

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
10 May 2012 (10.05.2012)

PCT

(10) International Publication Number  
**WO 2012/061689 A2**

(51) International Patent Classification:  
A61K 38/37 (2006.01)

(21) International Application Number:  
PCT/US2011/059297

(22) International Filing Date:  
4 November 2011 (04.11.2011)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:  
61/410,437 5 November 2010 (05.11.2010) US

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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ,

CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

**Declarations under Rule 4.17:**

— as to the identity of the inventor (Rule 4.17(i))

**Published:**

— without international search report and to be republished upon receipt of that report (Rule 48.2(g))

— with sequence listing part of description (Rule 5.2(a))



WO 2012/061689 A2

(54) Title: A NEW VARIANT OF ANTIHEMOPHILIC FACTOR VIII HAVING INCREASED SPECIFIC ACTIVITY

(57) Abstract: The present invention is in the field of hemophilia therapy. It relates to a new variant of antihemophilic factor VIII having increased specific activity in comparison to known factor VIII products.

**A new variant of antihemophilic factor VIII having increased specific activity**

FIELD OF THE INVENTION

- 5 The present invention is in the field of blood coagulation factors and hemophilia. It relates to a new variant of antihemophilic factor VIII (FVIII), designated herein rFVIIIv3, having an increased specific activity in comparison to known factor VIII products.

BACKGROUND OF THE INVENTION

- 10 Blood clotting begins when platelets adhere to the cut wall of an injured blood vessel at a lesion site. Subsequently, in a cascade of enzymatically regulated reactions, soluble fibrinogen molecules are converted by the enzyme thrombin to insoluble strands of fibrin that hold the platelets together in a thrombus. At each step in the cascade, a variant precursor is converted to a protease that cleaves the next variant  
15 precursor in the series. Cofactors are required at most of the steps.

- Factor VIII (also called antihemophilic factor VIII or FVIII) circulates as an inactive precursor in blood, bound tightly and non-covalently to von Willebrand factor. Factor VIII is proteolytically activated by thrombin or factor Xa, which dissociates it from von Willebrand factor and activates its procoagulant function in the cascade. In its active  
20 form, the variant factor VIIIa is a cofactor that increases the catalytic efficiency of factor IXa toward factor X activation by several orders of magnitude.

- Cloning of human FVIII has revealed that the variant contains 2332 amino acids organized within a number of domains with the sequence A1-A2-B-A3-C1-C2 (Vehar et al., 1984, Nature 312, 337-342; Toole et al., 1984, Nature 312, 342-347 and Wood  
25 et al., 1984, Nature 312, 330-337). Most of FVIII is heterodimeric in plasma, containing a light-chain and various heavy-chain derivatives. The heterodimeric structure is due to proteolytic cleavage of the precursor at arginine<sup>1648</sup>, resulting in heavy- and light-chains comprising A<sub>1</sub>-A<sub>2</sub>-B and A<sub>3</sub>-C<sub>1</sub>-C<sub>2</sub>, respectively. The heterogeneity within the heavy chain is explained by limited proteolysis within its  
30 carboxy-terminal B-domain.

In order to function as a co-factor for factor X activation, FVIII requires limited proteolysis by factor Xa or thrombin. This activation involves cleavage at arginine at positions 372 and 740 on the heavy-chain and at position 1689 on the light-chain. It has been established that in comparison with the inactive precursor, active FVIII

cofactor lacks a light chain fragment 1649-1689 and the whole B-domain (Mertens *et al.*, 1993).

People with deficiencies in factor VIII or antibodies against factor VIII who are not treated with factor VIII suffer uncontrolled internal bleeding that may cause a range of serious symptoms, from inflammatory reactions in joints to early death. Severe hemophiliacs, who number about 10,000 in the United States, can be treated with infusion of human factor VIII, which will restore the blood's normal clotting ability if administered with sufficient frequency and concentration. The classic definition of factor VIII, in fact, is the substance present in normal blood plasma that corrects the clotting defect in plasma derived from individuals with hemophilia A.

Several preparations of human plasma-derived factor VIII of varying degrees of purity are available commercially for the treatment of hemophilia A. These include a partially purified factor VIII derived from the pooled blood of many donors that is heat- and detergent treated for viruses but contain a significant level of antigenic variants; a monoclonal antibody purified factor VIII that has lower levels of antigenic impurities and viral contamination; and recombinant human factor VIII.

The development of antibodies ("inhibitors" or "inhibitory antibodies") that inhibit the activity of factor VIII is a serious complication in the management of patients with hemophilia.

Alloantibodies develop in approximately 20% of patients with hemophilia A in response to therapeutic infusions of factor VIII. In previously untreated patients with hemophilia A who develop inhibitors, the inhibitor usually develops within one year of treatment. Additionally, antibodies (autoantibodies) that inactivate factor VIII occasionally develop in individuals with previously normal factor VIII levels. If the inhibitor titer is low enough, patients can be managed by increasing the dose of factor VIII. However, often the inhibitor titer is so high that it cannot be overwhelmed by factor VIII. An alternative strategy is to bypass the need for factor VIII during normal hemostasis using activated prothrombin complex concentrate preparations (for example, KONYNE (Cutter Laboratories), FEIBA (Baxter Healthcare), PROPLEX (Baxter Healthcare)) or recombinant human factor VIIa. Additionally, since porcine factor VIII usually has substantially less reactivity with inhibitors than human factor VIII, a partially purified porcine factor VIII preparation (HYATE: C (IPSEN Pharma)) has been used. Many patients who have developed inhibitory antibodies to human factor VIII have been successfully treated with porcine factor VIII and have tolerated such treatment for long periods of time.

However, public health concerns regarding the risk of viruses or other blood-borne contaminants have limited the usefulness of porcine factor VIII purified from porcine blood. A recombinant porcine factor VIII variant has therefore been developed, which is designated "OBI-1" and described e.g. in WO 01/68109. OBI-1 is a partially B-domain deleted porcine FVIII. This molecule is presently in clinical development.

B-domain deleted factor VIII variants are known to keep the procoagulant and cofactor activity of factor VIII. In addition to this, Mertens et al. (British Journal of Haematology 1993, 85, 133-142) describe recombinant human factor VIII variants lacking the B-domain and the heavy-chain sequence spanning from Lysine 713 to Arginine 740.

Many hemophiliacs require daily replacement of factor VIII to prevent bleeding and the resulting deforming hemophilic arthropathy. In view of this, there is a need for a more potent factor VIII molecule.

#### SUMMARY OF THE INVENTION

In a first aspect, the invention relates to an isolated, recombinant, fully or partially B-domain deleted factor VIII (FVIII) variant, the FVIII variant being devoid of an up to 27 amino acid sequence corresponding to amino acids 716 to 742 of porcine factor VIII as depicted in Figure 2 or amino acids 714 to 740 of SEQ ID NO: 7. This 27 amino acid sequence, NTGDYYEDSYEDISAYLLSKNNAIEPR (SEQ ID NO:10), may be partially or completely deleted.

In one embodiment, the invention relates to an isolated, recombinant, fully or partially B-domain deleted FVIII variant, the FVIII variant being devoid of an up to 27 amino acid sequence corresponding to amino acids 716 to 742 of porcine factor VIII as depicted in Figure 2 or amino acids 714 to 740 of SEQ ID NO: 7, where 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 amino acids may be deleted. In another embodiment, 27, 26, 25, 24, 23, 22, 21 or 20 amino acids are deleted. In another embodiment, 27, 26, or 25 amino acids are deleted. In another embodiment, the 27 amino acids corresponding to DIGDYDNTYEDIPGFLLSGKNVIEPR (SEQ ID NO:10) are deleted.

In one embodiment, the invention relates to an isolated, recombinant, fully or partially B-domain deleted FVIII variant, the FVIII variant being devoid of an up to 27 amino acid sequence corresponding to amino acids 716 to 742 of human factor VIII as depicted in Figure 2 or amino acids 714 to 740 of SEQ ID NO: 8, where 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 amino acids may be deleted. In another embodiment, 27, 26, 25, 24, 23, 22, 21 or

20 amino acids are deleted. In another embodiment, 27, 26, or 25 amino acids are deleted. In another embodiment, the 27 amino acids corresponding to NTGDYYEDSYEDISAYLLSKNNAIEPR (SEQ ID NO:11) are deleted.

5 In another embodiment, the invention relates to an isolated, recombinant, fully or partially B-domain deleted porcine FVIII variant being completely devoid of the 27 amino acids corresponding to DIGDYYDNTYEDIPGFLLSGKNVIEPR (SEQ ID NO:10).

10 In another embodiment, the invention relates to an isolated, recombinant, fully or partially B-domain deleted human FVIII variant being completely devoid of the 27 amino acids corresponding to NTGDYYEDSYEDISAYLLSKNNAIEPR (SEQ ID NO:11).

15 In another embodiment, the invention relates to an isolated, recombinant, fully or partially B-domain deleted canine FVIII variant being completely devoid of the 27 amino acids corresponding to NIDDYYEDTYEDIPTLLNENNVIKPR (SEQ ID NO:12).

A second aspect of the invention relates to a polynucleotide encoding a polypeptide as defined in the first aspect and the embodiments described.

In a third aspect, the invention relates to an expression vector comprising a polynucleotide as defined in the second aspect.

20 A fourth aspect of the invention relates to a mammalian cell comprising an expression vector as defined in the third aspect.

In a fifth aspect, the invention relates to a method for producing a FVIII as defined in the first aspect, comprising the steps of:

- a. Culturing a mammalian cell according to the fourth aspect; and
- 25 b. Isolating from the mammalian cell the FVIII variant.

A sixth aspect of the invention relates to a pharmaceutical composition comprising a FVIII variant as defined in the first aspect.

30 In a seventh aspect, the invention relates to a method of treating a patient suffering from hemophilia comprising administering a therapeutically effective amount of FVIII variant according to the first aspect of the invention to a patient in need thereof, thereby treating the hemophilia in said patient.

In an eighth aspect, the invention relates to a FVIII variant according to the first aspect for use in treating hemophilia.

## BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows the amount of protein in  $\mu\text{g}$  per dose required for 5 known FVIII products (blank columns) and the new rpFVIIIv3 (hashed column). BDD=B-domain deleted; FL=full length; u= $\mu$ ;

5 Fig. 2 a-e are continuations of the same sequence. Fig 2a-e shows a sequence alignment between the factor VIII sequences from human (*homo sapiens*), pig (*sus scrofa*), mouse (*mus musculus*) and dog (*canis familiaris*). This sequence alignment is taken from HADB (aka HAMSTeRS) 2010, home page of the Haemophilia A Mutation Database and a resource site for work on factor VIII ([hadb.org.uk](http://hadb.org.uk)). The numbering follows the human sequence and is not identical to the amino acid numbers of the sequences in the Sequence Listing. The bold plus double underlined sequence depicts the sequence missing in the recombinant FVIIIv3 variants of the invention. The underlined sequence is the sequence of the B-domain. The sequence highlighted in grey is the portion from the B-domain which can be retained in the partially B-domain deleted FVIIIv3 sequences of the invention; and

10

15

Fig. 3 shows typical example of the SEC profile of the calibration standards and the linear regression of the calibration curve as described in Example 5.

