C L A I M S

1. Method for predicting whether an immunoglobulin single variable domain (ISV) or protein or polypeptide comprising at least one ISV at its C-terminal end will give rise to protein interference in an immunoassay or has increased tendency to give rise to protein interference in an immunoassay, or to predict whether said ISV or protein or polypeptide comprising at least one ISV will be bound by interference factor(s) present in the blood or serum of a human being, wherein said interference is caused by pre-existing proteins binding to the C-terminal end of the ISV, protein or polypeptide, said method comprising performing an immunoassay that at least comprises the steps of:
(i) contacting said ISV, protein or polypeptide with an antibody that has been obtained from a human subject and that has been selected or isolated based on its ability to recognize or bind to the C-terminal end of said ISV; and
(ii) determining whether said ISV, protein or polypeptide is bound by said antibody in said immunoassay.

2. Method for predicting according to claim 1, in which the ISV is either a VHH, sequence optimized VHH, humanized VHH or camelized VH, or is another ISV which is other than a VHH, sequence optimized VHH, humanized VHH or camelized VH that is a VH domain or that comprises a VH domain.

3. Method for predicting according to claim 1 or 2, in which the ISV is a VHH, sequence optimized VHH, humanized VHH or camelized VH.

4. Method for predicting according to any of claims 1 or 2, in which the fact that the ISV, protein or polypeptide binds

to said antibody in step (ii) means that the ISV, protein or polypeptide can give rise to or has a high or increased risk of giving rise to such protein interference.

5. Method for predicting according to any of claims 1 or 2, in which the antibody is a polyclonal antibody.

6. Method for predicting according to any of claims 1 or 2, in which the antibody is a polyclonal antibody that has been obtained, starting from a biological sample obtained from a human subject and suitable as a starting material for obtaining polyclonal antibodies, by a method that comprises at least one step of immunoaffinity chromatography in which affinity matrix is used that carries the ISV or protein or polypeptide comprising at least one ISV or in which the ISV or protein or polypeptide comprising at least one ISV is used as the affinity moiety or antigen.

7. Method for predicting according to claim 6, in which the ISV or protein or polypeptide comprising at least one ISV that is carried on the affinity matrix or that is used as the affinity moiety or antigen, is an ISV or protein or polypeptide comprising at least one ISV that ends at its C-terminal end with the amino acid sequence VTVSS (SEQ ID NO:33), or in which the ISV or protein or polypeptide comprising at least one ISV that is carried on the affinity matrix or that is used as the affinity moiety or antigen has at its C-terminal end an ISV that ends at its C-terminal end with the amino acid sequence VTVSS (SEQ ID NO:33).

8. Method for predicting according to claim 6, in which the ISV or protein or polypeptide comprising at least one ISV that is carried on the affinity matrix or that is used as

the affinity moiety or antigen is an ISV or protein or polypeptide comprising at least one ISV, that ends at its C-terminal end with the amino acid sequence VTVSS (SEQ ID NO:33) and that has a proline residue on position 14; or in which the ISV or protein or polypeptide comprising at least one ISV that is carried on the affinity matrix or that is used as the affinity moiety or antigen, has at its C-terminal end an ISV or protein or polypeptide comprising at least one ISV that ends at its C-terminal end with the amino acid sequence VTVSS (SEQ ID NO:33) and that has a proline residue on position 14.

9. Method for predicting according to claim 6, in which the ISV that is carried on the affinity matrix or that is used as the affinity moiety or antigen is a VHH or camelized VH, wherein said VHH or camelized VH is sequence-optimized or humanized; or in which the ISV-based drug that is carried on the affinity matrix or that is used as the affinity moiety or antigen has at its C-terminal end an ISVthat is a VHH or camelized VH, wherein said VHH or camelized VH is sequence-optimized or humanized VHH.

