

CLAIMS

1. An antibody preparation suitable for intravenous administration in humans comprising IgG, IgA and at least 5% IgM antibodies by weight of the total amount of antibodies, wherein the preparation is prepared from human plasma, wherein the antibody preparation has specific complement activating activity, and wherein in an in vitro assay with human serum suitable to determine the ability of the antibody preparation to activate complement unspecifically the antibody preparation generates substantially no C5a and/or substantially no C3a.
2. An antibody preparation according to claim 1 further comprising more than 5% IgA and more than 40% IgG by weight of the total amount of antibodies.
3. An antibody preparation according to claims 1 or 2 comprising at least 10% IgM by weight of the total amount of antibodies.
4. An antibody preparation according to claim 3 comprising at least 15% IgM by weight of the total amount of antibodies.
5. An antibody preparation according to any preceding claim wherein the antibody preparation adjusted to an IgM concentration of 1.72 mg/ml generates less than 200 ng/ml C5a after 60 minutes of the assay.
6. An antibody preparation according to any preceding claim wherein the antibody preparation adjusted to an IgM concentration of 1.72 mg/ml generates less than 6000 ng/ml C3a after 60 minutes of the assay.
7. An antibody preparation according to any preceding claim wherein in the in vitro serum assay the antibody preparation with human serum generates the same amount of C5a and/or C3a as human serum alone $\pm$ 70%.
8. An antibody preparation according to any preceding claim wherein the in vitro serum assay to determine that the antibody preparation generates substantially no C5a comprises the steps of:

- (a) adding an amount of the antibody preparation to 100 μ l human serum to create a reaction mixture containing 1.72mg/ml IgM and incubating the reaction mixture for 60 minutes at 37 °C with constant agitation;
- (b) preparing a set of dilutions of the reaction mixture suitable for an ELISA;
- (c) performing a sandwich ELISA on the set of dilutions of the reaction mixture utilizing a primary and a secondary antibody to C5a and a chromogenic substance, wherein the secondary antibody is conjugated to an enzyme and the chromogenic substance is the substrate of the enzyme; and
- (d) determining the amount of C5a in the reaction mixture based on a colour change obtained as a result of contacting the chromogenic substance with the enzyme bound to C5a via the secondary antibody.

9. An antibody preparation according to any preceding claim wherein the in vitro serum assay to determine that the antibody preparation generates substantially no C3a comprises the steps of:

- (a) adding an amount of the antibody preparation to 100 μ l human serum to create a reaction mixture containing 1.72mg/ml IgM and incubating the reaction mixture for 60 minutes at 37 °C with constant agitation;
- (b) preparing a set of dilutions of the reaction mixture suitable for an ELISA;
- (c) performing a sandwich ELISA on the set of dilutions of the reaction mixture utilizing a primary and a secondary antibody to C3a and a chromogenic substance, wherein the secondary antibody is conjugated to an enzyme and the chromogenic substance is the substrate of the enzyme; and
- (d) determining the amount of C3a in the reaction mixture based on a colour change obtained as a result of contacting the chromogenic substance with the enzyme bound to C3a via the secondary antibody.

10. An antibody preparation according to any preceding claim comprising less than 2% aggregates of 1200 kDa or above.

11. An antibody preparation according to claim 10 comprising less than 1.5% aggregates of 1200 kDa or above.

12. An antibody preparation according to any preceding claim wherein the anti-complementary activity of the preparation is less than 1.0 CH50/mg protein.

13. An antibody preparation according to claim 12 wherein the anti-complementary activity is less than 0.75 CH50/mg protein.
14. An antibody preparation according to any preceding claim comprising an immunoglobulin content of greater than 95% of the total protein content.
15. An antibody preparation according to any preceding claim prepared from human serum in the absence of a step of heat treatment at a temperature 40°C or above for more than 10 minutes.
16. An antibody preparation according to any preceding claim prepared from human serum in the absence of a step involving chemical or enzymatic modification of the antibodies.
17. An antibody preparation according to claim 16 wherein the step of chemical modification is a step of contacting the antibodies with β -propiolactone.
18. An antibody preparation according to any preceding claim prepared by a process which is capable of a more than 3log₁₀ removal of non-enveloped viruses.
19. An antibody preparation according to any preceding claim prepared from human plasma by a process comprising the steps of:
 - (a) preparing from the human plasma a plasma fraction as a solution containing immunoglobulins;
 - (b) mixing a C₇ to C₉ carboxylic acid with the solution and treating the mixed solution with a vibrating agitator to precipitate contaminating proteins;
 - (c) separating the precipitated proteins from the solution to yield the IgM containing immunoglobulin composition;
 - (d) incubating the IgM containing immunoglobulin composition at between pH3.5 and pH 4.5 to form an incubated solution;
 - (e) irradiating the incubated solution with UVC to form a UVC irradiated solution; and
 - (f) filtering the UVC irradiated solution under sterile conditions to form the antibody preparation suitable for intravenous administration in humans.