## BRIEF DESCRIPTION OF THE SEQUENCES

20 SEQ ID NO: 1 shows the sequence of porcine partially B-domain deleted FVIIIv3;  
 SEQ ID NO: 2 shows the sequence of a human B-domain deleted FVIIIv3;  
 SEQ ID NO: 3 shows the sequence of a canine B-domain deleted FVIIIv3;  
 SEQ ID NO: 4 shows the sequence from the porcine FVIII B-domain that can be present in a partially B-domain deleted FVIII molecule of the invention  
 25 (the so called "B-domain linker");  
 SEQ ID NO: 5 shows a B-domain linker sequence from the human FVIII B-domain that can be present in a partially B-domain deleted FVIII molecule of the invention;  
 SEQ ID NO: 6 shows a B-domain linker sequence from the canine FVIII B-domain  
 30 that can be present in a partially B-domain deleted FVIII molecule of the invention;  
 SEQ ID NO: 7 shows the amino acid sequence of full-length porcine FVIII;  
 SEQ ID NO: 8 shows the amino acid sequence of full-length human FVIII;

SEQ ID NO: 9 shows the amino acid sequence of full-length canine FVIII;

SEQ ID NO:10 shows the 27 peptide sequence which corresponds to amino acids 716 to 742 of porcine factor VIII as depicted in Figure 2 or amino acids 714 to 740 of SEQ ID NO: 7;

5 SEQ ID NO:11 shows the 27 peptide sequence which corresponds to amino acids 714 to 740 of human factor VIII as depicted in Figure 2 or amino acids 714 to 740 of SEQ ID NO: 8; and

SEQ ID NO:12 shows the 27 peptide sequence which corresponds to amino acids 714 to 740 of canine factor VIII as depicted in Figure 2 or amino acids  
10 708 to 734 of SEQ ID NO: 9.

#### DETAILED DESCRIPTION OF THE INVENTION

The invention is based on the finding of a variant of a partially B-domain deleted recombinant porcine factor VIII protein which contains a particular 27 amino acid deletion. This variant, which is herein designated rpFVIIIv3, has been shown to have  
15 an increased specific activity as compared to a similar protein containing the 27 amino acid stretch, namely a partially B-domain deleted recombinant porcine FVIII called "OBI-1". OBI-1, its amino acid sequence and polynucleotides encoding OBI-1 are known e.g. from US 6,458,563.

The invention relates to an isolated, recombinant, fully or partially B-domain deleted  
20 factor VIII (FVIII) variant, wherein the FVIII variant is devoid of an amino acid sequence corresponding to amino acids 716 to 742 of porcine factor VIII as depicted in Figure 2 or amino acids 714 to 740 of SEQ ID NO: 7.

Additionally, the invention relates to an isolated, recombinant, fully or partially B-domain deleted FVIII variant, the FVIII variant being devoid of a 27 amino acid  
25 sequence corresponding to amino acids 716 to 742 of porcine factor VIII as depicted in Figure 2 or amino acids 714 to 740 of SEQ ID NO: 7, where 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 amino acids may be deleted. In one variant, 27, 26, 25, 24, 23, 22, 21 or 20 amino acids are deleted. In another variant, 27, 26, or 25 amino acids are deleted. In the rpFVIIIv3  
30 variant, the 27 amino acids corresponding to DIGDYDNTYEDIPGFLLSGKNVIEPR (SEQ ID NO:10) are deleted.

Additionally, the invention relates to an isolated, recombinant, fully or partially B-domain deleted FVIII variant, the FVIII variant being devoid of a 27 amino acid sequence corresponding to amino acids 714 to 740 of human factor VIII as depicted

in Figure 2 or amino acids 714 to 740 of SEQ ID NO: 8, where 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 amino acids may be deleted. In one variant, 27, 26, 25, 24, 23, 22, 21 or 20 amino acids are deleted. In another variant, 27, 26, or 25 amino acids are deleted. In a human  
5 FVIIIv3 variant (rhFVIIIv3), the 27 amino acids corresponding to NTGDYYEDSYEDISAYLLSKNNAIEPR (SEQ ID NO:11) are deleted.

Additionally, the invention relates to an isolated, recombinant, fully or partially B-domain deleted FVIII variant, the FVIII variant being devoid of a 27 amino acid sequence corresponding to amino acids 714 to 740 of canine factor VIII as depicted  
10 in Figure 2 or amino acids 708 to 734 of SEQ ID NO: 9, where 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 amino acids may be deleted. In one variant, 27, 26, 25, 24, 23, 22, 21 or 20 amino acids are deleted. In another variant, 27, 26, or 25 amino acids are deleted. In a canine FVIIIv3 variant (rcFVIIIv3), the 27 amino acids corresponding to  
15 NIDDYYEDTYEDIPTLLNENNVIKPR (SEQ ID NO:12) are deleted.

Such variants have an elevated specific activity as compared to similar variants containing the intact 27 amino acid sequence. Preferably, the specific activity is increased by more than 25 % or 30 % or 35 % or 40 % or 45 % or 50 % or 55 % or 60 %.

20 The term "specific activity," as used herein, refers to the activity that will correct the coagulation defect of human factor VIII deficient plasma. Specific activity is measured in units of clotting activity per milligram total factor VIII variant in a standard assay in which the clotting time of human factor VIII deficient plasma is compared to that of normal human plasma. A suitable standard assay for measuring the potency of FVIII  
25 products, which is accepted by the FDA for high purity FVIII concentrates, is called the one-stage clotting assay (OSCA; see Example 5; Langdell RD, Wagner RH, Brinkhous KM., Effect of antihemophilic factor on one-stage clotting tests: a presumptive test for hemophilia and a simple one-stage antihemophilic assay procedure, J Lab Clin Med, 1953; 41:637-47; Brand JT, Measurement of factor VIII:  
30 a potential risk factor for vascular disease, Arch Pathol Lab Med, 1993; 117:48-51; Preston FE, Kitchen S, Quality control and factor VIII assays, Haemophilia, 1998; 4:651-3; National Committee for Clinical Laboratory Standards (NCCLS USA). Determination of factor coagulant activities; Approved guideline, NCCLS Document H-48-A 1997; 17:1-36.). The amount of FVIII protein present in a sample can be  
35 measured e.g. by SEC HPLC (size-exclusion high-performance liquid chromatography).

One unit of factor VIII activity is the activity present in one milliliter of normal human plasma. In the assay, the shorter the time for clot formation, the greater the activity of the factor VIII being assayed. Porcine factor VIII has coagulation activity in a human factor VIII assay.

- 5 In line with the present invention, the terms "protein" and "polypeptide" are being used interchangeably.

Being "devoid of an amino acid sequence corresponding to amino acids 716 to 742 as depicted in Fig. 2" means that amino acids 716 and 742, as well as the amino acids between these positions, are deleted, *i.e.* not included, in the FVIII variant of  
10 the invention. This stretch of amino acids consists thus of 27 amino acids, of which the FVIII variants of the invention are devoid.

The factor VIII variants of the invention are herein globally designated "FVIII variant(s)" or "FVIIIv3" or "rFVIIIv3". They can be of porcine, human, canine, murine or any other origin which is appropriate for human or animal therapy. Fig. 2 depicts a  
15 sequence alignment of FVIII sequences of human, porcine, murine and canine origin.

The sequence deleted in the FVIII variants of the invention is highlighted in bold and double underlined. This stretch of amino acids can be identified for the FVIII variants of other species by the person skilled in the art by aligning the FVIII sequences of further species with *e.g.* the porcine or human sequence and taking the amino acids  
20 that correspond to amino acids 716 to 742 as per Fig. 2 of the present patent application.

Embodiments of the invention relate to FVIII variants of porcine, human or canine origin.

In an embodiment, the FVIII variant according to the invention comprises the  
25 sequence of SEQ ID NO:1, or a variant thereof comprising a sequence being at least 90% identical to SEQ ID NO: 1. It is understood that the 10% variability concerns the sequences outside of the deleted stretch of amino acids, *i.e.* that the variant is completely devoid of DIGDYDNTYEDIPGFLLSGKNVIEPR (SEQ ID NO:10), an amino acid sequence corresponding to amino acids 716 to 742 of porcine factor VIII  
30 as depicted in Figure 2 or amino acids 714 to 740 of SEQ ID NO: 7.

In a further embodiment, the FVIII variant of the invention comprises the sequence of SEQ ID NO:2, or a variant thereof comprising a sequence being at least 90% identical to SEQ ID NO: 2. Again, the variant is understood to be completely devoid of an amino acid sequence NTGDYYEDSYEDISAYLLSKNNAIEPR (SEQ ID NO:11),

corresponding to amino acids 714 to 740 of human factor VIII as depicted in Fig. 2 or amino acids 714 to 740 of SEQ ID NO: 8.

In yet a further embodiment, the FVIII variant of the invention comprises the sequence of SEQ ID NO:3, or a variant thereof comprising a sequence being at least  
5 90% identical to SEQ ID NO: 3. Again, the variant is understood to be completely devoid of an amino acid sequence NIDYYEDTYEDIPTLLNENNVIKPR (SEQ ID NO:12) corresponding to amino acids 714 to 740 of canine factor VIII as depicted in Fig. 2 or amino acids 708 to 734 of SEQ ID NO: 9.

The FVIII variants of the invention can also comprise sequences that are at least 91,  
10 92, 93, 94, 95, 96, 97, 98, 99, 99.5, 99.6, 99.7, 99.8 or 99.9% identical to the sequences of SEQ ID NO: 7, 8 or 9.

To determine the percent identity of two polypeptides/proteins, the amino acid sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in the sequence of a first amino acid sequence for optimal alignment with  
15 a second amino acid sequence). The amino acid residues at corresponding amino acid positions are then compared. When a position in the first sequence is occupied by the same amino acid residue as the corresponding position in the second sequence, then the molecules are identical at that position. The percent identity between the two sequences is a function of the number of identical positions shared  
20 by the sequences (*i.e.*, % identity=# of identical positions/total # of positions (e.g., (overlapping positions)×100).

The determination of percent identity between two sequences can be accomplished using a mathematical algorithm. A preferred, non-limiting example of a mathematical algorithm utilized for the comparison of two sequences is the algorithm of Karlin and  
25 Altschul (1990) Proc. Natl. Acad. Sci. USA 87:2264–2268, modified as in Karlin and Altschul (1993) Proc. Natl. Acad. Sci. USA 90:5873–5877. Such an algorithm is incorporated into the NBLAST and XBLAST programs of Altschul, *et al.* (1990) J. Mol. Biol. 215:403–410. BLAST nucleotide searches can be performed with the NBLAST program, score=100, wordlength=12 to obtain nucleotide sequences  
30 homologous to a nucleic acid molecules of the invention. BLAST variant searches can be performed with the XBLAST program, score=50, wordlength=3 to obtain amino acid sequences homologous to a variant molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul *et al.* (1997) Nucleic Acids Res. 25:3389–3402. Alternatively,  
35 PSI-Blast can be used to perform an iterated search which detects distant

relationships between molecules. When utilizing BLAST, Gapped BLAST, and PSI-Blast programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) can be used. Another preferred, non-limiting example of a mathematical algorithm utilized for the comparison of sequences is the algorithm of Myers and Miller, (1988) CABIOS 4:11–17. Such an algorithm is incorporated into the ALIGN program (version 2.0) which is part of the GCG sequence alignment software package. When utilizing the ALIGN program for comparing amino acid sequences, a PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4 can be used. Yet another useful algorithm for identifying regions of local sequence similarity and alignment is the FASTA algorithm as described in Pearson and Lipman (1988) Proc. Natl. Acad. Sci. USA 85:2444–2448. When using the FASTA algorithm for comparing nucleotide or amino acid sequences, a PAM120 weight residue table can, for example, be used with a k-tuple value of 2.

