

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
27 December 2007 (27.12.2007)

PCT

(10) International Publication Number
WO 2007/148065 A2

(51) International Patent Classification: **Not classified**

(21) International Application Number:
PCT/GB2007/002271

(22) International Filing Date: 20 June 2007 (20.06.2007)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
0612456.4 23 June 2006 (23.06.2006) GB

(71) Applicant (for all designated States except US): **THE SECRETARY OF STATE FOR DEFENCE** [GB/GB]; DSTL, Porton Down, Salisbury, Wiltshire SP4 0JQ (GB).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **MAYERS, Carl, Nicholas** [GB/GB]; DSTL, Porton Down, Salisbury, Wiltshire SP4 0JQ (GB). **LOVE, Tracey, Elizabeth** [GB/GB]; DSTL, Porton Down, Salisbury, Wiltshire SP4 0JQ (GB). **PATEL, Vikesh** [GB/GB]; DSTL, Porton Down, Salisbury, Wiltshire SP4 0JQ (GB).

(74) Agent: **FARNSWORTH, Alastair, Graham**; D/IPR Formalities Section, Poplar 2, MOD Abbey Wood #2218, Bristol BS34 8JH (GB).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, 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, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

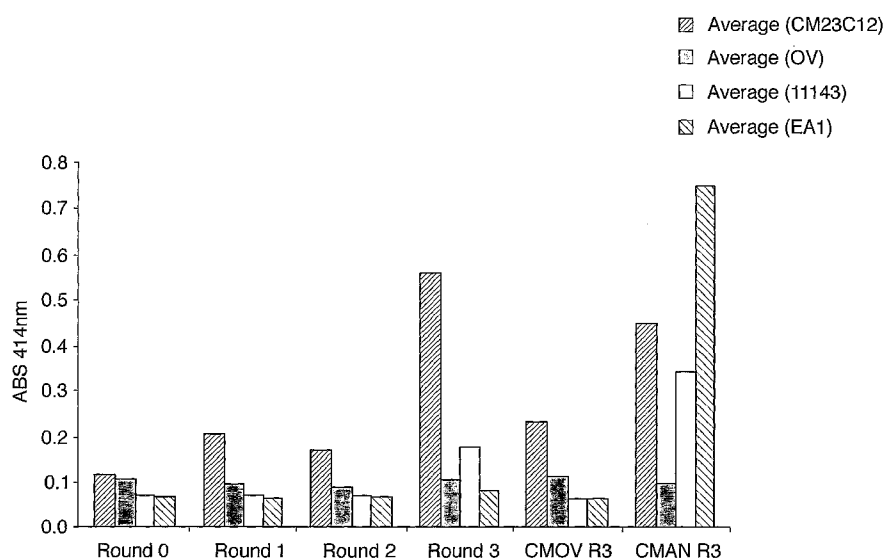
(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— without international search report and to be republished upon receipt of that report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: ANTIBODY FOR ANTHRAX



(57) Abstract: A method for the production of antibody specific for B. anthracis comprising the steps of i) generating an immune scFv phage-display library and ii) panning the library against immobilised B. anthracis spore in the presence of spore from one or more other Bacillus species.

WO 2007/148065 A2

ANTIBODY FOR ANTHRAX

The present invention is generally concerned with a method for the production of antibody which is specific for anthrax (*B. anthracis*). The invention is particularly, although not exclusively, directed to a recombinant antibody specific for anthrax
5 spore and uses thereof.

An immunoassay suitable for the detection of anthrax should preferably be based on an antibody which recognises the spore over the vegetative cell and other closely related, non-pathogenic species such as *B. cereus*. The identification of such a
10 specific antibody is, however, often difficult - especially when a unique antigen or epitope between species is not known or not immunodominant.

Traditional methods for the isolation of specific antibody are often based on screening of polyclonal antibody raised against whole cell or spores. These methods, although
15 sometimes successful, often require detailed knowledge of suitable target proteins and are time-consuming and expensive.

The use of whole antibody for detection often requires significant modification or development of existing detection platforms. Optimal detection may, for example,
20 require the use of additional reagents, optimisation of assay conditions or development of new technologies.

Recombinant antibody is more compatible with existing detection platforms. In addition, recombinant libraries offer the advantage that they can be designed for specificity between very similar proteins.

- 5 Methods for the production of recombinant antibody are well known to the art - see for example, Krebber, A., et al., (1997).

The present invention generally aims to produce antibody with high specificity and high affinity (nanomolar or above) for anthrax. As used herein, the term “specific” in
10 relation to antibody for anthrax will be understood to refer to antibody which preferably binds to anthrax in the presence of other *Bacillus* species. The antibody may, however, bind to the live spore and/or the vegetative cell.

The present invention also aims to provide an antibody specific for anthrax which is
15 suitable for use with existing detection platforms such as those based on surface plasmon resonance (SPR).

Zhou, B., et al. (2002) suggest an approach for the identification of human antibody specific to *B. anthracis*. The approach is based on a naïve, human single-chain Fv
20 (scFv) phage-display library.

The library is panned against a suspension of live spores of *B. subtilis* and positive scFv-phages selected against the immobilised spores by an enzyme-linked immunosorbent assay (ELISA).

Whilst specificity for the live *B. subtilis* spore over the vegetative cell is shown, the selected phage are cross-reactive with other *Bacillus* species - even following construction of a chain-shuffled Fab library and subtractive bio-panning.

- 5 UK patent application GB 0514319.3, incorporated herein by reference in its entirety, discloses an alternative approach for the production of recombinant antibody specific to *B. anthracis*.

10 An immune mouse scFv phage-display library is panned against immobilised (S-layer) protein EA1. Antibody specific for *B. anthracis* is not found - except when the library is first panned against immobilised protein EA1 in the presence of S-layer protein extracts from competing *Bacillus* species (competitive bio-panning).

15 It is believed that competitive bio-panning of more complex mixtures of antibody eliminates the majority of non-specific antibody from the library in a single, first (-VE selection) step and reveals antibody specific to EA1 *B. anthracis* which is lost in ordinary bio-panning.