20. An antibody preparation according to claim 19 wherein the process further comprises subjecting the incubated solution obtained from step (d) to nanofiltration prior irradiation in step (e).
21. An antibody preparation according to any preceding claim wherein the antibody preparation is capable of administration to cynomolgus monkeys at 115 mg IgM/kgBW/hr in the absence of a greater than 10% reduction in arterial pressure from pretreatment level.
22. An antibody preparation according to any preceding claim wherein at least 90% of the antibodies in the preparation are biologically active.
23. An antibody preparation according to any preceding claim wherein in an in vitro Rubella antigen based assay suitable to determine Fc function the activity of the Fc part of the antibodies of the antibody preparation is the same as that of a biological reference preparation $\pm 10\%$.
24. A method of producing an antibody preparation according to any preceding claim from human plasma comprising the steps of:
- (a) preparing from the human plasma a plasma fraction as a solution containing immunoglobulins;
 - (b) mixing a C₇ to C₉ carboxylic acid with the solution and treating the mixed solution with a vibrating agitator to precipitate contaminating proteins;
 - (c) separating the precipitated proteins from the solution to yield the IgM containing immunoglobulin composition;
 - (d) incubating the IgM containing immunoglobulin composition at between pH 3.5 and pH 4.5 to form an incubated solution;
 - (e) irradiating the incubated solution with UVC to form a UVC irradiated solution; and
 - (f) filtering the UVC irradiated solution under sterile conditions to form the antibody preparation suitable for intravenous administration in humans.
25. An antibody preparation according to any one of claims 1 to 23 for use in medicine.
26. An antibody preparation according to claim 25 for use in the treatment of an immunological disorders or a bacterial infection.

27. An antibody preparation according to claim 26 wherein the immunological disorder is an IgM deficiency disorder.
28. Use of an antibody preparation according to any one of claims 1 to 23 for the manufacture of a medicament for treatment of an immunological disorder or a bacterial infections.
29. A method of treatment of a patient comprising administering an antibody preparation according to any one of claims 1 to 23.
30. A method of treatment according to claim 29 wherein the patient is suffering from an immunological disorder or a bacterial infection.
31. A method of treatment according to claim 29 and 30 wherein the antibody preparation is administered to the patient intravenously.

Claims:

1. An antibody preparation suitable for intravenous administration in humans comprising IgG, IgA and at least 5% IgM antibodies by weight of the total amount of antibodies, wherein the preparation is prepared from human plasma, wherein the antibody preparation has specific complement activating activity, wherein the antibody preparation is prepared by a process which is capable of a more than $3\log_{10}$ removal of non-enveloped viruses and wherein in an in vitro assay with human serum suitable to determine the ability of the antibody preparation to activate complement unspecifically the antibody preparation generates: (i) substantially no C5a, such that the antibody preparation adjusted to an IgM concentration of 1.72 mg/ml generates less than 200 ng/ml C5a after 60 minutes of the assay; and/or (ii) substantially no C3a, such that the antibody preparation adjusted to an IgM concentration of 1.72 mg/ml generates less than 6000 ng/ml C3a after 60 minutes of the assay.
2. An antibody preparation according to claim 1 further comprising more than 5% IgA and more than 40% IgG by weight of the total amount of antibodies.
3. An antibody preparation according to claims 1 or 2 comprising at least 10% IgM by weight of the total amount of antibodies and preferably comprising at least 15% IgM by weight of the total amount of antibodies.
4. An antibody preparation according to any preceding claim wherein in the in vitro serum assay the antibody preparation with human serum generates the same amount of C5a and/or C3a as human serum alone $\pm 70\%$.
5. An antibody preparation according to any preceding claim wherein the in vitro serum assay to determine that the antibody preparation generates substantially no C5a comprises the steps of:
 - (a) adding an amount of the antibody preparation to 100 μ l human serum to create a reaction mixture containing 1.72mg/ml IgM and incubating the reaction mixture for 60 minutes at 37 $^{\circ}$ C with constant agitation;
 - (b) preparing a set of dilutions of the reaction mixture suitable for an ELISA;

(c) performing a sandwich ELISA on the set of dilutions of the reaction mixture utilizing a primary and a secondary antibody to C5a and a chromogenic substance, wherein the secondary antibody is conjugated to an enzyme and the chromogenic substance is the substrate of the enzyme; and

(d) determining the amount of C5a in the reaction mixture based on a colour change obtained as a result of contacting the chromogenic substance with the enzyme bound to C5a via the secondary antibody.