The percent identity between two sequences can be determined using techniques similar to those described above, with or without allowing gaps. In calculating percent identity, only exact matches are counted.

The FVIII variant of the invention can be partially or fully B-domain deleted. This means that either the whole B-domain is deleted, or a part of the B-domain is retained in the FVIII variant. The remaining (retained) part of the B-domain is e.g. selected from the 20, 15, 12, 10 or 5 N-terminal amino acids and the 20, 15, 12, 10 or 5 C-terminal amino acids of the B-domain, fused in frame with each other.

Hence, preferably, in the partially B-domain deleted FVIII variants of the invention, significant portions of the approximately 900 amino acid B-domain are being removed. In embodiments of the invention, less than 5% or less than 4% or less than 3% or less than 2 % or less than 1 % of the B-domain remain present in the FVIII variants.

In embodiments of the invention, the remaining portion of the B-domain is selected from a sequence consisting of SEQ ID NO: 4 or SEQ ID NO:5 or SEQ ID NO: 6.

In a further aspect, the invention relates to a polynucleotide encoding a polypeptide/protein as described above. A polynucleotide can be an RNA or DNA molecule whose nucleotide sequence embodies coding information to a host cell for the amino acid sequence of the variant of the invention, according to the known relationships of the genetic code.

In a further aspect, the invention relates to an expression vector comprising a polynucleotide as described above.

An "expression vector" is a DNA element, often of circular structure, having the ability to replicate autonomously in a desired host cell, or to integrate into a host cell genome and also possessing certain well-known features which permit expression of a coding DNA inserted into the vector sequence at the proper site and in proper orientation. Such features can include, but are not limited to, one or more promoter sequences to direct transcription initiation of the coding DNA and other DNA elements such as enhancers, polyadenylation sites and the like, all as well known in the art. The term "expression vector" is used to denote both a vector having a DNA coding sequence to be expressed inserted within its sequence, and a vector having the requisite expression control elements so arranged with respect to an insertion site that it can serve to express any coding DNA inserted into the site, all as well-known in the art. Thus, for example, a vector lacking a promoter can become an expression vector by the insertion of a promoter combined with a coding DNA. An expression vector, as used herein, can also be a viral vector.

Expression of the recombinant FVIII variants of the invention is preferably carried out in mammalian cell culture.

Therefore, in a further aspect, the invention relates to a mammalian cell comprising an expression vector as described above. For instance, CHO (Chinese hamster ovary) cells and BHK cells (baby hamster kidney cells) are mammalian cells that are suitable host cells in the context of the present invention.

In accordance with another aspect of the invention, a method for producing a FVIII variant of the invention comprises the steps of:

- a. Culturing a mammalian cell as described above; and
- b. Isolating from the mammalian cell the FVIII variant.

In an embodiment, the method further comprises the step of

- c. Formulating the factor VIII variant together with appropriate excipients into a pharmaceutical composition.

Excipients suitable for human or animal administration are e.g. pharmaceutical stabilization compounds, preservatives, delivery vehicles, and/or carrier vehicles.

One suitable formulation for factor VIII products is e.g. described in WO 03/080108, which is incorporated by reference herein.

In a further aspect, the invention relates to a pharmaceutical composition comprising a FVIII variant of the invention.

A further aspect of the invention relates to a method of treating a patient suffering from factor VIII deficiency comprising administering a therapeutically effective amount of FVIII variant of the invention to a patient in need thereof, thereby treating the factor VIII deficiency in said patient.

- 5 The term "therapeutically effective amount" as used herein, means the level of FVIII in the plasma of a patient having FVIII deficiency, who has received a pharmaceutical composition of FVIII variant, that is sufficient to exhibit a measurable improvement or protective effect in the patient (e.g., to stop bleeding). The patients having FVIII deficiency are typically congenital hemophilia A patients but also include those
- 10 subjects diagnosed with "acquired hemophilia", a condition in which those who are not congenital hemophiliacs spontaneously develop inhibitory antibodies to their FVIII, creating a serious FVIII deficiency.

The invention also relates to a FVIII variant of the invention for use in treating factor VIII deficiency.

- 15 Treatment can take the form of a single intravenous administration of the composition or periodic or continuous administration over an extended period of time, as required. Preferably, administration of the FVIII variant is by intravenous route.

- "Factor VIII deficiency," as used herein, includes deficiency in clotting activity caused by production of defective factor VIII, by inadequate or no production of factor VIII, or
- 20 by partial or total inhibition of factor VIII by inhibitors. Hemophilia A is a type of factor VIII deficiency resulting from a defect in an X-linked gene and the absence or deficiency of the factor VIII variant it encodes.

- The FVIII variants of the invention are used to treat uncontrolled bleeding due to factor VIII deficiency (e.g., intraarticular, intracranial, or gastrointestinal hemorrhage)
- 25 in hemophiliacs.

In an embodiment of the invention, the factor VIII deficiency is hemophilia A.

In another embodiment, the factor VIII deficiency is acquired hemophilia.

In an embodiment, the factor VIII deficiency is treated in patients having developed human FVIII antibodies.

- 30 As mentioned above, the FVIII variants of the invention have increased specific activity. It is therefore possible to administer reduced amounts of FVIII variant in order to treat VIII deficiency in accordance with the present invention. The amounts of FVIII variant can be reduced by at least 30%, 35%, 40%, 45%, 50%, 55%, 60% as

compared to therapy with a FVIII product that does not contain the up to 27 amino acid deletion of the invention.

Reducing the amount of FVIII variant therapy has the advantage of expected reduced immunogenicity, *i.e.* it is expected that the probability of generation of inhibitory antibodies against the FVIII replacement therapy in the patient is significantly reduced.

In an embodiment, a factor VIII variant of the invention is administered in an amount of no more than 200 µg/dose or 150 µg/dose or 145 µg/dose or 140 µg/dose or 136 µg/dose or 130 µg/dose.

Having now fully described this invention, it will be appreciated by those skilled in the art that the same can be performed within a wide range of equivalent parameters, concentrations and conditions without departing from the spirit and scope of the invention and without undue experimentation.

While this invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications. This application is intended to cover any variations, uses or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth as follows in the scope of the appended claims.

All references cited herein, including journal articles or abstracts, published or unpublished U. S. or foreign patent application, issued U. S. or foreign patents or any other references, are entirely incorporated by reference herein, including all data, tables, figures and text presented in the cited references. Additionally, the entire contents of the references cited within the references cited herein are also entirely incorporated by reference.

Reference to known method steps, conventional methods steps, known methods or conventional methods is not any way an admission that any aspect, description or embodiment of the present invention is disclosed, taught or suggested in the relevant art.

The foregoing description of the specific embodiments will so fully reveal the general nature of the invention that others can, by applying knowledge within the skill of the art (including the contents of the references cited herein), readily modify and/or adapt for various application such specific embodiments, without undue experimentation,

without departing from the general concept of the present invention. Therefore, such adaptations and modifications are intended to be within the meaning a range of equivalents of the disclosed embodiments, based on the teaching and guidance presented herein. It is to be understood that the phraseology or terminology herein is for the purpose of description and not of limitation, such that the terminology or phraseology of the present specification is to be interpreted by the skilled artisan in light of the teachings and guidance presented herein, in combination with the knowledge of one of ordinary skill in the art.

EXAMPLES

10 Example 1: Expression of recombinant porcine partially B-domain deleted FVIII (OBI-1) and isolation of three variant proteins (rpVIIIv1, v2 and v3)

Expression of OBI-1 in BHK cells was carried out essentially as described in US 6,458,563. Three variants were detected in the produced material (rpVIIIv1, v2 and v3). The variants were isolated and purified to >95% purity ion exchange chromatography. For purification, an AKTA Explorer system (AKTA Explorer 10, GE Healthcare Lifesciences # 17-5167-01) using a MonoQ HR10/10 semi-preparative anion exchange column (now Mono Q 10/100 GL ,GE Healthcare Lifesciences # 17-5167-01) was used. Elution was carried out on a gradient with the two following buffers:

20 Buffer A: 10 mM TRIS, pH 7.0, 0.01% Polysorbate 80

Buffer B: Buffer A and 1 M sodium chloride.

The purification method characteristics are summarized in Table 1.

**Table 1: rpFVIII Variant Purification Method**

AKTA Method:	LoopMQ1010	
Flow:	4 ml/min	
Equilibrium Column	8 CV	
Load flow	4ml/min	
Sample Volume	40 - 1000 ml	
Wash Column	10CV	
Gradient	Column Volume (CV)	%B
	7	45
	4	50
	4	50
	6	52
	4	52
	2	55
	7	60
	1	65

	5	65
	1	70
	5	70
	1	100
	7	100
	1	10
	4	10
Total Run	59 CV	
Column Temp	Not controlled	

Example 2: Variant 3 (rpVIIIv3) has a significantly higher specific activity than variants 1 and 2

The specific activity of each variant was assessed by dividing the potency assessed by one stage coagulation assay (OSCA) by the protein concentration as measured by the SEC HPLC per method. The OSCA and SEC HPLC methods were carried out as described in "Materials and Methods" Example 5.

RESULTS

The results for the specific activities are indicated in Table 2.

10 **Table 2: Specific Activities (OSCA method)for Purified Variants 1, 2 and 3 obtained from two different lots of rpFVIII**

Sample based on SEC	OSCA units/ml	%RSD*	Specific activity units/ml	%RSD*
V1 – lot 1	1060	1.5	11956	1.7
V2 – lot 1	715	2.7	17267	3.3
V3 – lot 1	216	1.2	25926	2.8
OBI-1	513	1	18150	1.3
V1 – lot 2	980	1.2	12532	1.5
V2 – lot 2	613	0.6	16089	1.3
V3 – lot 2	280	2.9	19718	4.1
OBI-1	565	4.0	14427	4.4

\*relative standard deviation

The observation of significantly higher specific activity for variant 3 in the OSCA assay is distinct and consistent. Since the OSCA method is predictive and representative for the coagulation process in humans, it is expected that variant 3 is a product of significant value. It is expected that 1.5-2.0 fold increase in potency reduce the amount of FVIII administered to patients and might therefore indirectly reduce the occurrence of immunological side effects.

Example 3: Sequence determination of rpFVIIIv3

The sequence of purified v3 was determined by peptide map followed by LCMS (liquid chromatography mass spectrometry) and LCMS/MS (liquid chromatography mass spectrometry/mass spectrometry) on a Q-TOF Ultima Mass Spectrometer, running MassLynx 4.0 (Waters Corporation, Milford, MA).

