectable in the antigen EIA. One specimen containing $7·2 \times 10^8$ copies per ml B19 DNA, which was B19 IgM reactive, tested borderline positive (IV = 1·0) in the antigen EIA. All remaining specimens, which contained less than $1·9 \times 10^7$ copies per ml B19 DNA and either B19 IgM or IgG or both, were unreactive in the antigen EIA.

Detection of the B19 antigen in the presence of specimen-derived B19-specific IgG or IgM is essential to avoid false negativity. Table 2 clearly illustrates that specimen-derived B19 antigen is detectable in the presence of both B19 IgG and IgM (n = 8), IgM only (n = 2) or IgG only (n = 3). Furthermore, B19 antigen is also detectable in specimens Bt72 and Bt73, which contained B19 IgM (Fig. 2). It is clear, therefore, that only B19 levels greater than 4×10^7 B19 DNA copies per ml are detectable in the antigen EIA and that the presence or absence of IgM or IgG in the specimen does not affect detection of the B19 antigen (Fig. 2 and Table 2). A specimen containing erythrovirus genotype 2 (specimen Bt81) was detected as well as erythrovirus genotype 1 (specimens Bt72, Bt73 and Bt80) in the antigen EIA (Fig. 2). Furthermore, erythrovirus genotype

Table 1 Parvovirus B19 detection by antigen enzyme immunoassay (EIA) and serological analysis (B19 IgM and IgG) of specimens previously quantified by polymerase chain reaction (PCR) (copies per ml). For the antigen EIA an index value (IV) ≥ 1·0 is positive (+) and < 1·0 is deemed negative (–). For both the B19 IgM and IgG EIA IV > 1·1 is positive; IV < 0·9 is negative; and IV between < 1·1 and IV > 0·9 is deemed equivocal (*eq*)

Sample identifier	IgM EIA	IV	IgG EIA	IV	qPCR (copies per ml)	Antigen EIA	IV
Cut-off calibrator	6·77	+	0·99	*eq*	$1·3 \times 10^{9}$	1·00	+
W P	0·80	–	0·14	–	$6·9 \times 10^{11}$	18·7	+
C4	0·26	–	0·06	–	$6·0 \times 10^{11}$	> 3·0	+
PL19	0·59	–	0·07	–	$5·6 \times 10^{11}$	> 3·0	+
C7	0·58	–	0·06	–	$5·5 \times 10^{11}$	> 3·0	+
C1	0·13	–	0·04	–	$4·8 \times 10^{11}$	> 3·0	+
C2	0·08	–	0·06	–	$4·6 \times 10^{11}$	> 3·0	+
C6	0·24	–	0·05	–	$3·3 \times 10^{11}$	> 3·0	+
C3	0·08	–	0·09	–	$3·9 \times 10^{11}$	> 3·0	+
PL9	0·11	–	0·06	–	$1·4 \times 10^{11}$	> 11·0	+
C5	2·02	+	0·17	–	$7·2 \times 10^{8}$	1·0	+
E R	3·0	+	8·1	+	$1·9 \times 10^{7}$	0·03	–
PL1	6·3	+	1·95	+	$1·6 \times 10^{7}$	0·39	–
C8	0·15	–	2·56	+	$2·6 \times 10^{4}$	0·04	–
D T	2·3	+	6·2	+	$7·4 \times 10^{3}$	0·07	–
R S	6·6	+	6·8	+	$8·9 \times 10^{3}$	0·42	–
PL20	0·11	–	4·78	+	550	0·42	–
PL16	0·2	–	4·80	+	200	0·39	–

Table 2 Effect of B19 IgM and IgG in plasma on the detection of B19 antigen. B19 antigen enzyme immunoassay (EIA) and serology results for plasma from patients with suspected B19 infection. For the antigen EIA an index value (IV) ≥ 1·0 is positive (+) and < 1·0 is deemed negative (–). For both the B19 IgM and IgG EIA an IV > 1·1 is positive; IV < 0·9 is negative; and IV between < 1·1 and > 0·9 is deemed equivocal (*eq*)