20 Competitive bio-panning (also known as pre-adsorption or subtractive screening) has been used for isolating other targets. However, it normally follows conventional bio-panning.

Krebs, B. et al., (2004) describe a method for the identification of specific sc-Fv antibody in which pre- and post-adsorption steps are utilised to screen out antibody
25 that bind to cross reacting species.

The preparation of anti-melanoma antibodies by pre-adsorption to melanocytes has been reported by Cai, X. and Garen, A. (1995).

Surprisingly, it has now been found that antibody of high specificity and high affinity
5 for anthrax can be produced by panning an immune library against immobilised *B. anthracis* spore in the presence of spore from competing *Bacillus* species.

Accordingly, in a first aspect, the present invention provides a method for the production of antibody specific for *B. anthracis* comprising the steps of i) generating
10 an immune scFv phage-display library and ii) panning the library against immobilised *B. anthracis* spore in the presence of spore from one or more other *Bacillus* species.

In a preferred embodiment, the library is raised against whole spore (irradiated) *B. anthracis*. The library may, in particular, comprise a library of positive scFv phage
15 selected by ELISA against immobilised *B. anthracis* spore.

In one embodiment, the one or more other species comprise *B. cereus* and/or *B. thuringiensis*. Most preferably, however, the one or more other species comprise *B. cereus*, particularly *B. cereus* 11143.
20

Other cross-reacting species which may be used in the invention comprise *B. alvei*, *B. pumilus*, *B. brevis*, *B. circulans*, *B. licheniformis*, *B. megaterium*, *B. polymyxa*, *B. sphaericus* and *B. subtilis*.

In a preferred embodiment, step ii) is performed using an amount of the one or more other *Bacillus* species which is in excess of the amount of immobilised spore. The amount of the other *Bacillus* species may, in particular, comprise up to a 10-fold excess.

5

In a highly preferred embodiment, step ii) is the first panning step and is performed on the positive library. In this embodiment, subsequent rounds of non-competitive or competitive bio-panning may be used. Most preferably, step ii) is repeated two or three times.

10

In a second aspect, the present invention provides for an antibody specific for *B. anthracis* spore comprising at least one of the amino acid sequences SEQ ID 2, SEQ ID 3, SEQ ID 4, SEQ ID 15, SEQ ID 19, SEQ ID 20, SEQ ID 24, SEQ ID 30, SEQ ID 31, SEQ ID 32, SEQ ID 33, SEQ ID 34, SEQ ID 35, SEQ ID 36, SEQ ID 37, SEQ ID 38, SEQ ID 39 or a variant or a fragment thereof.

15

In a preferred embodiment, the antibody comprises any one of amino acid SEQ ID 1 to 45 or a variant or fragment thereof.

20 In another preferred embodiment, the hypervariable region of the antibody are characterised in that CDR-L1 comprises either SEQ ID 19, SEQ ID 31 or SEQ ID 32; CDR-L2 comprises either SEQ ID 2, SEQ ID 20 or SEQ ID 33; CDR-L3 comprises either SEQ ID 3, SEQ ID 15 or SEQ ID 34; CDR-H1 comprises either SEQ ID 4 or SEQ ID 35; CDR-H2 comprises SEQ ID 23, SEQ ID 36 or SEQ ID 37 and CDR-H3
25 comprises SEQ ID 24, SEQ ID 38 or SEQ ID 39.

Most preferably, however, the antibody comprises SEQ ID 1 to 6; or SEQ ID 7 to 12; or SEQ ID 13 to 18; or SEQ ID 19 to 24; or SEQ ID 2 to 4 and 25 to 27 or SEQ ID 2 to 5 and 28 to 30.

- 5 The term “variant” as used in relation to amino acid sequences refers to sequences which differ from the original sequence from which they are derived in that one or more amino acids within the sequence are substituted for other amino acids but which retain the ability of the original sequence to bind to anthrax with affinities (K_A) of order of magnitude 10^9 or above. Amino acid substitutions may be regarded as
- 10 “conservative” where an amino acid is replaced with a different amino acid with broadly similarly properties. Non-conservative substitutions are where amino acids are replaced with amino acids of a different type. Generally, fewer non-conservative substitutions will be possible without altering the biological activity of the polypeptide. Suitably, variants will be at least 60% identical, preferably at least 70%
- 15 identical, most preferably at least 90% identical to the original sequence.

Identity in this instance can be judged, for example, using the BLAST programme or the algorithm of Lipman and Pearson (1985), with ktuple: 2, gap penalty 4, gap length penalty 12, standard PAM scoring matrix.

20

The term “fragment” refers to any portion of the given amino acid sequence, which has the same activity as the complete amino acid sequence. Fragments will suitably comprise at least 5 and preferably at least 10 consecutive amino acids from the base sequence.

25

In a third aspect, the present invention describes a polynucleotide encoding an antibody of the present invention. Preferably, the polynucleotide comprises any of SEQ ID 46 to 51 or a variant thereof.

5 The term variant in relation to a polynucleotide sequence means any substitution, variation, modification, replacement, deletion or addition of one or more nucleic acid(s) from or to a polynucleotide sequence providing the resultant protein sequence encoded by the polynucleotide exhibits the same properties as the protein encoded by the original sequence. The term therefore includes allelic variants and also a
10 polynucleotide which hybridises to the basic polynucleotide sequence. Preferably such hybridisation occurs at, or between low and high stringency conditions. In general terms, low stringency conditions can be defined as 3 x SSC at about ambient temperature to about 55°C and high stringency conditions can be defined as 0.1 x SSC at about 65°C. SSC is the name of the buffer of 0.015M tri-sodium citrate and 0.15 M
15 NaCl. 3 x SSC is three times as strong as SSC and so on.

Typically, variants have 60% or more of the nucleotides in common with the polynucleotide sequence of the present invention, more typically 65%, preferably 70%, even more preferably 80% or 85% and, especially preferred are 90%, 95%, 98%
20 or 99% or more identity.