Approximately 250 µg of Variant 3 was concentrated using an Amicon, Centricon YM-10 concentrator with a 10,000 MWCO filter (Millipore Corporation, Billerica, MA) to a volume of < 100 µL. The samples were mixed with 450 µL of 6 M guanidine HCl/ 0.002 M EDTA (ethylenediaminetetraacetic acid) /0.02 M Tris buffer pH 8 and transferred to 2.0 mL polypropylene tubes. 1 M DTT (dithiothreitol) was added to each sample to a final concentration of 10 mM and incubated for 1 hour at 37 °C. After reduction, 2 M iodoacetamide was added to each tube to a final concentration of 20 mM and incubated for an additional hour at room temperature. The reduced and alkylated samples were transferred to dialysis cassettes and dialyzed for 1 hour against 1 L of 50 mM ammonium bicarbonate dialysis buffer containing 1.0 M urea, pH 8. The samples were then dialyzed against 1 L of dialysis buffer overnight at room temperature while maintaining constant stirring. After dialysis the final volumes were approximately 0.5 mL each. Samples were divided into two 125 µg aliquots.

After dialysis, the protein sample was contained in a matrix that is optimal for proteolytic digestion with trypsin. Trypsin was added to each sample at an enzyme to substrate ratio of 1:20 (w:w) and incubated for 8 hours at 37 °C. All samples were transferred to HPLC vials and analyzed by HPLC-MS.

Reduced and alkylated protein was injected onto a Vydac C18 reverse phase column (Grace, Deerfield, IL). Prior to the sample injection, a blank sample (Mobile Phase A; deionized water containing 0.2% (v:v) formic acid) was analyzed to equilibrate the column and demonstrate the absence of interfering peaks.

Mass spectrometry data was collected on a Q-TOF API-US mass spectrometer or a Q-TOF Ultima mass spectrometer (Waters Corporation, Milford, MA) using electrospray ionization (ESI) in the positive ion mode. Prior to sample analysis, the mass spectrometer was calibrated using a 5th order fit on the fragment ions of Glu-Fibrinogen peptide covering a range of 175 to 1285 m/z. For the calibration to pass specifications, a RMS (root mean square) error for the mass of the peptide fragments less than 5 ppm was required. The software package Masslynx 4.0™ SP2 and SP4 (Waters Corporation, Milford, MA) were used for data acquisition and analysis.

Both MS and MS/MS data were collected using a single liquid chromatography (LC) run. Full mass spectrometer (MS) survey scans were collected from 200-1950 m/z.

The sequence of rpFVIII was determined to correspond to SEQ ID NO: 1. This sequence contains a 27 amino acid deletion in comparison with the OBI-1 sequence.

5 Example 4: rpFVIIIv3 has a higher specific activity than other known FVIII products

The specific activity of rpFVIIIv3 was compared to three commercially available FVIII products, namely Xynthia® (a recombinant, B-domain deleted human FVIII from Wyeth Pharmaceuticals (Philadelphia, PA)), Kogenate FS® (a full-length human FVIII from Bayer Healthcare (Tarrytown, NY)) and Advate® (a full-length human FVIII from Baxter (Westlake Village, CA)), as well as to OBI-1 (a recombinant partially B-domain deleted porcine FVIII), which is presently under clinical development, and a B-domain deleted canine factor VIII (Denise E. Sabatino *et.al.*, Recombinant canine B-domain-deleted FVIII exhibits high specific activity and is safe in the canine hemophilia A model, Blood, 12 November 2009, Vol. 114, No. 20, pp. 4562-4565).

15 The specific activity of OBI-1 and rpFVIIIv3 was determined by the methods as described in Example 5.

The results are depicted in Table 3 and Fig. 1. Due to its increased specific activities, lower amounts of prVIIIv3 per dose can be administered to a patient. It is expected that this decrease in dose will lead to a reduced immunogenicity, *i.e.* result in a lower probability that patients develop inhibitory antibodies.

**Table 3: Specific activities of FVIII products as compared to rpVIIIv3**

	Specific Activity in IU/mg Protein	Amt protein µg/dose <sup>^</sup>
Xynthia	7500	400
Kogenate FS	4000	750
Advate	4000	750
OBI-1	11000	273
rpVIIIv3	22000	136
Canine BDD* FVIII	34000	88

5        ^Amt Protein = xxxx µg/dose. The numbers shown in the column for rpVIII3 mean that an amount of 136 µg per dose will be required. One dose is the required amount to raise the blood factor VIII level from 0% to 100%, i.e. the higher the specific activity (XXX units/mg), the less amount (in terms of µg) is required. Note that 1 mg = 1000µg. Given that a typical dose is 3000 units per patient, the amount of actual protein required =  $3000/22000 = 0.136$  mg = 136 µg.

\*BDD stands for B-domain deleted and FL stands for full length

10        In conclusion, these results show that rpVIIIv3 has a significantly higher specific activity than the tested products that are presently commercialized, or under clinical development, for FVIII replacement therapy.

In particular, it is highly surprising a deletion of 27 amino acids, present in rpVIIIv3 but not in OBI-1, leads to a 50% increase in specific activity (as measured by the OSCA assay).

15        Example 5: Materials and methods

#### ONE-STAGE COAGULATION ASSAY - OSCA - METHOD

1. Dilute Reference Standard in Assay Buffer (10% Factor VIII deficient plasma + Owren's Veronal Buffer, per liter, 28 mmol of sodium barbital and 125 mmol of NaCl, pH 7.35) to target potency of 1.0 units/mL.
- 20        2. Dilute check standards, activity controls and samples in Assay Buffer to the target potency.
3. Load the calibration standard (reference) and reagents into the appropriate wells inside a Symex instrument. (Sysmex CA-1500, Coagulation Analyzer, Dade Behring # B4260-1500 (Siemens Corporation, Deerfield, IL)).
- 25        4. Load check standard, controls and samples into racks outside of the Sysmex instrument.
5. Assay sequence occurs automatically inside the instrument as follows:
  - a. 5 uL of sample is diluted into 45 ul of assay buffer inside the reaction tube
  - 30        b. 50ul of factor VIII deficient plasma is added to the reaction tube
  - c. 50ul of Dade Actin FS (purified soy phosphatides in  $1.0 \times 10^{-4}$ M Ellagic acid activator, Dade Behring, Liederbach, Germany) activated partial thromboplastin time reagent is added to the reaction tube and incubated for 60 seconds.

- d. 50ul of Calcium Chloride (0.025M, Dade Behring, Liederbach, Germany) is added to the reaction tube and incubated for 240 seconds.
  - e. The clot time is measured up to a maximum time of 300 seconds.
- 5      6. The clot time is then correlated to the potency generated by the reference standard calibration curve.

#### SIZE-EXCLUSION (SEC) HPLC METHOD

10      An Agilent 1100 (Agilent Technologies, Santa Clara, CA ) or a Waters Bioalliance HPLC system (Waters Corporation, Milford, MA) was and is typically used for the size exclusion method for determination of protein concentration. The typical size exclusion column used was a Superdex 200 from GE (Superdex 200 10.300 GL, 10 X 300mm, Cat # 17-5175-01 (GE Healthcare Lifesciences, Piscataway, NJ)). A general assay protocol is as follows:

- 15      1. A typical mobile phase was prepared that contained 400mM NaCl with 20mM TRIS, pH 7.4 and 0.01% polysorbate 80.
2. The mobile phase was run through the HPLC system and column until equilibration of the baseline was observed.
- 20      3. Standards were run to establish system suitability with a typical run time of 30 minutes for each sample.
4. Samples were typically loaded onto an auto sampler with a control chamber temperature of 4°C.
- 25      5. A calibration standard of known concentrations containing various amounts of standard samples, typically from 1, 3, 5, 10 and 25 µg of protein were loaded onto the column in volumes between 10 to 50 µl.
6. Samples of unknown concentrations were also loaded into the columns typically in volumes between 30 to 50 µl.
7. The HPLC system was programmed to run the samples automatically according to the sequence set up.
- 30      8. The peak area of the calibration standards and the unknown samples were determined based on fluorescence detection with excitation and emission at 280nm and 340nm respectively.

9. A linear regression of the calibration standards was generated and the concentration of the unknown sample was determined against this calibration curve.

A typical example of the SEC profile of the calibration standards and the linear regression of the calibration curve is shown in Figure 3.

#### Example 6: Binding to von Willebrand Factor

Von Willebrand factor (vWF) is a multifunctional glycoprotein which circulates in plasma as a multimeric form complexed with Factor VIII (FVIII/vWF complex). The FVIII/vWF complex serves to protect the bound FVIII from early proteolysis in circulation *in vivo*.

Size exclusion chromatography (SEC) is used to characterize the binding in FVIII/vWF complexes. A Superose 6 column (Superose 6 10/300GL, GE Healthcare #17-5172-01, flow rate = 0.5 mL/min) was selected for its known biocompatibility and high exclusion limit of 40 million Daltons (Mobile Phase formulation buffer: 2 mM CaCl<sub>2</sub>, 10 mM Tris pH 7.0, 300 mM NaCl, 0.01% PSB-80, 11 mM sucrose, 10 mM trisodium citrate). The differences in molecular weight between von Willebrand factor (dimeric @ ~ 500KD, Fitzgerald Cat# 30C-CP4003U, Lot # A09121050, Monomer Mol. Wt = ~260kD, Conc. = 77 µg/mL) and rpFVIII (~ 160 KD) is sufficient to resolve the two species.

The stoichiometry of binding was determined by titration of constant amount of vWF with increasing amounts of rpFVIII. The profile of the complexation formation between rpFVIII and vWF was determined from the SEC chromatogram and integration of the appropriate peaks. The soluble form of the resultant mixtures in the supernatant were used to determine the end point of the complexation from titration with increasing amounts of rpFVIII. The larger, stable complexes formed between rpFVIII and vWF migrate from the normal retention time to the exclusion limit of the column. When saturation occurs, increasing amounts of unbound rpFVIII will be observed in the SEC chromatogram. This method is used to distinguish differences and similarities in the properties of the variants.

#### Example 7: Thrombin Digestion Kinetics

##### *SDS-PAGE*

Recombinant porcine factor VIII (rpFVIII) molecules are heterodimers of approximately 160kD composed of a 78.5kD molecular weight light chain (A3-C1-C2, 765-1448) and a heavy chain ranging in molecular weight from 86.7kD (A1-A2). The

heavy chain of rpFVIII is heterogeneous and composed of three main variants which are formed upon secretion from the cell and may be cleaved by membrane bound proteases of the subtilisin family designated PACE (Paired Basic Amino Acid Cleavage Enzyme).