Sample Identifier	IgM EIA	IV	IgG EIA	IV	Antigen EIA IV
Cut-off calibrator	6·77	+	0·99	*eq*	1
931	0·14	–	0·70	–	18·6
420	0·16	–	0·90	*eq*	18·3
981	1·73	+	1·50	+	18·1
410	0·25	–	0·90	*eq*	18·1
375	0·14	–	0·70	–	18·1
939	0·30	–	0·80	–	18·0
889	4·99	+	1·70	+	17·9
976	0·17	–	1·20	+	17·8
441	3·40	+	0·80	–	17·6
973	0·28	–	1·28	+	17·3
966	1·92	+	1·46	+	17·3
936	1·21	+	1·40	+	16·3
444	0·86	–	1·00	*eq*	15·4
980	0·71	–	1·70	+	12·0
427	2·06	+	0·80	–	11·9
929	2·74	+	1·40	+	11·2
888	0·25	–	1·10	+	8·2
925	1·32	+	1·50	+	6·76
416	6·89	+	2·80	+	1·3
895	6·02	+	1·90	+	1·0

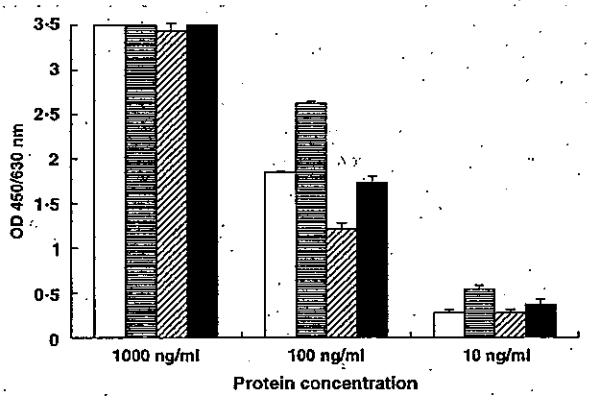

Fig. 3 Comparison of erythrovirus genotype 1 and 3 VP2 reactivity in the antigen enzyme immunoassay (EIA). Genotype 1 (clear and horizontal lined bars) and genotype 3 (diagonal lined and filled bars) recombinant VP2 was decimally diluted in either Tris-buffered saline Tween-20 (TBST) (clear and diagonal lined bars) or the proprietary low pH buffer (horizontal lined and filled bars). Error bars represent the standard deviation from the mean.

3 recombinant VP2 capsids exhibit indistinguishable reactivity in the assay to genotype 1 recombinant VP2 (Fig. 3).

Donor sample evaluation

During an 18-month period, approximately 1·4 million donations were tested for B19 DNA in The Netherlands [14], and 70 cases of asymptomatic donors (0·005%) with levels of B19 DNA greater than 10^{6} IU/ml were identified. Of these, 49/70 (70%) tested positive on the antigen EIA assay for B19

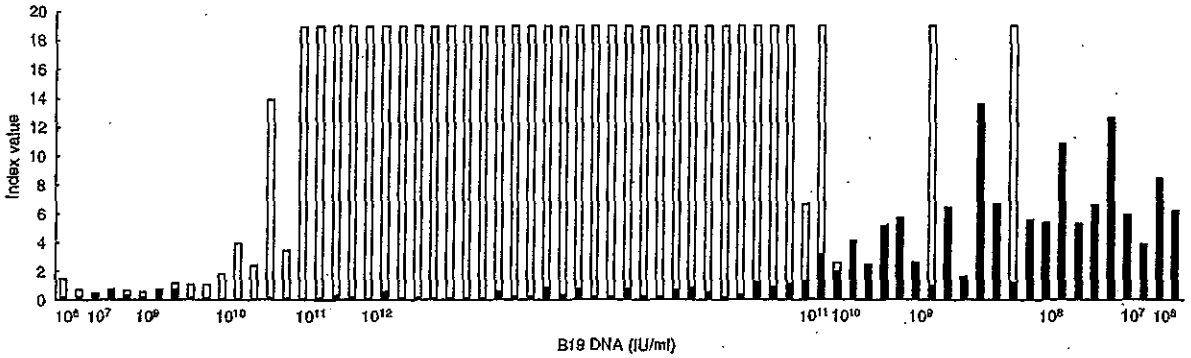

Fig. 4. A summary of the B19 antigen enzyme immunoassay (EIA) and immunoglobulin M (IgM) EIA reactivity of the panel of viraemic donors. An index value (IV) > 1·1 (denoted by line) is considered positive on both the B19 IgM EIA (filled bars) and antigen EIA (clear bars). The *y*-axis was truncated for clarity.