When comparing nucleic acid sequences for the purposes of determining the degree of identity, programs such as BESTFIT and GAP (both from Wisconsin Genetics Computer Group (GCG) software package) can be used. BESTFIT, for example,
25 compares two sequences and produces an optimal alignment of the most similar

segments. GAP enables sequences to be aligned along their whole length and finds the optimal alignment by inserting spaces in either sequence as appropriate. Suitably, in the context of the present invention when discussing identity of nucleic acid sequences, the comparison is made by alignment of the sequences along their whole
5 length.

In a fourth aspect, the present invention provides a method for detection of anthrax comprising the step of determining the binding of an antibody according to the invention to the spore.

10

The present invention also contemplates the vaccination and/or treatment against anthrax by a humanised antibody according to the invention as well as the manufacture of medicaments therefor.

15 In particular, the scFv antibodies of the present invention may be used to produce an anti-idiotypic antibody ("surrogate antigen") according to conventional techniques which may produce a protective immune response in humans and/or animals.

Alternatively, the administration of humanised form of scFv antibody as described by
20 Zhou, B., (2002) may prevent or enhance protection against anthrax infection in humans and/or animals.

The present invention will now be described by reference to the following results and drawings in which

Figure 1 is a graph showing a polyclonal ELISA of a vpap library binding to *B. anthracis* UM23CL2, *B. cereus* 11143 and EA1 following competitive biopanning; and

Figure 2 is a phylogenetic tree showing the relationship between the specific
5 antibodies of the present invention and a range of other non-specific antibodies.

Materials & Methods

Bacterial strains and plasmids

Strain RBA91 (PXO1⁻, PXO2⁻ *B. anthracis* Sap mutant) was provided by the Pasteur
10 Institute (25,28 rue du Docteur Roux, Paris). *B. alvei* (NCTC 6352), *B. cereus*
(NCTC 11143, NCTC 9946, NCTC 2559, NCTC 6474, NCTC 7464, NCTC 8035,
NCTC 9939, NCTC 9945, NCTC 9946, NCTC 10320 and NCTC 11145), *B. pumilus*
(NCTC 10337), *B. brevis* (NCTC 2611), *B. coagulans* (NCTC 10334), *B. circulans*
(NCTC (2610), *B. licheniformis* (NCTC 10341), *B. megaterium* (NCTC 10342), *B.*
15 *pasteuri* (NCTC 4822), *B. polymyxa* NCTC 10343, *B. sphaericus* (NCTC 10338) *B.*
subtilis (NCTC 3610), *B. subtilis* (10073), *B. subtilis* var. *niger* (NCTC 10073), *B.*
thuringiensis var. *kurstaki* and *B. thuringiensis* var. *israelensis* were obtained from
NCTC (PHLS, 61 Colindale Avenue, London).

20 Plasmids pAK100 (for phage display) and pAK300 (for production of soluble scFv)
were a kind gift from Dr A. Plückthun (University of Zürich, Switzerland) and were
used as described by Krebber et al. (1997).

Spore production

Spores were prepared using New Sporulation agar (3.0 g/litre Difco Tryptone; 6.0 g/litre Oxoid bacteriological peptone; 3.0g/litre Oxoid yeast extract: 1.5 g/litre Oxoid Lab Lemco; 1 ml 0.1% $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$; 25 g/litre Difco Bacto agar) and incubated at 37°C until the cultures contained >95% phase bright spores. The spores were harvested by release from the solid media using ice cold sterile distilled water and subsequently centrifuged at 10000g for 10 minutes at 4°C and then washed 10 times in ice cold sterile distilled water to remove vegetative cells and debris. Preparations were examined using phase contrast microscopy to confirm that they contained >95 % phase bright spores.

Construction and use of immune mouse scFv library

Six 12 week old female Balb/c mice were immunised with irradiated *B. anthracis* Ames spores. Each immunisation consisted of 1×10^7 spores in Freund's incomplete adjuvant. Mice were immunised 4 times at intervals of three weeks, and killed by cervical dislocation once they showed a sufficiently high titre (>1:100 000) to the spores by endpoint ELISA. Spleens were removed from the killed mice and splenic mRNA isolated using Trizol reagent (Invitrogen, Fountain Road, Inchinnan Business Park, Paisley, UK.).

The total RNA from the immunised mice was used to produce the immune scFv library: PCR amplification of antibody sequences, overlap extension PCR, cloning of the assembled scFv sequence into pAK100 and production of phage-displayed scFv was carried out as described by Krebber et al. (1997).

Biopanning

Immunotubes (Nunc, BRL, Life Technologies Ltd., Trident House, Washington Road, Paisley, UK) were coated with 1ml of purified γ -irradiated *B. anthracis* Ames spores at 1×10^8 cfu/ml in PBS overnight at 4 °C and blocked with 2 % (w/v) milk powder PBS (MPBS). 100 μ l of scFv-phage were mixed with 900 μ l MPBS, incubated for one hour at room temperature, and added to the coated immunotubes. After incubation at room temperature (2 h), the immunotubes were washed 10 times with PBS 0.1 % (v/v) Tween 20. Bound phage was eluted with 100mM triethylamine and neutralised with 500 μ l 1 M Tris HCl pH 7.5. Eluted phage was infected into log phase XL1-Blue *E. coli*, plated on a 24 cm square 2 x YT 1% (w/v) glucose 30 μ g/ml chloramphenicol agar plate and incubated overnight at 30 °C. The procedure was repeated for each round of panning carried out.

Competitive panning was carried out in an identical fashion, adding *B. cereus* 11143 spores to the scFv-phage MPBS solution for 1 hour before panning. The concentrations of antigen used for competitive panning was up to a 10 fold excess of the *B. anthracis* spores, that was 1×10^9 cfu/ml.

DNA fingerprinting of scFv clones

The scFv sequences from selected clones were amplified by PCR primers surrounding the scFv sequence (scfor and scback) by the method of Krebber et al. (1997) and subjected to BstN1 digestion to determine the diversity of the original library and each consecutive round of selection. Restriction digest products were resolved on 4% E-gels (Invitrogen) using 25bp markers. PCR and DNA fingerprinting were carried out

on 10 randomly selected scFv from rounds 1 and 2 and 50 scFv clones from round three.