5 Like the human factor VIII, rpFVIII is transformed into an active form by limited proteolysis from thrombin. Thrombin activation of rpFVIII is specific for the cleavage site of the heavy chain at Arg(372)-Ser(373). The cleavage region for the Light Chain at Arg(805)-Ser(806) liberates a small 40 amino acid peptide fraction ranging from 765-804 (~4.4kD). The combination of these initial cleavages by  
10 thrombin form the activated recombinant porcine factor VIII as a heterotrimer of subunits designated as A1 (~50kD), A2 (~40kD), and A3-C1-C2 (~70kD). The cleaved rpFVIII A1 and A3-C1-C2 subunits retain the divalent metal ion-dependent linkage, whereas the A2 subunit is weakly associated with the A1-A3-C1-C2 dimer by primarily electrostatic interactions. In SDS-PAGE, these subunits are displayed  
15 as three distinct bands. The efficiency in conversion of the rpFVIII (2 peptide units) to three peptide units is characteristic of thrombin activation of the product. This method distinguishes the similarity and differences between the individual variants.

Two other orthogonal methods can be and were used to map the kinetics of thrombin digestion of FVIII. Denaturing reverse phase HPLC may be used; the peak  
20 profiles are expected to be similar to that of SDS-PAGE. Anion exchange chromatography, which retains the native form of the peptides, may also be used.

#### *Reverse Phase HPLC*

##### Equipment:

1. HPLC 1100 series: Agilent Technologies, Santa Clara, CA, USA; Model No. 25 61312A, Serial No. DE10907753.
2. Poroshell 5um 2.1X75mm column: Agilent Technologies, Santa Clara, CA, USA; Part No. 660750-909, Serial No. USVV001988.

##### Reagents:

1. Buffer A: 0.1% TFA (JT B Baker, Phillisburg, NJ; Cat No. 94700-00, Lot 30 No. J23J00) in water
2. Buffer B: 0.1% TFA in Acetonitrile (JT Baker, Phillisburg, NJ; Cat No. 9017-03, Lot No. J38807).

##### Procedure:

1. Equilibrate the column for 60 minutes using 99%A and 1%B at 1ml/min. 35
2. Sample injections were 50µl in volume; 25 µl of reference standard was used as control.

3. An example of the method used to run the samples is shown Table 4.

Table 4:

Injection	Time (min)	Flow (ml/min)	%A	%B
1	0.00	2	99	1
2	2.00	2	99	1
3	3.00	2	64	36
4	3.50	2	64	36
5	4.00	2	61	39
6	5.00	2	61	39
7	5.10	2	58	42
8	6.50	2	58	42
9	6.51	2	56	44
10	7.20	2	56	44
11	7.21	2	40	60
12	8.50	2	40	60
13	8.51	2	10	90
14	9.00	2	10	90
15	9.10	2	99	1
16	10.00	2	99	1

#### 5 Anion Exchange Chromatography

##### Equipment:

- AllianceBio HPLC, Waters corporation, Milford, MA, USA; Model No. 2796; Serial No M08BA1199M.
- Protein Pak Hi Res Q 5um 4.6X100mm column: Waters Corporation, Milford, MA, USA; Part No. 186004931, Serial No. 502N112561VE04.

##### Reagents:

##### Buffer A:

- 10mM Tris base (1.211g/L)
- 15 2mM Calcium Chloride (0.588g/L)
- 0.01% polysorbate 80 (1ml of 10% PS-80/L)
- Filtered water up to 1L, pH 7.0

##### 20 Buffer B:

- 10mM Tris base (1.211g/L)
- 2mM Calcium Chloride (0.588g/L)
- 0.01% polysorbate 80 (1ml of 10% PS-80/L)
- 1M Sodium Chloride (58.44g/L)

Filtered water upto 1L, pH 7.0

Procedure:

1. Equilibrate the column for 60min using 70%A and 30%B at 0.5ml/min
- 5 2. Sample injections were 50µl in volume; 10 µl of reference standard was used as control.
3. An example of the method used to run the samples is shown Table 5.

Table 5:

Injection	Time (min)	Flow (ml/min)	%A	%B
1	0.01	0.5	70	30
2	0.50	0.5	70	30
3	3.50	0.5	60	40
4	5.00	0.5	60	40
5	10.00	0.5	30	70
6	10.10	0.5	1	99
7	12.00	0.5	1	99
8	12.10	0.5	70	30
9	17.00	0.5	70	30

10

The skilled artisan would know and appreciate that these and other methods may be used to arrive at understanding recombinant Factor VIII proteins as described herein.

## CLAIMS

1. An isolated, recombinant, fully or partially B-domain deleted factor VIII (FVIII)  
5 variant, the FVIII variant being devoid of an amino acid sequence corresponding to  
amino acids 716 to 742 of porcine factor VIII as depicted in Figure 2 or amino acids  
714 to 740 of SEQ ID NO: 7.
2. The FVIII variant according to claim 1, comprising a sequence being at least  
90% identical to SEQ ID NO: 1, the variant being devoid of the amino acid sequence  
10 corresponding to amino acids 716 to 742 of porcine factor VIII as depicted in Figure 2  
or amino acids 714 to 740 of SEQ ID NO: 7.
3. The FVIII variant according to claim 2, wherein said variant shares at least 95,  
96, 97, 98, 99, 99.5, 99.6, 99.7, 99.8 or 99.9% identity to SEQ ID NO:1.
4. The FVIII variant according to claim 3, wherein said variant shares 99.5, 99.6,  
15 99.7, 99.8 or 99.9% identity to SEQ ID NO:1.
5. The FVIII variant according to claim 4, wherein said variant is SEQ ID NO:1.
6. An isolated, recombinant, fully or partially B-domain deleted factor VIII (FVIII)  
variant, the FVIII variant being devoid of an amino acid sequence corresponding to  
amino acids 716 to 742 of human factor VIII as depicted in Figure 2 or amino acids  
20 714 to 740 of SEQ ID NO: 8.
7. The FVIII variant according to claim 6, comprising a sequence being at least  
90% identical to SEQ ID NO: 1, the variant being devoid of the amino acid sequence  
corresponding to amino acids 716 to 742 of porcine factor VIII as depicted in Figure 2  
or amino acids 714 to 740 of SEQ ID NO: 8.
- 25 8. The FVIII variant according to claim 7, wherein said variant shares at least 95,  
96, 97, 98, 99, 99.5, 99.6, 99.7, 99.8 or 99.9% identity to SEQ ID NO:2.
9. The FVIII variant according to claim 8, wherein said variant shares 99.5, 99.6,  
99.7, 99.8 or 99.9% identity to SEQ ID NO:2.
10. The FVIII variant according to claim 9, wherein said variant is SEQ ID NO:2.
- 30 11. The FVIII variant according to any one of the preceding claims, wherein the  
FVIII variant is partially B-domain deleted and the remaining portion of the B-domain  
is selected from a sequence consisting of SEQ ID NO: 4 or SEQ ID NO:5.

12. A polynucleotide encoding a polypeptide according any one of the preceding claims.
13. An expression vector comprising a polynucleotide according to claim 12.
14. A mammalian cell comprising an expression vector according to claim 13.
- 5 15. A method for producing a FVIII variant according to any one of claims 1 to 10, comprising the steps of :
  - a. Culturing a mammalian cell according to claim 14; and
  - b. Isolating from the mammalian cell the FVIII variant.
16. The method according to claim 15, further comprising the step of
  - 10 c. Formulating the factor VIII variant together with appropriate excipients into a pharmaceutical composition.
17. A pharmaceutical composition comprising a factor VIII variant according to any one of claims 1 to 10.
18. A method of treating a patient suffering from hemophilia comprising
  - 15 administering a therapeutically effective amount of FVIII variant according to any one of claims 1 to 10 to a patient in need thereof, thereby treating the hemophilia in said patient.
19. A FVIII variant according to any one of claims 1 to 10 for use in treating hemophilia.
- 20 20. The method or use according to claim 18 or 19, wherein said hemophilia is hemophilia A.
21. The method or use according to claim 18 or 19, wherein said hemophilia is acquired hemophilia.
22. The method or use according to any one of claims 18 to 21, wherein said
  - 25 hemophilia is treated in patients having developed human FVIII antibodies.
23. The method or use according to any one of claims 18 to 22, wherein the factor VIII is administered in an amount of no more than 200 µg/dose or 150 µg/dose or 140 µg/dose.