(range: $3{\cdot}1 \times 10^6$–$3{\cdot}2 \times 10^{12}$ IU/ml; mean: $1{\cdot}1 \times 10^{12}$ IU/ml, median: $1{\cdot}2 \times 10^{12}$ IU/ml B19 DNA) (Fig. 4). Thus, Fig. 4 depicts the combined B19 IgM and antigen EIA data of the 70 viraemic specimens, and the *x*-axis is arranged to show the rise (10^6–10^{12} IU/ml) and subsequent drop in viraemia with the development of B19-specific IgM antibodies (10^{12}–10^6 IU/ml). Testing further revealed that the panel of viraemic specimens was either pre- or early antibody seroconversion as none contained B19 IgG (data not shown).

There was a positive correlation (correlation coefficient $r = 0{\cdot}81$) between the level of B19 DNA (qPCR) and the level of B19 antigenemia (antigen EIA), but this relationship was not directly proportional. Concordance between qPCR and the antigen EIA was highest when viraemia titres were high ($> 1 \times 10^{11}$ IU/ml). Of the viraemic donor specimens, 27 (38·6%) tested positive (IV > 1·1) or borderline positive (two specimens were equivocal: IV ≤ 1·1, IV ≥ 0·9) for B19 IgM (Fig. 4). The specimens that were equivocal for IgM reactivity reacted strongly in the antigen EIA (IV > 19). The overlap between the two groups was considerable and 17% of the specimens tested positive for both B19 IgM and antigen (Fig. 4). Significantly, 91% of the viraemic donors were positive for either B19 IgM or antigen. Thus, these data clearly demonstrate that the combined implementation of a screening algorithm for B19 IgM and antigen readily facilitates the detection of specimens containing greater than 10^6 IU/ml B19 DNA equivalents.

Discussion

Here we describe a B19 antigen EIA for the direct detection of B19 antigen in human plasma. The detection limit of the assay was 0·01 ng/ml of purified recombinant VP2 capsids (which theoretically corresponds to $1{\cdot}9 \times 10^6$ viral particles per ml). Using dilutions of viraemic serum, the sensitivity was estimated at between 4×10^7 and 10^8 copies per ml B19 DNA equivalents. The antigen EIA was capable of detecting both erythrovirus genotypes 2 (virus) and 3 (recombinant capsids). When the antigen assay was used to test B19 viraemic donations, 70% tested positive of which had viral loads between $3{\cdot}1 \times 10^6$ and $3{\cdot}2 \times 10^{12}$ IU/ml.

B19 detection in plasma was greatly enhanced by specimen acidification. The low pH conditions may act by disrupting the viral capsid into its structural subunits, making it more accessible to the capture antibody. Although it was previously thought that B19V was highly resistant to physicochemical treatments, more recent work has shown the susceptibility of B19V to low pH treatment [24]. Boschetti *et al.* [24] showed that B19V was inactivated by greater than 5 logs after 2 h at pH 4 and that infectivity also decreased.

When the antigen assay was performed at physiological pH, the specimens that gave the highest absorbance values were B19 IgM negative, implying immune complexes hinder detection. However, when specimens were prepared in low pH conditions, neither the presence of IgM nor IgG, even at high levels, affected the detection of B19 (Table 2). It is probable that acidification caused the dissociation of any immune complexes present. False-negative results due to immunocomplexes present a problem for B19 RHA assays, which exploit the binding of a B19V receptor to red blood cells [11]. Hence, the RHA assay is ineffective for antigen detection in specimens that have seroconverted a problem resolved by the B19 antigen EIA.

B19 detection by PCR has a greater sensitivity, but such assays have many disadvantages (e.g. potential cross-contamination) not shared with an EIA. First, although erythrovirus genotypes may diverge significantly at the genomic level [25,26], requiring primer optimization [13], there does not appear to be any antigenic or immunological differences between the genotypes. The antigen EIA could identify genotype 2 erythrovirus and genotype 3 recombinant VP2 capsids at the same sensitivity as genotype 1. This is supported by the fact that all three erythrovirus genotypes can haemagglutinate human red blood cells and also infect myeloid cells with equal efficiency [27]. Second, the significance of DNA in plasma postviraemia

is unclear as low levels of B19 DNA can persist for several years post-infection, even after IgM is lost and IgG reactivity has been established [28]. A virus detection assay, however, allows simultaneous testing of hundreds of specimens, is suitable for large-scale screening, is more economical and has a shorter time to result.