DNA sequencing

DNA sequencing was undertaken by MWG (Sweden) the DNA sequences were translated using the ExpAsy translation tool. From this the CDR regions were determined using the Kabat definition as described by Martin (2001).

ELISA

50 µl of *Bacillus* species spore (1×10^8 cfu/ml) were coated onto Immulon2 plates (Nunc) and incubated overnight at 37°C, appropriate control antigens were coated onto Immulon2 plates (Nunc.). PEG-purified phage-displayed scFv were diluted with MPBS, and an anti-M13 HRP conjugated antibody (Sigma, Fancy Road, Poole, UK) used to detect bound phage. Bound phage was quantified by measuring the conversion of ABTS substrate to coloured product based on A405 readings in an automated ELISA reader (Anthos 2001, Anthos Labtec Instruments, Salzburg, Austria).

Detection of whole spore

Antibodies were immobilised to resonant mirror (T70, Thermo Labsystems, Saxon Way, Bar Hill, Cambridge, UK) low molecular weight carboxymethylated dextran (CMD) cuvette surface by standard EDC/NHS coupling. The binding of anthrax spore passed at various concentrations during 10 mins. was detected by a shift in the angle of incident light for resonance. The surface could be regenerated by exposure to 20 mM KOH during 3 mins.

Results

Referring now to Figure 1, a polyclonal ELISA shows the specificity of a the vpap fraction of the sc-Fv phage library against *B. anthracis* UM23CL2, *B. cereus* and surface antigen protein EA1 following (three) successive rounds of competitive bio-panning (sample/MPBS dilution 50% (v/v) for EA1). An anti-ovalbumin sc-Fv was used as a -VE control and bound phage detected using anti-M13 HRP conjugated antibody. Assays were performed in triplicate; error bars show tow standard deviations from the means. A positive result is defined as higher than the average of the background signal plus three standard deviations of the mean background sample.

As may be seen, the vpap library is highly specific for *B. anthracis* after three (3) rounds of competitive bio-panning compared to other parts of the library (mvov, cman).

The specificity of individual scFv phage determined using a monoclonal ELISA against *B. anthracis* spore led to the selection of 50 individual clones. The clones were isolated and their cross reactivity to *B. cereus* 11143, identified as a competing species by non-competitive panning, was determined.

<u>Antibody</u>	<u>CDR</u>	<u>Sequence</u>	<u>SEQ ID #</u>
vpap10	L1	rssqsivhsngntyle	1
vpap10	L2	vsnrfs	2
vpap10	L3	fqqshvpwt	3
vpap10	H1	nsams	4
vpap10	H2	sitsgg-ityypdsv	5

vpap10	H3	gdyryae-gafayw	6
vpap34	L1	kasqnv-----tnva	7
vpap34	L2	asyrys	8
vpap34	L3	qqhysippt	9
vpap34	H1	sytmh	10
vpap34	H2	yinpnagythynqkf	11
vpap34	H3	---ws--dramdyw	12
vpap37	L1	kasqnvr-----sava	13
vpap37	L2	asnrht	14
vpap37	L3	lqhwnypla	15
vpap37	H1	syvmh	16
vpap37	H2	yinpyndgtkynekf	17
vpap37	H3	gllrl--qyamdyw	18
vpap43	L1	sasssvs-----ymh	19
vpap43	L2	tsklas	20
vpap43	L3	qqwssnppt	21
vpap43	H1	syams	22
vpap43	H2	tissggsytypdsv	23
vpap43	H3	gvtr-----whgll	24
vpap44	L1	rssqiialsngntyle	25
vpap44	L2	vsnrfs	2
vpap44	L3	fqgshvpwt	3
vpap44	H1	nsams	4
vpap44	H2	sitsgg-ityypdsa	26

vpap44	H3	gdhrvqq-ggfayw	27
vpap47	L1	rssqsialsngntyle	28
vpap47	L2	vsnrfs	2
vpap47	L3	fqqshvpwt	3
vpap47	H1	nsams	4
vpap47	H2	sitsgg-ityypgsa	29
vpap47	H3	gdyryde-gafayw	30

Table I

Subsequent DNA fingerprinting and DNA sequencing identified 6 unique clones referred to vpap10, vpap34, vpap37, vpap43, vpap44 and vpap47. Table I shows the amino-acid sequences (- = unknown amino acid).

Review of table reveals that sequences for particular CDRs may be grouped together according to common features:

For example SEQ ID 1, 25 and 28 each of which represents the CDR-L1 region of an antibody include the common sequence rssq---sngntyle (SEQ ID 31), where - = any amino acid. Alternatively, SEQ ID 7 and 13 each of which represents the CDR-L1 region of an antibody include the common sequence kasqnv-----va (SEQ ID 32).

Similarly, SEQ ID 8 and 14 each of which represents the CDR-L2 region of an antibody included the common sequence as-r-- (SEQ ID 33).

Further, SEQ ID 9 and 21 each of which represents the CDR-L3 region of an antibody include the common sequence qq---ppt (SEQ ID 34).

Similarly, SEQ ID 10, 16 and 22 each of which represents the CDR-H1 region of an antibody include the common sequence sy-m- (SEQ ID 35).

Again, SEQ ID 5, 26 and 29 each of which represents the CDR-H2 region of an antibody include the common sequence sitsgg-ityyp-s- (SEQ ID 36). Alternatively, SEQ ID 11 and 17 each of which represents the CDR-H2 region of an antibody include the common sequence yinp----t-yn-kf (SEQ ID 37)

Again, SEQ ID 6, and 27 each of which represent the CDR-H3 region of an antibody include the common sequence g-fayw (SEQ ID 38). Alternatively, SEQ ID 12 and 18 each of which represent the CDR-H3 region of an antibody include the common sequence amdyw (SEQ ID 39).