6. An antibody preparation according to any preceding claim wherein the in vitro serum assay to determine that the antibody preparation generates substantially no C3a comprises the steps of:

(a) adding an amount of the antibody preparation to 100 μ l human serum to create a reaction mixture containing 1.72mg/ml IgM and incubating the reaction mixture for 60 minutes at 37 °C with constant agitation;

(b) preparing a set of dilutions of the reaction mixture suitable for an ELISA;

(c) performing a sandwich ELISA on the set of dilutions of the reaction mixture utilizing a primary and a secondary antibody to C3a and a chromogenic substance, wherein the secondary antibody is conjugated to an enzyme and the chromogenic substance is the substrate of the enzyme; and

(d) determining the amount of C3a in the reaction mixture based on a colour change obtained as a result of contacting the chromogenic substance with the enzyme bound to C3a via the secondary antibody.

7. An antibody preparation according to any preceding claim comprising less than 2% aggregates of 1200 kDa or above, and preferably comprising less than 1.5% aggregates of 1200 kDa or above.

8. An antibody preparation according to any preceding claim wherein the anti-complementary activity of the preparation is less than 1.0 CH50/mg protein, and preferably wherein the anti-complementary activity is less than 0.75 CH50/mg protein.

9. An antibody preparation according to any preceding claim, wherein:

(i) the antibody preparation comprises an immunoglobulin content of greater than 95% of the total protein content; and/or

- (ii) the antibody preparation is prepared from human serum in the absence of a step of heat treatment at a temperature 40°C or above for more than 10 minutes; and/or
- (iii) the antibody preparation is prepared from human serum in the absence of a step involving chemical or enzymatic modification of the antibodies, and preferably wherein the antibody preparation is prepared in the absence of a step of chemical modification which is a step of contacting the antibodies with β -propiolactone.

10. An antibody preparation according to any preceding claim prepared from human plasma by a process comprising the steps of:

- (a) preparing from the human plasma a plasma fraction as a solution containing immunoglobulins;
- (b) mixing a C₇ to C₉ carboxylic acid with the solution and treating the mixed solution with a vibrating agitator to precipitate contaminating proteins;
- (c) separating the precipitated proteins from the solution to yield the IgM containing immunoglobulin composition;
- (d) incubating the IgM containing immunoglobulin composition at between pH3.5 and pH 4.5 to form an incubated solution;
- (e) irradiating the incubated solution with UVC to form a UVC irradiated solution; and
- (f) filtering the UVC irradiated solution under sterile conditions to form the antibody preparation suitable for intravenous administration in humans.

11. An antibody preparation according to claim 10 wherein the process further comprises subjecting the incubated solution obtained from step (d) to nanofiltration prior irradiation in step (e).

12. An antibody preparation according to any preceding claim wherein:

- (i) the antibody preparation is capable of administration to cynomolgus monkeys at 115 mg IgM/kgBW/hr in the absence of a greater than 10% reduction in arterial pressure from pretreatment level; and/or
- (ii) at least 90% of the antibodies in the preparation are biologically active; and/or

(iii) in an in vitro Rubella antigen based assay suitable to determine Fc function the activity of the Fc part of the antibodies of the antibody preparation is the same as that of a biological reference preparation $\pm 10\%$.

13. An antibody preparation comprising at least 15% IgM, more than 5% IgA and more than 40% IgG as percentages of the total amount of antibodies, and comprising less than 1.5% aggregates of 1200kDa or above of the total immunoglobulin content as determined by high performance size exclusion chromatography.

14. An antibody preparation according to claim 13, which is substantially free of non-enveloped virus, which has an anti-complementary activity of less than 0.75 CH50/mg protein, and wherein at least 90% of the antibodies in the preparation are biologically active, as determined by the Eur. Ph. 2.7.9. Test for Fc Function of Immunoglobulin.

15. A method of producing an antibody preparation according to any preceding claim from human plasma comprising the steps of:

(a) preparing from the human plasma a plasma fraction as a solution containing immunoglobulins;

(b) mixing a C₇ to C₉ carboxylic acid with the solution and treating the mixed solution with a vibrating agitator to precipitate contaminating proteins;

(c) separating the precipitated proteins from the solution to yield the IgM containing immunoglobulin composition;

(d) incubating the IgM containing immunoglobulin composition at between pH3.5 and pH 4.5 to form an incubated solution;

(e) irradiating the incubated solution with UVC to form a UVC irradiated solution; and

(f) filtering the UVC irradiated solution under sterile conditions to form the antibody preparation suitable for intravenous administration in humans.

16. An antibody preparation according to any one of claims 1 to 14 for use in medicine.

17. An antibody preparation according to claim 16 for use in the treatment of an immunological disorders or a bacterial infection, preferably wherein the immunological disorder is an IgM deficiency disorder.