Combined B19 antigen and IgM EIA analysis of the viraemic donor specimens revealed that 91% of the donor specimens could be diagnosed as acute infection using this screening algorithm. Previously, clinical samples taken from individuals with a suspected B19 infection, which had a level of B19 DNA greater than 10^5 IU/ml, were shown to be positive for specific IgM also [20]. This was not the case with the Dutch donor specimens herein, as this panel was from asymptomatic individuals whose infection was detected due to routine screening. Donor specimens, therefore, would be from all stages post-infection including the preseroconversion stage. Experimental infection has shown that B19 infection has two phases [29], characterized by symptom-free initial high viraemia (~10^{11} copies per ml serum) followed by detectable IgM antibody and appearance of symptoms such as rash and arthralgia. IgM seroconversion causes a rapid decline of viral titre. The 70 viraemic specimens identified in this study showed a typical viraemia and IgM seroconversion pattern (Fig. 4), confirming that the donor samples are representative of all stages of acute infection.

It is important to confirm the diagnosis of acute B19 infection in a public health setting where an outbreak could lead to serious medical consequences, especially for pregnant women and immunocompromised patients. In addition, B19 screening of blood donors prior to donation would avoid the risk of contaminating blood products. The B19 antigen EIA in conjunction with specific B19 IgM detection offers an effective method of detecting acute infection.

Acknowledgements

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References

1 Kurtzman GJ, Cohen BJ, Meyers P, Amunullah A, Young NS: Persistent B19 parvovirus infection as a cause of severe chronic anemia in children with acute lymphocytic leukaemia. *Lancet* 1988; 2:1159–1162

2 Wildig J, Michon P, Siba P, Mellombo M, Ura A, Mueller I, Cossart Y: Parvovirus B19 infection contributes to severe anemia in young children in Papua New Guinea. *J Infect Dis* 2006; 194:146–153

3 Enders M, Weidner A, Zoellner I, Searle K, Enders G: Fetal morbidity and mortality after acute human parvovirus B19 infection in pregnancy: prospective evaluation of 1018 cases. *Prenat Diagn* 2004; 24:513–518

4 European Pharmacopoeia: *European Pharmacopoeia Monograph of Human anti-D Immunoglobulin*. European Pharmacopoeia, Strasbourg, France, 2004; Document 01:0557

5 Groeneveld K, van der Noordaa J: Blood products and parvovirus B19. *Neth J Med* 2003; 61:154–156

6 Prowse C, Ludlam CA, Yap PL: Human parvovirus B19 and blood products. *Vox Sang* 1997; 72:1–10

7 Santagostino E, Mannucci PM, Gringeri A, Azzi A, Morfini M, Musso R, Santoro R, Schiavoni M: Transmission of parvovirus B19 by coagulation factor concentrates exposed to 100 degrees C heat after lyophilization. *Transfusion* 1997; 37:517–522

8 Laub R, Stengers P: Parvovirus B19 and blood products. *Pathol Biol* 2002; 50:339–348

9 Hausler M, Scheithauer S, Ritter K, Kleines M: Molecular diagnosis of Epstein-Barr virus. *Expert Rev Mol Diagn* 2003; 3:81–92

10 Baylis SA, Shah N, Minor PD: Evaluation of different assays for the detection of parvovirus B19 DNA in human plasma. *J Virol Methods* 2004; 121:7–16

11 Sakata H, Ihara H, Sato S, Kato T, Ikeda H, Sekiguchi S: Efficiency of donor screening for human parvovirus B19 by the receptor-mediated hemagglutination assay method. *Vox Sang* 1999; 77:197–203

12 Koppelman M, Cuypers T, Emrich T, Zaaijer H: Quantitative real-time detection of parvovirus B19 DNA in plasma. *Transfusion* 2004; 44:97–103

13 Aubin JT, Defer C, Vidaud M, Maniez Montreuil M, Flan B: Large-scale screening for human parvovirus B19 DNA by PCR: application to the quality control of plasma for fractionation. *Vox Sang* 2000; 78:7–12

14 Wong S, Brown KE: Development of an improved method of detection of infectious parvovirus B19. *J Clin Virol* 2006; 35:407–413

15 Takeda Y, Wakisaka A, Noguchi K, Murozuka T, Katsubayashi Y, Matsumoto S, Tomono T, Nishioka K: Receptor-mediated haemagglutination screening and reduction in the viral load of parvovirus B19 DNA in immunopurified Factor VIII concentrate (Cross Eight M). *Vox Sang* 2001; 81:266–268