Thus each of the antibodies may be described as CDR-L1 comprising either SEQ ID 19, SEQ ID 31 or SEQ ID 32; CDR-L2 comprising either SEQ ID 2, SEQ ID 20 or SEQ ID 33; CDR-L3 comprising either SEQ ID 3, SEQ ID 15 or SEQ ID 34; CDR-H1 comprising either SEQ ID 4 or SEQ ID 35; CDR-H2 comprising SEQ ID 23, SEQ ID 36 or SEQ ID 37 and CDR-H3 comprising SEQ ID 24, SEQ ID 38 or SEQ ID 39.

The full amino acid sequences of the selected sc-Fv antibody-phage are as follows (linker sub-unit GGGGS):

vpap10 (1 linker)

divmtqspslpvsldqasiscrssqsihsngntylewylqkpgqspklliykvsnrfsqvpdrfsgsgsgtdftlkisr
 veaedlgvyycfqgshvpwtfgggkcleikrgggsgggsgsdvkvvessggglvkpggsklscaasgftfsnsams
 wgrqtpekrlewwasitssggityypdsvkgrftisrddarnilylqmsslrstedamyycargdyryaegafaywgqgt

5 lvtvsa (SEQ ID 40)

vpap34 (4 linkers)

divmtqsqkfmstsvgdrsvtckasqnvgtvawyyqkpgqspkaliysasyrysgvpdrftgsgsgteftfissv
 qaedlavyyccqghysipptfgggkcleikrgggsgggsgggsgggsgggsevlqqsgaelarpgasvkmsckasg
 10 ytfstymhwwkqrpqglewigyinpnagythynqkfkekatltadkssstvymqlssltedsavyycarwsdra
 mdywgqgtsvtvss (SEQ ID 41)

vpap37 (2 linkers)

diqmnqsqkfmsasvgrvsitckasqnrvsawwyqkpgqspkaliylasnrhtgvpdrftgsgsgtdftltisnvq
 15 sedladyfclqhwnyplafgagtkleikrgggsgggsgggsgggsgggsgvqlkesgpelvkpgasvkmsckasgyt
 ftsyvmhwwkqkpgqglewigyinpyndgtkynefkkgkatltsdkssstaymelssltedsavyycargllrlqya
 mdywgqgtsvtvss (SEQ ID 42)

vpap43 (1 linker)

20 divltqspaimsaspgkevmtmtcsasssvsymhwyqqksgtspkrwiatsklasgvparfsgsgsgtsysltissvea
 edaatyyccqwssnpptfgggkcleikrgggsgggsgggsgggsgggsgvqlkesgpelvkpgasvkmsckasgyt
 ewvatissggsytypdsvkgrftisrdnakntlylqmsslrstedahvllcktgvttrwhgllgssnlsqrlglgeg
 (SEQ ID 43)

vpap44 (3 linkers)

divmtqtplslpvsldqasiscrssqiaalsngntylewylqkpgqspklliykvsnrfsrvpdrfsgsgsgtdftlkitrv
 eaedlgvyyfcqshvpwtfgggtklelkrgggsgggsgggsgggsggggpevkvvvesggglvkpggslklscaasgft
 fsnsamswarqtpekrlewqvasitsggityypdsakgrftisrddarnilylamnslrsedtalyycargdhrvqqggf
 5 aywgqgtlgtgagfagaqipkliseenl (SEQ ID 44)

vpap47 (2 linkers)

dilmtqtplslpvsldqasiscrssqsiaalsngntylewylqkpgqspklliykvsnrfsrvpdrfsgsgsgtdftlkisrv
 eaedlgvyyfcqgshvpwtfgggtkleikrgggsgggsgggsgggsggggseqlvesggglvkpggslklscaasgftfsnsamswa
 10 rqtpekrlewvasitsggityypgsakgrftisrddarnilylqmnsrsedtahvflckrgl (SEQ ID 45)

The cross reactivity of each selected scFv antibody against closely related *Bacillus* species as determined by ELISA is reported in Table II.

<i>Bacillus</i>	Reactivity					
	vpap10	vpap34	vpap37	vpap43	vpap44	vpap47
<i>B. anthracis</i>	+++	+++	+++	+++	+++	++
<i>B. cereus</i> 11143	-	-	-	-	-	-
<i>B. cereus</i> 11145	-	-	-	-	-	-
<i>B. thuringiensis</i> (var. kurstki)	-	-	-	-	-	-
<i>B. thuringiensis</i> (var. israelensis)	-	-	-	-	-	-

Table II

As may be seen, each sc-Fv antibody showed high specificity for *B. anthracis* viz., no cross-reactivity.

Results are expressed as the % of the maximum signal seen in the assay. +++ indicates 60-100% of the maximum, ++ indicates 20-59% of the maximum and + indicates a signal greater than the detection threshold (defined as the background signal plus 3 standard deviations from the mean background signal). – indicates a signal below the detection threshold.

The full DNA sequences encoding the vpap library were determined to be as follows:

10 vpap10

GACTACAAAGACATTGTGATGACCCAGTCTCCACTCTCCCTGCCTGTCAGT
CTTGGAGATCAAGCCTCCATCTCTTGCAGATCTAGTCAGAGCATTGTACAT
AGTAATGGAAACACCTATTTAGAATGGTACCTGCAGAAACCAGGCCAGTC
TCCAAAGCTCCTGATCTACAAAGTTTCCAACCGATTTTCTGGGGTCCCAGA
15 CAGGTTTCAGTGGCAGTGGATCAGGGACAGATTTCACTCAAGATCAGCA
GAGTGGAGGCTGAGGATCTGGGAGTTTATTACTGCTTTCAAGGTTACAT
GTTCCGTGGACGTTTCGGTGGAGGCACCAAGCTGGAAATCAAACGTGGTGG
TGGTGGTTCTGGTGGTGGTGGTTCTGGCGGCGGCGGCTCCGGTGGTGGTG
GATCCGGCTTAGTGAAGCCTGGAGGGTCCCTGAAACTCTCCTGTGCAGCC
20 TCTGGATTCACTTTCAGTAACTCTGCCATGTCTTGGGGTCGCCAGACTCCA
GAGAAGAGGCTGGAGTGGGTTCGCATCCTTACTAGTGGTGGTATCACCTAC
TATCCAGACAGTGTGAAGGGCCGATTACCATCTCCAGAATGATGCCCCGA
CATCCCTGTTTTGCAAAGAGCAGTCTGAGGTTTGAGGACACGGCCATGTTT