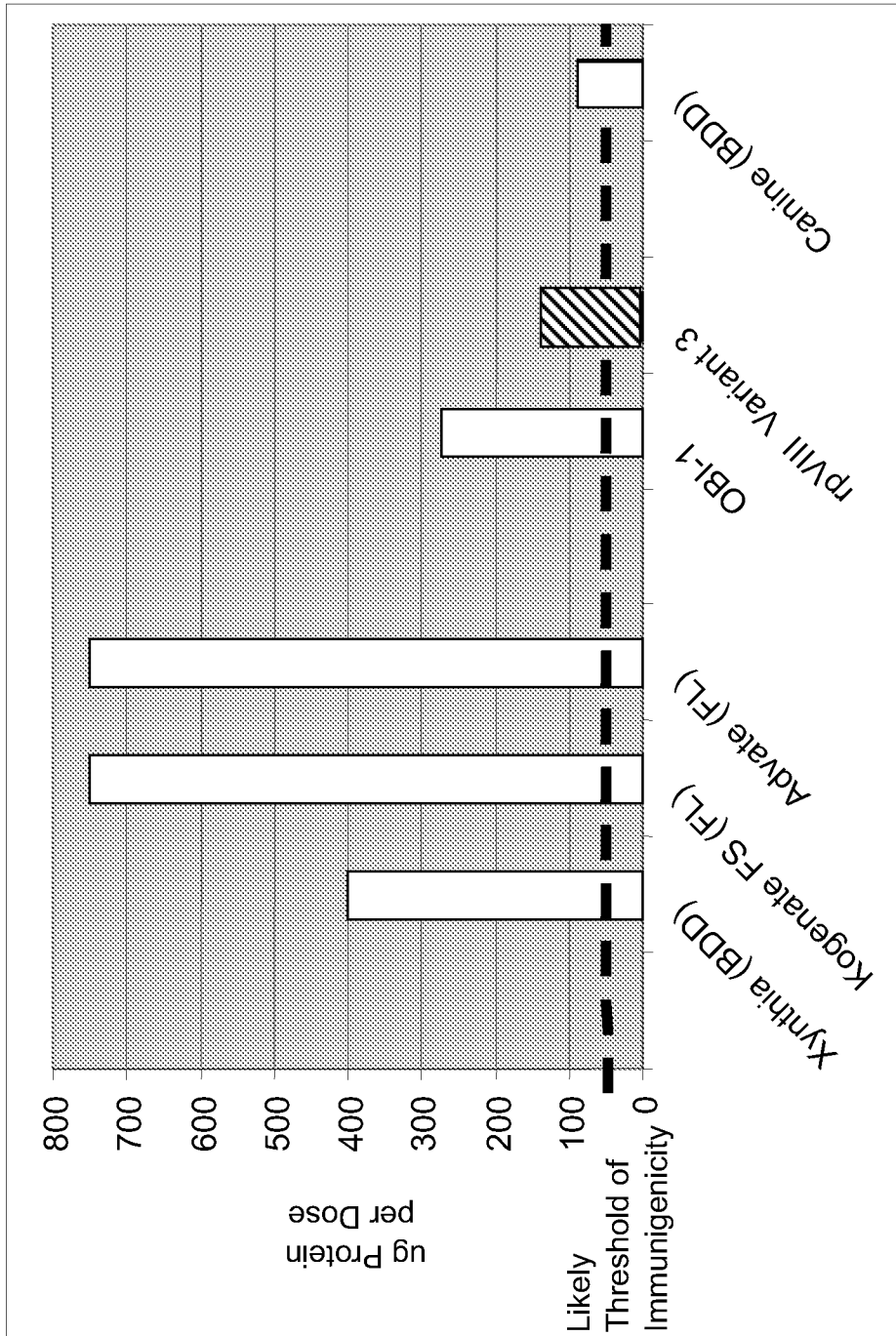


Fig. 1

2/7

	1				50
HUMFVIII	ATRRYYLGAV	ELSWDYMQSD	.LGELPVDAR	FPPRVPKSFP	FNTSVVYKKT
PIGFVIII	AIRRYLGAV	ELSWDYRQSE	LLRELHVDTR	FPATAPGALP	LGPSVLYKKT
MURFVIII	AIRRYLGAV	ELSWNYIQSD	LLSVLHTDSR	FLPRMSTSFP	FNTSIMYKKT
CANFVIII	ATRKYYLGAV	ELSWDYMQSD	LLSALHADTS	FSSRVPGSLP	LTTSVTYRKT
	51				100
HUMFVIII	LFVEFTDHLF	NIAKPRPPWM	GLLGPTIQAE	VYDTVVITLK	NMASHPVSLH
PIGFVIII	VFVEFTDQLF	SVARPRPPWM	GLLGPTIQAE	VYDTVVVTLK	NMASHPVSLH
MURFVIII	VFVEYKDQLF	NIAKPRPPWM	GLLGPTIWTE	VHDTVVITLK	NMASHPVSLH
CANFVIII	VFVEFTDDL	NIAKPRPPWM	GLLGPTIQAE	VYDTVVIVLK	NMASHPVSLH
	101				150
HUMFVIII	AVGVSYWKAS	EGAHEYDDQTS	QREKEDDKVF	PGGSHTYVWQ	VLKENGPMAS
PIGFVIII	AVGVSPWKSS	EGAHEYDHTS	QREKEDDKVL	PGKSQTYVWQ	VLKENGPTAS
MURFVIII	AVGVSYWKAS	EGDEYEDQTS	QMEKEDDKVF	PGESHTYVWQ	VLKENGPMAS
CANFVIII	AVGVSYWKAS	EGAHEYDQTS	QKEKEDDNVI	PGESHTYVWQ	VLKENGPMAS
	151				200
HUMFVIII	DPLCLTYSYL	SHVDLVKDLN	SGLIGALLVC	REGSLAKEKT	QTLHKFILLF
PIGFVIII	DPPCLTYSYL	SHVDLVKDLN	SGLIGALLVC	REGSLTRERT	QNLHEFVLLF
MURFVIII	DPPCLTYSYM	SHVDLVKDLN	SGLIGALLVC	KEGSLSKERT	QMLYQFVLLF
CANFVIII	DPPCLTYSYF	SHVDLVKDLN	SGLIGALLVC	KEGSLAKERT	QTLQEFVLLF
	201				250
HUMFVIII	AVFDEGKSWH	SETKNSLMQD	RDAASARAWP	KMHTVNGYVN	RSLPGLIGCH
PIGFVIII	AVFDEGKSWH	SARNSWTRA	MDPAPARAQP	AMHTVNGYVN	RSLPGLIGCH
MURFVIII	AVFDEGKSWH	SETNDSYTQS	MDSASARDWP	KMHTVNGYVN	RSLPGLIGCH
CANFVIII	AVFDEGKSWH	SETNASLTQ.	.....AEAQH	ELHTINGYVN	RSLPGLTVCH
	251				300
HUMFVIII	RKSVYWHVIG	MGTTPEVHSI	FLEGHTFLVR	NHRQASLEIS	PITFLTAQTL
PIGFVIII	KKSVYWHVIG	MGTSPEVHSI	FLEGHTFLVR	HHRQASLEIS	PLTFLTAQTF
MURFVIII	RKSVYWHVIG	MGTTPEIHSI	FLEGHTFFVR	NHRQASLEIS	PITFLTAQTL
CANFVIII	KRSVYWHVIG	MGTTPEVHSI	FLEGHTFLVR	NHRQASLEIS	PITFLTAQTF
	301				350
HUMFVIII	LMDLQGFLLF	CHISSHQHDG	MEAYVKVDSC	PEEPQLRMK.	NNEEAEDYDD
PIGFVIII	LMDLQGFLLF	CHISSHHGG	MEAHVRVESC	AEEPQLRRK.	ADEE.EDYDD
MURFVIII	LIDLQGFLLF	CHISSHKHDG	MEAYVKVDSC	PEESQWQKKN	NNEEMEDYDD
CANFVIII	LMDLQGFLLF	CHIPSHQHDG	MEAYVKVDSC	PEEPQLRMK.	NNED.KDYDD
	351				400
HUMFVIII	DLTDEMDVV	RFDDDNSPSF	IQIRSVAKKH	PKTWVHYIAA	EEEDWDYAPL
PIGFVIII	NLYDSDMDVV	RLDGDDVSPF	IQIRSVAKKH	PKTWVHYISA	EEEDWDYAPA
MURFVIII	DLY.SEMDMF	TLDYD.SSPF	IQIRSVAKKY	PKTWIHYISA	EEEDWDYAPS
CANFVIII	GLYDSDMDVV	SFDDSSSPF	IQIRSVAKKH	PKTWVHYIAA	EEEDWDYAPS
	401				450
HUMFVIII	VLAPDRSYK	SQYLNGPQR	IGRKYKKVRF	MAYTDETFKT	REAIQHESGI
PIGFVIII	VPSPDRSYK	SLYLNSGPQR	IGRKYKKARF	VAYTDVTFKT	RKAIPYESGI
MURFVIII	VPTSDNGSYK	SQYLSNGPHR	IGRKYKKVRF	IAYTDETFKT	RETIQHESGL
CANFVIII	GTPPNDRSHK	NLYLNGPQR	IGKYYKKVRF	VAYTDETFKT	REAIQYESGI
	451				500
HUMFVIII	LGPLLYGEVG	DTLLIIFKNQ	ASRPYNIYPH	GITDVRPLYS	RRLPKGVKHL
PIGFVIII	LGPLLYGEVG	DTLLIIFKNK	ASRPYNIYPH	GITDVSALHP	GRLLKGWKHL
MURFVIII	LGPLLYGEVG	DTLLIIFKNQ	ASRPYNIYPH	GITDVSPLHA	RRLPRGIKHV
CANFVIII	LGPLLYGEVG	DTLLIIFKNQ	ASRPYNIYPH	GINYVTPLHT	GRLPKGVKHL
	501				550

Fig. 2a

HUMFVIII	KDFPILPGEI	FKYKWTVTVE	DGPTKSDPRC	LTRYYSFVN	MERDLASGLI
PIGFVIII	KDMPILPGET	FKYKWTVTVE	DGPTKSDPRC	LTRYYSIN	LEKDLASGLI
MURFVIII	KDLPIHPGEI	FKYKWTVTVE	DGPTKSDPRC	LTRYYSFIN	PERDLASGLI
CANFVIII	KDMPILPGEI	FKYKWTVTVE	DGPTKSDPRC	LTRYYSFIN	LERDLASGLI
	551				600
HUMFVIII	GPLLCYKES	VDQRGNQIMS	DKRNVILFSV	FDENRSWYLT	ENIQRFLPNP
PIGFVIII	GPLLCYKES	VDQRGNQMMS	DKRNVILFSV	FDENQSWYLA	ENIQRFLPNP
MURFVIII	GPLLCYKES	VDQRGNQMMS	DKRNVILFSI	FDENQSWYIT	ENMQRFLPNA
CANFVIII	GPLLCYKES	VDQRGNQMMS	DKRNVILFSV	FDENRSWYLT	ENMQRFLPNA
	601				650
HUMFVIII	AGVQLEDPEF	QASNIMHSIN	GYVFDSLQLS	VCLHEVAYWY	ILSIGAQTFD
PIGFVIII	DGLQPQDPEF	QASNIMHSIN	GYVFDSLQLS	VCLHEVAYWY	ILSVGAQTFD
MURFVIII	AKTQPQDPGF	QASNIMHSIN	GYVFDSLELT	VCLHEVAYWH	ILSVGAQTFD
CANFVIII	DVVQPHDPEF	QLSNIMHSIN	GYVFDNLQLS	VCLHEVAYWY	ILSVGAQTFD
	651				700
HUMFVIII	LSVFFSGYTF	KHKMVEYEDTL	TLFPFSGETV	FMSMENPGLW	ILGCHNSDFR
PIGFVIII	LSVFFSGYTF	KHKMVEYEDTL	TLFPFSGETV	FMSMENPGLW	VLGCHNSDLR
MURFVIII	LSLFFSGYTF	KHKMVEYEDTL	TLFPFSGETV	FMSMENPGLW	VLGCHNSDFR
CANFVIII	LSVFFSGYTF	KHKMVEYEDTL	TLFPFSGETV	FMSMENPGLW	VLGCHNSDFR
	701				750
HUMFVIII	NRGMTALLKV	SSCDK	<u>NTGDY YEDSYEDISA YLLSKNNAIE PR</u>	<u>STSONSRH</u>	
PIGFVIII	NRGMTALLKV	YSCDR	<u>DIGDY YDNTYEDIPG FLLSGKNVIE PR</u>	<u>STFAONSRE</u>	
MURFVIII	KRGMTALLKV	SSCDK	<u>STSDY YEEIYEDIPT OLVNENNVID PR</u>	<u>STFQNTNH</u>	
CANFVIII	NRGMTALLKV	SSCNR	<u>NIDDY YEDTYEDIPT PLLNENNVIK PR</u>	<u>STSONSRH</u>	
	751				800
HUMFVIII	<u>ESTRQKQFNA</u>	TTIPENDIEK	TDPWFAHRTP	MPKIQNVSSS	DLLMLLRQS.
PIGFVIII	<u>ESTRQKQFQT</u>	ITSPEDDVE.	LDPQSGERTQ	ALEELVPSG	DGSMLLGN.
MURFVIII	<u>ESTRKKKFKD</u>	STIPKNDMEK	IEPQFEEIAE	MLKVQSVSVS	DMLMLLGQSH
CANFVIII	<u>ESTREKQLKA</u>	TTTPENDIEK	IDLQSGERTQ	LIKAQSVSSS	DLLMLLGN.
	801				850
HUMFVIII	<u>PTPHGLSLSD</u>	LQEAKEYETFS	DDPSPGAIDS	NNSLSEMTHF	RPQLHHSGDM
PIGFVIII	<u>PAPHGSSSSD</u>	LQEARNE..A	DDYLPGARER	NTAPSAAARL	RPELHHSUER
MURFVIII	<u>PTPHGLFLSD</u>	GQEAIYEAIH	DDHSPNAIDS	NEGPSKVTQL	RPESHHSSEKI
CANFVIII	<u>PTPRGLFLSD</u>	LREA..TDRA	DDHSRGAIER	NKGPPEVASL	RPELRHSEDR
	851				900
HUMFVIII	<u>VFTPESEGLQL</u>	RLNEKLGTTA	ATELKKLDFK	VSSTSNNLI.	.STIPSDNLA
PIGFVIII	<u>VLTPEPE...</u>	.....	.KELKKLDSK	MSSSDLLKT	SPTIPSDTLS
MURFVIII	<u>VFTPQPGQLQL</u>	RSNKSLETTI	EVKWKKLGLQ	VSSLPSNLMT	.TTILSDNLK
CANFVIII	<u>EFTPEPELQL</u>	RLNENLGTNT	TVELKKLDLK	ISSSDSLMT	SPTIPSDKLA
	901				950
HUMFVIII	<u>AGTDNTSSLG</u>	PPSMPVHYDS	QLDITLFGKK	SSPLTESGGP	LSLSEENNDS
PIGFVIII	<u>AETERTHSLG</u>	PPHPQVNFERS	QLGAIVLGKN	SSHFIGAGVP	LGSTEED...
MURFVIII	<u>ATEKTDSSG</u>	FPDMPVHSSS	KLSTAFGKK	AYSLVGSHVP	LNASEENSDDS
CANFVIII	<u>AATEKTGSLG</u>	PPNMSVHFNS	HLGTIVFGNN	SSHLIQSGVP	LELSEEDNDS
	951				1000
HUMFVIII	<u>KLLEAGLMNS</u>	QESSWGKNVS	STESGRLFKG	KRAHGPAALT	KDNALFKVSI
PIGFVIII	<u>.....</u>	HESSLGENVS	PVESDGIFEK	ERAHGPAALT	KDDVLFKVINI
MURFVIII	<u>NILDSTLMYS</u>	QESLPRDNIL	SIENDRLRE	KRFHGIALLT	KDNTLFDKDNV
CANFVIII	<u>KLLEAPLMNI</u>	QESSLRENVL	SMESNRLFKE	ERIRGPASLI	KDNALFKVINI