16 Doyle S, Corcoran A: The immune response to parvovirus B19 exposure in previously seronegative and seropositive individuals. *J Infect Dis* 2006; 194:154–158

17 O'Keeffe S, O'Leary D, Doyle S, Kilty C, Kerr S: The detection of parvovirus B19 in human sera using antigen-capture EIA. Poster presented at the Society for General Microbiology (Irish Branch), National University of Ireland, Maynooth, Co. Kildare, Ireland, 24–25 April 2003

18 Lowin T, Raab U, Schroeder J, Franssila R, Modrow S: Parvovirus B19 VP2-proteins produced in *Saccharomyces cerevisiae*: comparison with VP2-particles produced by baculovirus-derived vectors. *J Vet Med B Infect Dis Vet Public Health* 2005; 52:348–352

19 Doyle S, Kerr S, O'Keeffe G, O'Carroll D, Daly P, Kilty C: Detection of parvovirus B19 IgM by antibody capture enzyme immunoassay: receiver operating characteristic analysis. *J Virol Methods* 2000; 90:143–152

20 Beersma MF, Claas EC, Sopaheluakan T, Kroes AC: Parvovirus B19 viral loads in relation to VP1 and VP2 antibody responses in diagnostic blood samples. *J Clin Virol* 2005; 34:71–75

21 Kerr S, O'Keeffe G, Kilty C, Doyle S: Undenatured parvovirus B19 antigens are essential for the accurate detection of parvovirus IgG. *J Med Virol* 1999; 57:179–185

22 Hermanson GT: Heterobifunctional Cross-Linkers; in *Bioconjugate Techniques.* San Diego, Academic Press, 1996:228–286

23 Saldanha J, Lelie N, Yu MW, Heath A: B19 Collaborative Study Group: Establishment of the first World Health Organization International Standard for human parvovirus B19 DNA nucleic acid amplification techniques. *Vox Sang* 2002; 82:24–31

24 Boschetti N, Niederhauser I, Kempf C, Stuhler A, Lower J, Blumel J: Different susceptibility of B19 virus and mice minute virus to low pH treatment. *Transfusion* 2004; 44:1079–1086

25 Nguyen QT, Wong S, Heegaard ED, Brown K: Identification and characterisation of a second novel human erythrovirus variant, A6. *Virology* 2002; 301:374–380

26 Servant A, Laperche S, Lallemand F, Marinho V, De Saint Maur G, Meritet JF, Garbarg-Chenon A: Genetic diversity within human erythroviruses: identification of three genotypes. *J Virol* 2002; 76:9124–9134

27 Hokynar K, Ekman A, Kantola K, Kakkola L, Hedman L, Bonden H, Aberham C, Gessner M, Garbarg-Chenon A, Hedman K, Soderlund-Venermo M: Biological and immunological relation of the three variants of human parvovirus B19. Poster presented at the XIth Parvovirus Conference, Les Diablerets, Switzerland, 27–31 August 2006

28 Cassinotti P, Siegl G: Quantitative evidence for persistence of human parvovirus B19 DNA in an immunocompetent individual. *Eur J Clin Microbiol Infect Dis* 2000; 19:886–887

29 Anderson MJ, Higgins PG, Davis LR, Willman JS, Jones SE, Kidd IM, Pattison JR, Tyrrell DA: Experimental parvovirus infection in humans. *J Infect Dis* 1985; 152:257–265

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識別番号・報告回数		回	報告日 年　月　日	第一報入手日 2007 年 12 月 5 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	Standardization of nucleic acid amplification technique (NAT)-based assays for different genotypes of parvovirus B19: a meeting summary. Baylis, S.A. Vox Sanguinis, 94, 74-80 (2008)	公表国 英国	
販売名（企業名）						