TACTGTGCAAGAGGGGACTATTGGGTCCCCCAAGGGGGTTTTGTTTCCTGG
GCCAAGGGACTCTGGTCACTGTTTCTCGG

vpap34

GACTACAAAGATATTGTAATGACCCAGTCTCAAAAATTCATGTCCACATC
5 AGTAGGAGACAGGGTCAGCGTCACCTGCAAGGCCAGTCAGAATGTGGGT
ACTAATGTAGCCTGGTATCAACAGAAACCAGGGCAATCTCCTAAAGCACT
GATTTACTCGGCATCCTACCGGTACAGTGGGGTCCCTGATCGCTTCACTGG
CAGTGGATCTGGGACGGAATTCACCTTTCACCATCAGCAGTGTGCAGGCTG
AAGACCTGGCAGTTTATTACTGTCAGCAACATTATAGTATTCCTCCGACGT
10 TCGGTGGAGGCACCAAGCTGGAAATCAAACGTGGTGGTGGTGGTTCTGGT
GGTGGTGGTTCTGGCGGCGGCGGCTCCGGTGGTGGTGGATCCGAGGTGCA
GCTGCAGCAGTCTGGGGCTGAACTGGCAAGACCTGGGGCCTCAGTGAAGA
TGTCTTGCAAGGCTTCTGGCTACACCTTCACTAGCTACACGATGCACTGGG
TAAACAGAGGCCTGGACAGGGTCTGGAATGGATTGGATACATTAATCCT
15 AACGCTGGTTATACTCATTACAATCAGAAGTTCAAGGAGAAGGCCACATT
GACTGCAGACAAATCCTCCAGCACAGTCTACATGCAACTGAGCAGCCTGA
CATCTGAGGACTCTGCAGTCTATTACTGTGCTAGATGGTCCGACCGTGCTA
TGGACTACTGGGGTCAAGGAACCTCAGTCACCGTCTCCTCG

vpap37

20 GACTACAAAGATATTCAGATGAACCAGTCTCAAAAATTCATGTCCGCATC
AGTAGGAGACAGGGTCAGCATCACCTGCAAGGCCAGTCAGAATGTTTCGTT
CTGCTGTAGCCTGGTATCAACAGAAACCAGGGCAGTCTCCTAAAGCACTG
ATTTACTTGGCATCCAACCGGCACACTGGAGTCCCTGATCGCTTACAGGC

AGTGGATCTGGGACAGATTTCACTCTCACCATTAGCAATGTTCAATCTGAG
GACCTGGCAGATTATTTCTGTCTGCAACATTGGAATTATCCTCTCGCGTTC
GGTGCTGGGACCAAGCTGGAAATAAAACGTGGTGGTGGTGGTTCTGGTGG
TGGTGGTTCTGGCGGCGGCGGCTCCGGTGGTGGTGGATCCGAGCAGCTGC
5 AGGAGTCTGGACCTGAGCTGGAAAAGCCTGGGGCTCTAATGAGAGAGCTC
TGCAACAGTTTTTTGGATACACAGTGGCTATGCTAAACAATACACTCTGCTA
TGAAGAAGCCTGTGTTATGGCTTACCTGGAACCTGACCTATTAACACTTACG
AAACCTGTCCTGACACATTGATTAATGAATAGGCCAAGCCACAGTGAGAG
GGACAAATCCTCCAGCACAGCCTACATGGAGCTCAGCAGCCTGACCTCTG
10 AGGACTCTGCGGTCTATTACTGTGCAAGAGGGTTACTACTACAGTTTCAAN
ATGCTGTCCACTCTGGGTTGCCCCAAACGGCAAAAACCTTTTTTATGGGCT
GGAAGGGCAGGAATAACAAAAAC

vpap43

CGCGGCCAGATTATGAAATATCTGCTGCCGACCGCGGCGGCGGGCCTGCT
15 GCTGCTGGCGGCGCAGCCGGCGATGGCGGATTATAAAGATATTGTGCTGA
CCCAGAGCCCGGCGATTATGAGCGCGAGCCCGGGCGAAAAAGTGACCAT
GACCTGCAGCGCGAGCAGCAGCGTGAGCTATATGCATTGGTATCAGCAGA
AAAGCGGCACCAGCCCGAAACGCTGGATTTATGCGACCAGCAAACCTGGCG
AGCGGCGTGCCGGCGCGCTTTAGCGGCAGCGGCAGCGGCACCAGCTATAG
20 CCTGACCATTAGCAGCGTGGAAGCGGAAGATGCGGCGACCTATTATTGCC
AGCAGTGGAGCAGCAACCCGCCGACCTTTGGCGGCGGCACCAAACCTGGA
AATTAAACGCGGCGGCGGCGGCAGCGAAGTGAAAGTGGTGGAAAAAAA
GAAGGCCTGGTGAAACCGGGCGGCAGCCTGAAACTGAGCTGCGCGGCGA
GCGGCTTTACCTTTAGCAGCTATGCGATGAGCTGGGTGCGCCAGACCCCG

GAAAAACGCCTGGAATGGGTGGCGACCATTAGCAGCGGCGGCAGCTATA
CCTATTATCCGGATAGCGTGAAAGGCCGCTTTACCATTAGCCGCGATAAC
GCGAAAAACACCCTGTATCTGCAGATGAGCAGCCTGCGCAGCGAAGATAC
CGCGCATGTGCTGCTGTGCAAAACCGGCGTGACCACCCGCTGGCATGGCC
5 TGCTGGGCAGCAGCAACCTGAGCCAGCGCCTGCTGGGCCTGGGCGAAGGC