Fig. 2b

	1001		1050
HUMFVIII	<u>SLLKTNKTSN NSATNRKTHI DGPSLLIENS PSVWQNI.LE SDTEFKKVTP</u>		
PIGFVIII	<u>SLVKTNKARV YLKTNRKIHI DDAALLTENR ASA.....</u>		
MURFVIII	<u>SLMKTNKTYN HSTTNEKLHT ESPT.SIENS TTDLQDAILK VNSEIQEVTA</u>		
CANFVIII	<u>SSVKTNRAPV NLTTNRKTRV AIPTLLIENS TSVWQDIMLE RNTEFKEVTS</u>		
	1051		1100
HUMFVIII	<u>LIHDRMLMDK NATALRLNHM SNKTTSSKNM EMVQOKKEGP IPPDAQNPDM</u>		
PIGFVIII	<u>.....TFMDK NTTASGLNHV SN.....</u>		
MURFVIII	<u>LIHDGTLGK NSTYLRLNHM LNRTTSTKNK DIFHRKDEDP IPQDEENTIM</u>		
CANFVIII	<u>LIHNETFMDR NTTALGLNHV SNKTTLSKNV EMAHQKKEDP VPLRAENPDL</u>		
	1101		1150
HUMFVIII	<u>SFFKMLFLPE SARWIQRTHG KNSLNSGQGP SPKQLVSLGP EKSVEGQNFL</u>		
PIGFVIII	<u>.....WIKGPLG KNPLSSERGP SPELLTSSGS GKSVKQSSG</u>		
MURFVIII	<u>PFSKMLFLSE SSNWFKKTNG NNSLNSEQEH SPKQLVYLMF KKYVKNQSF</u>		
CANFVIII	<u>SSSKIPFLPD WI....KTHG KNSLSSEQRP SPKQLTSLGS EKSVKDQNFL</u>		
	1151		1200
HUMFVIII	<u>SEKNVVGK GEFTKDVGLK EMVFPSSRNL FLTNLDNLHE NNTHNQEKKI</u>		
PIGFVIII	<u>QGRIRVAVEE EELSKG...K EMLPNSLT FLTNSADVQG NDTHSQGKS</u>		
MURFVIII	<u>SEKNKVTEQ DGFTKNIGL DMAFPHMSI FLTTLNVHE NGRHNQEKNI</u>		
CANFVIII	<u>SE,EKVVGE DEFTKDTLQ E,IFPNKSI FFANLANVQE NDTYNQEKKS</u>		
	1201		1250
HUMFVIII	<u>QEEIEKKETL IQENVVLPQI HTVTGTKNFM KNLFLLSTRQ NVEGSYDGAY</u>		
PIGFVIII	<u>REEMERREKL VQEKVDLPQV YTATGTKNFL RNIFHQSTEP SVEGFDGGSH</u>		
MURFVIII	<u>QEEIE,KEAL IEEKVVLPQV HEATGSKNFL KDILILGTRQ NISLYE..VH</u>		
CANFVIII	<u>PEEIERKEKL TQENVALPQA HTMIGTKNFL KNLFLLSTKQ NVAGLEEQPY</u>		
	1251		1300
HUMFVIII	<u>APVLQDFRSL NDSTNRKTHI TAHFSKKG.. EENLEGLGN QTKQIVEKYA</u>		
PIGFVIII	<u>APVPQDSRSL NDSAERAETH IAHFSAIR.. EEAPLEAPGN RT.....</u>		
MURFVIII	<u>VPVLQNTSI NNSTNTVQIH MEHFFKRRKD KETNSEGLVN KTREMVKNY.</u>		
CANFVIII	<u>TPILQDTRSL NDSPHSEGIH MANFSKIR.. EENLEGLGN QTNQMVERFP</u>		
	1301		1350
HUMFVIII	<u>CTRISPNTS QQNFVTQRSK RALKQFRLPL EETELEKRII VDDTSTQWSK</u>		
PIGFVIII	<u>.....GPG PRSAVPRRVK QSLKQIRLPL EEIKPERGVV LNATSTRWS.</u>		
MURFVIII	<u>.....PS QKNITTQRSK RALGQFRL.. ..... STQWLK</u>		
CANFVIII	<u>STTRMSSNAS QH.VITQRGK RSLKQFRLSQ GEIKFERKVI ANDTSTQWSK</u>		
	1351		1400
HUMFVIII	<u>NMKHLTPSTL TQIDYNEKEK GAITQSPLSD CLTRSHSIPQ ANRSPLPIAK</u>		
PIGFVIII	<u>.....</u>		
MURFVIII	<u>TINCSTQCII QKIDHSKEMK KFITKSSLS SSVIK.STTQ TNSSDSHIVK</u>		
CANFVIII	<u>NMNYLAQGTL TQIEYNEKEK RAITQSPLSD CSMRNHVITQ MNDSALPVAK</u>		
	1401		1450
HUMFVIII	<u>VSSFPSIRPI YLTRVLFQDN SSSLPAAS...YRKKDSGV QESSHFLQGA</u>		
PIGFVIII	<u>.....ESSPILQGA</u>		
MURFVIII	<u>TSAFP...PI DLKRSPFQNK FSHVQASSYI YDFKTKSSRI QESNNFLKET</u>		
CANFVIII	<u>ESASPSVRHT DLTKIPSQHN SSSLPASACN YTFRERTSGV QEGSHFLQEA</u>		
	1451		1500
HUMFVIII	<u>KKNNLSLAIL TLEMTGDQRE VGSLGTSATN SVTYKKVENT VLPKPDLPKT</u>		
PIGFVIII	<u>KRNNSLSPFL TLEMAGGQGK ISALGKSAAG PLASGKLEKA VLSSAGLSEA</u>		
MURFVIII	<u>KINNSLAIL PWNMFIDQGK FTSPGKSNTN SVTYKKRENI IFLKPTLPEE</u>		
CANFVIII	<u>KRNNSLAFV TLGITEGQGK FSSLGKSATN QPMYKKLENT VLLQFGLSET</u>		
	1501		1550

Fig. 2c

HUMFVIII	<u>SGKVELLPKV HIYQKDLFPT ETSNGSPGHL DLVEGSLLOQ TEGAIKWNEA</u>			
PIGFVIII	<u>SGKAEFLPKV RVHREDLLPQ KTSNVSCAHG DLGQEIFLQK TRGPVNLNKK</u>			
MURFVIII	<u>SGKIELLPQV SIQEEEEILPT ETSNGSPGHL NLMKEVFLQK IQGPTKWNKA</u>			
CANFVIII	<u>SDKVELLSQV HVDQEDSFPT KTSNDSPGHL DLMGKIFLQK TQGPVKMNKT</u>			
	1551			1600
HUMFVIII	<u>NRPGKVPFLR VATESSAKTP SKLLDPLAWD NHYGTQIPKE EWKSQEKSPK</u>			
PIGFVIII	<u>NRPG.....RTP SKLLGPPM..PK EWESLEKSPK</u>			
MURFVIII	<u>KRHGES..IK GKTESSKNTR SKLLNHAWD YHYAAQIPKD MWKSKEKSPK</u>			
CANFVIII	<u>NSPGKVPFLK WATESSEKIP SKLLGVLAWD NHYDTQIPSE EWKSQKKSQT</u>			
	1601			1650
HUMFVIII	<u>KTAFKKKDTI .LSLNACESN HAIAAINEGQ NKPEIEVTWA KQGRTERLC</u>			
PIGFVIII	<u>STALRTKDII SLPLDRHESN HSIAAKNEGQ AETQREAAWT KQGGPGRLC</u>			
MURFVIII	<u>IISIKQEDTI .LSLRPHGNS HSIGA.NEQ NWPQRETTWV KQGOTORTC</u>			
CANFVIII	<u>NTAFKRKDTI .LPLGPCENN DSTAAINEGQ DKPQREAMWA KQGEPPGRLC</u>			
	1651			1700
HUMFVIII	<u>QNEFVLRKHC REITRRTLQS DQEEIDYDDT ISVEMKKEDF DIYDEDENQS</u>			
PIGFVIII	<u>QNEFVLRKHC RDISLPTFQP EEDKMDYDDI FSTETKGEDF DIYGEDENQD</u>			
MURFVIII	<u>QNEFVLRKHC EELS..AFQS EQEATDYDDA ITIETI.EDF DIYSEDIKQG</u>			
CANFVIII	<u>QNEFVSKKHC KEITVTTLQP EEDKFEYDDT FSIEMKREDF DIYGDYENQG</u>			
	1701			1750
HUMFVIII	<u>PRSFQKKTRH YFIAAVERLW DYGMSSSPHV LRNRAQSGSV PQFKKVVFQE</u>			
PIGFVIII	<u>PRSFQKRTRH YFIAAVEQLW DYGMSESPRA LRNRAQNGEV PRFKKVVFRE</u>			
MURFVIII	<u>PRSFQKKTRH YFIAAVERLW DYGMSTS.HV LRNRYQSDNV PQFKKVVFQE</u>			
CANFVIII	<u>LRSFQKKTRH YFIAAVERLW DYGMSRSPHI LRNRAQSGDV QQFKKVVFQE</u>			
	1751			1800
HUMFVIII	<u>FTDGSFTQPL YRGELNEHLG LLGPYIRAEV EDNIMVTFRN QASRPYSFYS</u>			
PIGFVIII	<u>FADGSFTQPS YRGELNKHLG LLGPYIRAEV EDNIMVTFKN QASRPYSFYS</u>			
MURFVIII	<u>FTDGSFSQPL YRGELNEHLG LLGPYIRAEV EDNIMVTFKN QASRPYSFYS</u>			
CANFVIII	<u>FTDGSFTQPL YRGELNEHLG LLGPYIRAEV EDNIVVTFKN QASRPYSFYS</u>			
	1801			1850
HUMFVIII	<u>SLISYEEDQR QGAEPKRFV KPNETKTYFW KVQHMAPTK DEFDCAWAY</u>			
PIGFVIII	<u>SLISYPDDQE QGAEPKRFV QPNETRYFW KVQHMAPTE DEFDCAWAY</u>			
MURFVIII	<u>SLISYKEDQ. RGEPPRRFV KPNETKIYFW KVQHMAPTE DEFDCAWAY</u>			
CANFVIII	<u>SLISYDEDEG QGAEPKRFV NPNETKIYFW KVQHMAPTK DEFDCAWAY</u>			
	1851			1900
HUMFVIII	<u>FSDVDLEKDV HSGLIGPLLV CHTNTLNPAH GRQVTVQEFA LFFTIFDETK</u>			
PIGFVIII	<u>FSDVDLEKDV HSGLIGPLLI CRANTLNAAH GRQVTVQEFA LFFTIFDETK</u>			
MURFVIII	<u>FSDVDLERDM HSGLIGPLLI CHANTLNPAH GRQVSVQEFA LFFTIFDETK</u>			
CANFVIII	<u>FSDVDLEKDV HSGLIGPLLI CRSNTLNPAH GRQVTVQEFA LVFTIFDETK</u>			
	1901			1950
HUMFVIII	<u>SWYF TENMER NCRAPCNIQM EDPTFKENYR FHAINGYIMD TLPGLVMAQD</u>			
PIGFVIII	<u>SWYF TENVER NCRAPCHLQM EDPTLKENYR FHAINGYVMD TLPGLVMAQN</u>			
MURFVIII	<u>SWYF TENVCR NCKTPCNFQM EDPTLKENYR FHAINGYVMD TLPGLVMAQD</u>			
CANFVIII	<u>SWYF TENLER NCRAPCNVQK EDPTLKENFR FHAINGYVKD TLPGLVMAQD</u>			
	1951			2000
HUMFVIII	<u>QRIRWYLLSM GSNENIHSIH FSGHVFTVRK KEEYKMAVYN LYPGVFETVE</u>			
PIGFVIII	<u>QRIRWYLLSM GSNENIHSIH FSGHVFSVRK KEEYKMAVYN LYPGVFETVE</u>			
MURFVIII	<u>QRIRWYLLSM GNNENIQSIH FSGHVFTVRK KEEYKMAVYN LYPGVFETLE</u>			
CANFVIII	<u>QKVRWYLLSM GSNENIHSIH FSGHVFTVRK KEEYKMAVYN LYPGVFETVE</u>			
	2001			2050
HUMFVIII	<u>MLPSKAGIWR VECLIGEHLH AGMSTLFLVY SNKCQTPLGM ASGHIRDFQI</u>			