研究報告の概要

ヒト血液製剤のパルボウイルス B19 汚染に関するゲノム増幅法の標準化についての国際ワーキンググループ会議（2007 年 3 月開催）で協議された議題の要約である。
パルボウイルス B19 の新規の遺伝子型が発見されていることから，規制基準を満たすためには核酸増幅法（NAT）による血漿プールのスクリーニング手順を更新する必要がある。そのため，本会議は，管理分析機関及び血漿分画製剤の製造者間でのパルボウイルス B19 の種々の遺伝子型の検出及び定量結果を統一する方法を見いだす目的で開催された。パルボウイルス B19 の全 3 種の遺伝子型は極めて似通っており，in vitro 試験で感染性の差は認められなかった。遺伝子型 1 及び 2 は，熱又は低 pH 条件に対し同等に不活化されることが知られている。さらに，先に示した文献［BYL-2008-0297］に記載の結果も本会議で提示された；米国人及びヨーロッパ人の血漿ドナーにおける遺伝子型 2 及び 3 の保有率は非常に低く，ガーナにおけるパルボウイルス B19 感染は大部分が遺伝子型 3 に起因していた。
本会議では，特性が十分に明らかになっている標準物質を用いたアッセイの標準化について合意が得られた。これにより，パルボウイルス B19 の種々の遺伝子型を示す血漿検体パネルが作成されることが示唆される。また，会議中，パルボウイルス B19 株の新規 DNA 配列がある場合はデータベースに蓄積し，閲覧可能な状態にしておくべきであることも強調されていた。

報告企業の意見	今後の対応
パルボウイルス B19 の新規の遺伝子型が発見されており，規制基準に対応するためには，パルボウイルス B19 の検出アッセイを新たに開発するか，又は高感度，或いは特異的なアッセイにする必要があると考えられる。 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。	引き続き，パルボウイルス B19 の亜型の検出や疫学に関する研究の進展の情報収集に努める。

使用上の注意記載状況・その他参考事項等
BYL-2008-0304

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REPORT

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Standardization of nucleic acid amplification technique (NAT)-based assays for different genotypes of parvovirus B19: a meeting summary

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An extraordinary meeting of the International Working Group on the Standardization of Genome Amplification Techniques for the safety testing of blood, tissues and organs for blood borne pathogens was held on 2 March 2007, at the National Institute for Biological Standards and Control. The aim of the meeting was to investigate ways to harmonize results obtained for the detection and quantification of different genotypes of parvovirus B19 (B19V) DNA by control laboratories and manufacturers of plasma derivatives. The meeting explored issues of B19V such as the classification of B19V strains, the prevalence and distribution of different genotypes, the clinical and biological significance of different genotypes, the detection of different genotypes in plasma-derived products, and their susceptibility to virus-inactivation procedures. At this meeting and through subsequent studies, high titre, high volume samples have been identified representing different genotypes of B19V, which will be evaluated by collaborative study to prepare reference panels for the purposes of assay validation.

Key words: erythrovirus B19V, plasma screening, B19V variants.

Introduction

Since 2004, European regulatory requirements have meant that plasma used in the production of anti-D immunoglobulin and pooled human plasma treated for virus inactivation must be screened to ensure that levels of parvovirus B19 (B19V) DNA do not exceed 10 IU/μl [1–3]. Plasma donations containing high titres of B19V are removed by the manufacturers of plasma derivatives, and the appropriate pools are tested by a group of European Official Medicines Control Laboratories (OMCLs) for subsequent batch release. Screening is performed using nucleic acid amplification technique (NAT)-based assays for B19V DNA. The introduction of these regulatory requirements was underpinned by the establishment of the first World Health Organization (WHO) International Standard for B19V DNA (NIBSC code 99/800) [4]. The discovery that B19V was more genetically diverse than was originally thought, forming three genotypes [5] has led to a review of testing procedures. Strains, representing each of the two more recently identified genotypes, have now been formally classified as B19V by the International Committee on the Taxonomy of Viruses (ICTV) [6]. This classification has led to regulatory issues. The guidelines for validation of quantitiative NAT assays for B19V, due to be published in the European Pharmacopoeia (Ph. Eur.), recommend that all genotypes of B19V should be detected. Recent Proficiency Testing Schemes (PTS), run by the European Directorate for the Quality of Medicines (EDQM), who coordinate the OMCL network, have highlighted discrepant results, when samples representing different genotypes of B19V have been included in the panels [7]. This was discussed further at a meeting held at the EDQM in Strasbourg on 9 November 2006, which focused on some of the issues with the types of commercial NAT assays available for the detection and quantification of B19V DNA. In an effort to harmonize results obtained by control laboratories and plasma fractionators, an extraordinary meeting of Standardization of Genome Amplification Techniques (SoGAT) was held at National Institute for Biological Standards and Control (NIBSC) on 2 March 2007. The aim of the meeting was to identify ways to provide appropriate reference materials, to support the

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