vpap44

GACTACAAAGATATTGTGATGACCCAACTCCACTCTCCCTGCCTGTCAGT
CTTGGAGATCAAGCCTCCATCTCTTGCAGATCTAGTCAGATCATTGCACTT
AGTAATGGAAACACCTATTTAGAATGGTATTTGCAGAAACCAGGCCAGTC
10 TCCAAAGCTCCTGATCTACAAAGTTTCCAACCGATTTTCTGGGGTCCCAGA
CAGGTTCAGTGGCAGTGGATCAGGGACAGATTTCACTCAAGATCACCA
GAGTGGAGGCTGAGGATCTGGGAGTTTATTACTGCTTTCAAGGTTACAT
GTTCCGTGGACGTTTCGGTGGAGGCACCAAGCTGGAGCTGAAACGTGGTGG
TGGTGGTTCTGGTGGTGGTGGTTCTGGCGGCGGCGGCTCCGGTGGTGGTG
15 GACCCGAGGTGAAGGTGGTGGAGTCTGGGGGAGGCTTAGTGAAGCCTGG
AGGGTCCCTGAACTCTCCTGTGCAGCCTCTGGATTCACTTTCAGTAACTC
TGCCATGTCTTGGGCTCGTCAGACTCCAGAGAAGAGGCTGGAATGGGTGCG
CATCCATTACTAGTGGGGGTATCACCTACTATCCAGACAGTGCGAAGGGC
CGATTACCATCTCCAGAGATGATGCCCCGGAACATCCTGTACCTTGCAATG
20 AACAGTCTGAGGTCTGAGGACACGGCCCTGTATTACTGTGCAAGAGGGGA
CCATAGGGTCCAACAAGGGGGCTTTGCTTACTGGGGGCAAGGGACTCTTG
GTACT

vpap47

CGCGGCCAGATTATGAAATATCTGCTGCCGACCGCGGCGGGCCTGCT
GCTGCTGGCGGCGCAGCCGGCGATGGCGGATTATAAAGATATTCTGATGA
CCCAGACCCCGCTGAGCCTGCCGGTGAGCCTGGGCGATCAGGCGAGCATT
5 AGCTGCCGCAGCAGCCAGAGCATTGCGCTGAGCAACGGCAACACCTATCT
GGAATGGTATCTGCAGAAACCGGGCCAGAGCCCGAAACTGCTGATTTATA
AAGTGAGCAACCGCTTTAGCGGCGTGCCGGATCGCTTTAGCGGCAGCGGC
AGCGGCACCGATTTTACCCTGAAAATTAGCCGCGTGGAAGCGGAAGATCT
GGGCGTGTATTATTGCTTTCAGGGCAGCCATGTGCCGTGGACCTTTGGCGG
10 CGGCACCAAACCTGGAAATTAAACGCGGCGGCGGCGGCAGCGGCGGCGGC
GGCAGCGAAGTGCAGCTGGTGGAAAGCGGCGGCGGCCTGGTGAACCGG
GCGGCAGCCTGAAACTGAGCTGCGCGGCGAGCGGCTTTACCTTTAGCAAC
AGCGCGATGAGCTGGGCGCGCCAGACCCCGGAAAAACGCCTGGAATGGG
TGGCGAGCATTACCAGCGGCGGCATTACCTATTATCCGGGCAGCGCGAAA
15 GGCCGCTTTACCATTAGCCGCGATGATGCGCGCAACATTCTGTATCTGCAG
ATGAACAGCCTGCGCAGCGAAGATAACGCGCATGTGTTTCTGTGCAAACG
CGGCCTG

Referring now to Figure 2, there is shown a phylogenetic tree describing the
20 relationship between the entire sequence of the selected antibodies (marked by dot)
and a range of other non-specific antibodies.

As may be seen, many of these antibodies show significant similarity. Further
examination of the CDR-H3 region in the closely related antibodies vpap10 and vpap

44 reveals that a 38% variation in a single CDR is tolerated without loss of specificity (gdyryaegafayw differs from gdhrvqqggfayw in 5 out of 13 amino acids).

References

5 The following references, referred to throughout the specification, are incorporated by reference in their entirety herein.

1. Zhou B., Wirsching P. and Janda K.D. "Human Antibodies against Spores of the Genus Bacillus: A Model Study for Detection of and Protection against Anthrax
10 and the Bioterrorist Threat", PNAS, 2002, 99, 5241-5246.

2. Krebber A., Bornhauser S., Burmester J., Honegger A., Willuda J., Bosshard H.R. and Pluckthun A., "Reliable Cloning of Functional Antibody Variable Domains from Hybridomas and Spleen Cell Repertoires Employing a Reengineered Phage
15 Display System", Journal of Immunological Methods, 1997, 201, 35-55.

3. Krebs B., Rauchenberger, R., Reiffert, S., Rothe, C., Tesar, M., Thomassen, E., Cao, M., Dreier, T., Fischer, Hoss, A., "High-Throughput generation and Engineering of Recombinant Human Antibodies", J. Immunol. Methods, 2004, 254,
20 67-84.

4. Cai X. and Garen, A. "Anti-Melanoma Antibodies from Melanoma Patients Immunised with Genetically Autologous Tumour Cells: Selection of Specific Antibodies from Single-Chain Fv Fusion Phage Libraries", Proc. Natl. Acad. Sci.
25 USA, 1995, 92, 6537-6541.

5. Lipman D.J. and Pearson, W.R., "Rapid and Sensitive Protein Similarity Searches", Science, 1985, 227, 1435-1441.
- 5 6. Martin, A.C.R. "Protein Sequence and Structure Analysis of Antibody Variable Domains, Antibody Engineering Laboratory Manual", Eds. Kontermann, R. and Dubel, S., Springer-Verlag, Heidelberg, pp 423-439.

CLAIMS

1. A method for the production of antibody specific for *B. anthracis* comprising the steps of i) generating an immune scFv phage-display library and ii) panning the
5 library against immobilised *B. anthracis* spore in the presence of spore from one or more other *Bacillus* species.

2. A method according to Claim 1, in which the scFv library is positive against immobilised *B. anthracis* spore.

10

3. A method according to Claim 2, in which the one or more other species comprise *B. cereus*.

15

4. A method according to any preceding Claim, in which step ii) is the first panning step.

5. A method according to any preceding Claim, in which step ii) is repeated up to three times.

20

6. A *B. anthracis* specific antibody comprising at least one of SEQ ID 2, SEQ ID 3, SEQ ID 4, SEQ ID 15, SEQ ID 19, SEQ ID 20, SEQ ID 24, SEQ ID 30, SEQ ID 31, SEQ ID 32, SEQ ID 33, SEQ ID 34, SEQ ID 35, SEQ ID 36, SEQ ID 37, SEQ ID 38, SEQ ID 39 or a variant or a fragment thereof.