Fig. 2d

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PIGFVIII	MLPSKVGIWR	IECLIGEHLQ	AGMSTTFLVY	SKECQAPLGM	ASGRIRDFQI
MURFVIII	MIPSRAGIWR	VECLIGEHLQ	AGMSTLFLVY	SKQCQIPLGM	ASGSIRDFQI
CANFVIII	MLPSQVGIWR	IECLIGEHLQ	AGMSTLFLVY	SKKCQTPLGM	ASGHIRDFQI
	2051				2100
HUMFVIII	TASQYQQWA	PKLARLHYSG	SINAWSTKEP	FSWIKVDLLA	PMIIHGIKTQ
PIGFVIII	TASQYQQWA	PKLARLHYSG	SINAWSTKDP	HSWIKVDLLA	PMIIHGIMTQ
MURFVIII	TASGHYQWA	PNLARLHYSG	SINAWSTKEP	FSWIKVDLLA	PMIVHGIKTQ
CANFVIII	TASQYQQWA	PKLARLHYSG	SINAWSTKDP	FSWIKVDLLA	PMIIHGIMTQ
	2101				2150
HUMFVIII	GARQKFSSLY	ISQFIIMYSL	DGKKWQTYRG	NSTGTLMVFF	GNVDSSGIKH
PIGFVIII	GARQKFSSLY	ISQFIIMYSL	DGRNWQSYRG	NSTGTLMVFF	GNVDASGIKH
MURFVIII	GARQKFSSLY	ISQFIIMYSL	DGKKWLSYQG	NSTGTLMVFF	GNVDSSGIKH
CANFVIII	GARQKFSSLY	VSQFIIMYSL	DGNKWHYSYRG	NSTGTLMVFF	GNVDSSGIKH
	2151				2200
HUMFVIII	NIFNPPIIAR	YIRLHPTHYS	IRSTLRMELM	GCDLNCSMP	LGMESKAISD
PIGFVIII	NIFNPPIVAR	YIRLHPTHYS	IRSTLRMELM	GCDLNCSMP	LGMQNKAISD
MURFVIII	NSFNPPIIAR	YIRLHPTHSS	IRSTLRMELM	GCDLNCSIP	LGMESKVISD
CANFVIII	NIFNPPIIAQ	YIRLHPTHYS	IRSTLRMELL	GCDFNCSMP	LGMESKAISD
	2201				2250
HUMFVIII	AQITASSYFT	NMFATWSPSK	ARLHLQGRSN	AWRPQVNNPK	EWLQVDFQKT
PIGFVIII	SQITASSHLS	NIFATWSPSQ	ARLHLQGRTN	AWRPRVSSAE	EWLQVDLQKT
MURFVIII	TQITASSYFT	NMFATWSPSQ	ARLHLQGRTN	AWRPQVNDPK	QWLQVDLQKT
CANFVIII	AQITASSYLS	SMLATWSPSQ	ARLHLQGRTN	AWRPQANNPK	EWLQVDFRKT
	2251				2300
HUMFVIII	MKVTGVTQG	VKSLLTSMYV	KEFLISSSQD	GHWTLFFQN	GKVKVFQGNQ
PIGFVIII	VKVTGITQG	VKSLLSMYV	KEFLVSSSQD	GRRWTLFLQD	GHTKVFQGNQ
MURFVIII	MKVTGIITQG	VKSLFTSMFV	KEFLISSSQD	GHHWTQILYN	GKVKVFQGNQ
CANFVIII	MKVTGITQG	VKSLLSMYV	KEFLISSSQD	GHNWTLFLQN	GKVKVFQGNR
	2301				2345
HUMFVIII	DSFTPVVNSL	DPPLLTRYLR	IHPQSWVHQI	ALRMEVLGCE	AQDLY
PIGFVIII	DSSTPVMNAL	DPPLFTRYLR	IHPTSQAQHI	ALRLEVLGCE	AQDLY
MURFVIII	DSSTPMMNSL	DPPLLTRYLR	IHPQIWEHQI	ALRLEILGCE	AQQQY
CANFVIII	DSSTPVRNRL	EPPLVARYVR	LHPQSWAHHI	ALRLEVLGCD	TQQPA

Fig. 2e

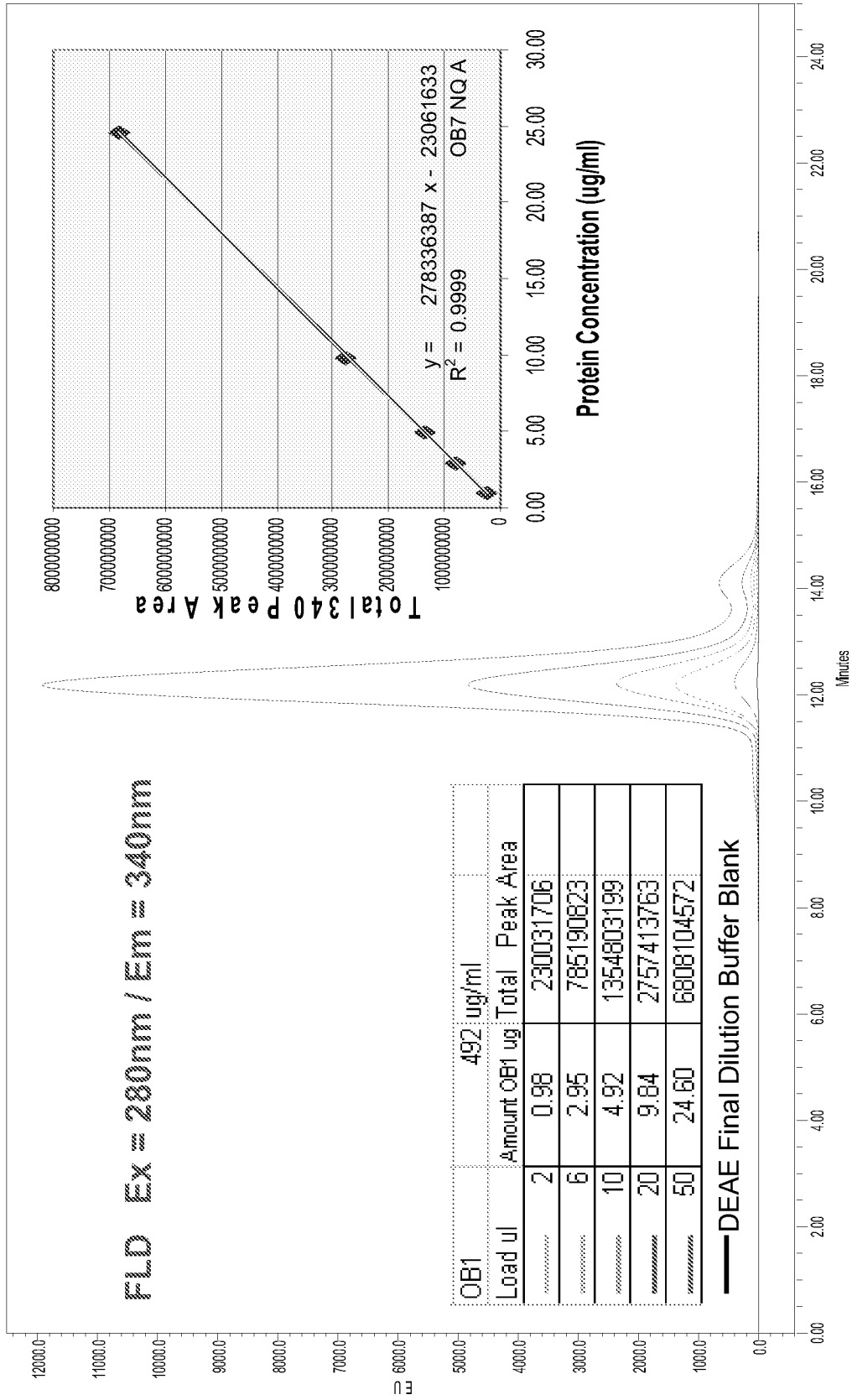


Fig. 3