7. A *B. anthracis* specific antibody according to Claim 6, in which CDR-L1 comprises any one of SEQ ID 19, SEQ ID 31 and SEQ ID 32; CDR-L2 comprises any one of SEQ ID 2, SEQ ID 20 and SEQ ID 33; CDR-L3 comprises any one of SEQ ID 3, SEQ ID 15 and SEQ ID 34; CDR-H1 comprises any one of SEQ ID 4 or SEQ ID 35; CDR-H2 comprises any one of SEQ ID 23, SEQ ID 36 and SEQ ID 37; and CDR-H3 comprises any one of SEQ ID 24, SEQ ID 38 and SEQ ID 39.
8. A *B. anthracis* specific antibody according to Claim 6, comprising any one of SEQ ID 1 to 6 or SEQ ID 7 to 12, or SEQ ID 13 to 18 or SEQ ID 19 to 24 or SEQ ID 2 to 4 and 25 to 27 or SEQ ID 2 to 5 and 28 to 30.
9. A *B. anthracis* specific antibody according to Claim 6, comprising any one of SEQ ID 1 to 45 or a variant or a fragment thereof.
10. A method for detecting anthrax comprising detecting the binding of *B. anthracis* spores to an antibody according to any of Claims 6 to 9.
11. A biosensor comprising an antibody according to any of Claims 6 to 9.
12. A polynucleotide encoding an antibody according to any of Claims 6 to 9.
13. A pharmaceutical composition comprising a humanised antibody according to any of Claims 6 to 9.

14. A composition according to Claim 13, which composition comprises a vaccine.

15. An antibody according to any of Claims 6 to 9, for use in therapy.

5

16. Use of an antibody according to any of Claims 6 to 9 in the preparation of a medicament for the treatment of anthrax infection.

17. An anti-idiotypic antibody to an antibody according to any of Claims 6 to 9.

1/2

- Average (CM23C12)
- Average (OV)
- Average (11143)
- Average (EA1)

Fig.1.

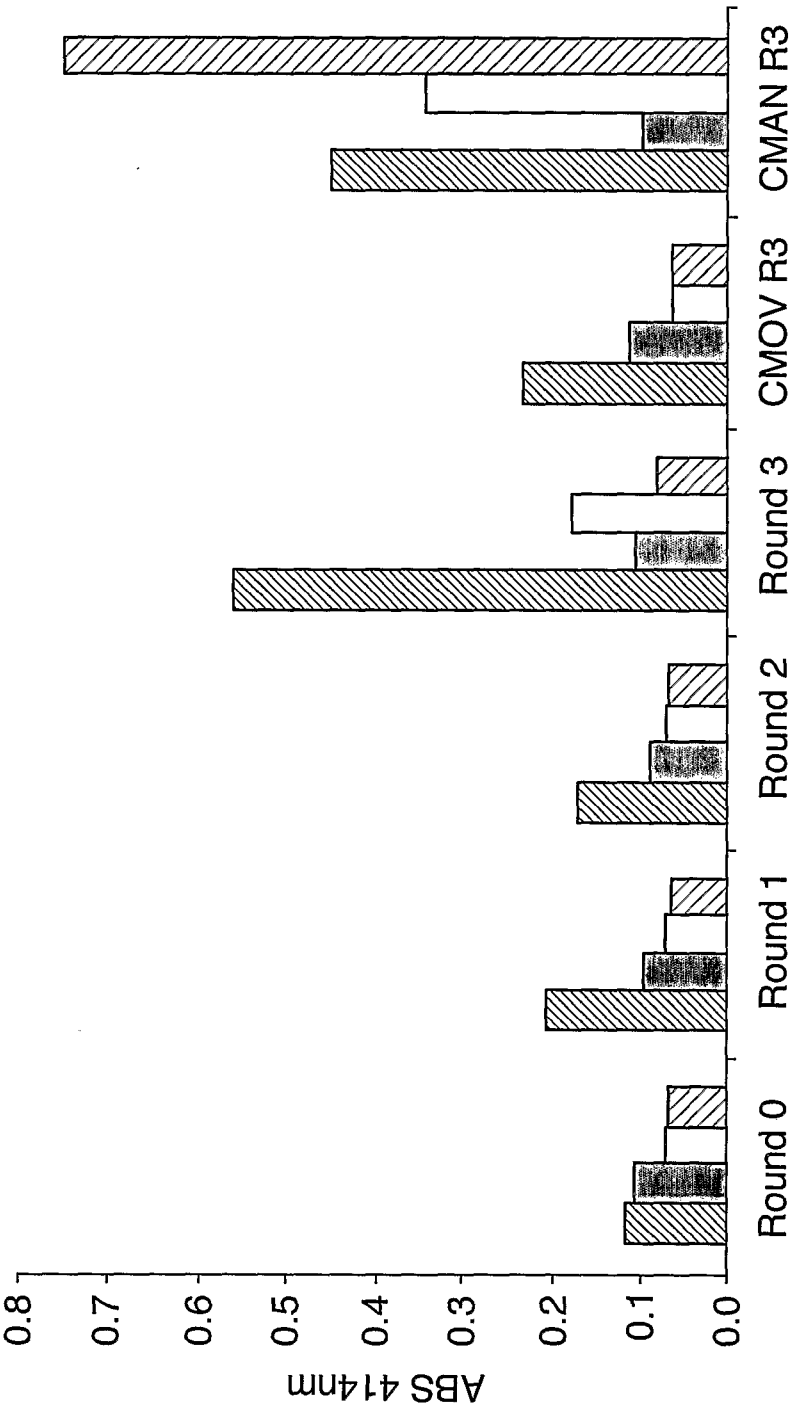


Fig.2.