10. Method for predicting according to claim 8, in which the ISV that is carried on the affinity matrix or that is used as the affinity moiety or antigen is a VHH or camelized VH, wherein said VHH or camelized VH is sequence-optimized or humanized, that ends at its C-terminal end with the amino acid sequence VTVSS (SEQ ID NO:33) and that has a proline residue on position 14 which has been introduced as part of the humanization or sequence optimization of the corresponding naturally occurring VHH; or in which the ISV-based drug that is carried on the affinity matrix or that is used as the affinity moiety or antigen has at its C-terminal end a VHH or camelized VH, wherein said VHH or

camelized VH is sequence-optimized or humanized, that ends at its C-terminal end with the amino acid sequence VTVSS (SEQ ID NO:33) and that has a proline residue on position 14 which has been introduced as part of the humanization or sequence optimization of the corresponding naturally occurring VHH.

11. Method for predicting according to any of claims 1 or 2, in which the antibody is a monoclonal antibody.

12. Method for predicting according to any of claims 1 or 2, in which the antibody is a monoclonal antibody that has been obtained starting from a biological sample from a human subject and suitable as a starting material for obtaining monoclonal antibodies, by a method that comprises at least one screening or selection step in which an ISV or ISV-based drug is used for screening and selecting a monoclonal antibody that binds to said ISV or ISV-based drug.

13. Method for predicting according to claim 12, in which the ISV that is used in the screening or selection step ends at its C-terminal end with the amino acid sequence VTVSS (SEQ ID NO:33), or in which the ISV-based drug that is used in the screening or selection step has at its C-terminal end an ISV that ends at its C-terminal end with the amino acid sequence VTVSS (SEQ ID NO:33).

14. Method for predicting according to claim 12, in which the ISV that is used in the screening or selection step ends at its C-terminal end with the amino acid sequence VTVSS (SEQ ID NO:33) and has a proline residue at position 14, or in which the ISV-based drug that is used in the screening or selection step has at its C-terminal end an ISV that ends at its C-terminal end with the amino acid sequence VTVSS

(SEQ ID NO:33) and that has a proline residue at position 14.

15. Method for predicting according to claim 12, in which the ISV that is used in the screening or selection step is a VHH or camelized VH, wherein said VHH or camelized VH is sequence-optimized or humanized; or in which the ISV-based drug that is used in the screening or selection step has at its C-terminal end an ISVthat is a VHH or camelized VH, wherein said VHH or camelized VH is sequence-optimized or humanized.

16. Method for predicting according to claim 14, in which the ISV that is used in the screening or selection step is a VHH or camelized VH, wherein said VHH or camelized VH is sequence-optimized or humanized, that ends at its C-terminal end with the amino acid sequence VTVSS (SEQ ID NO:33) and that has a proline residue on position 14 which has been introduced as part of the humanization or sequence optimization of the corresponding naturally occurring VHH; or in which the ISV-based drug that is used in the screening or selection step has at its C-terminal end a VHH or camelized VH, wherein said VHH or camelized VH is sequence-optimized or humanized, that ends at its C-terminal end with the amino acid sequence VTVSS (SEQ ID NO:33) and that has a proline residue on position 14 which has been introduced as part of the humanization or sequence optimization of the corresponding naturally occurring VHH.

17. Method according to claim 1, for predicting whether an ISV or protein or polypeptide comprising at least one ISV at its C-terminal end will give rise to or has increased tendency to give rise to protein interference in an immunoassay or to predict whether said ISV or protein or

polypeptide comprising at least one ISV at its C-terminal end will be bound by interference factor(s) present in the blood or serum of a human being, said method comprising performing an immunoassay that at least comprises the steps of:

(i) contacting said ISV with the monoclonal antibody 21-4 as expressed by the hybridoma cell line LMBP-9680-CB; and

(ii) determining whether said ISV is bound by said monoclonal antibody 21-4 in said immunoassay.

18. Method for predicting according to claim 17, in which the ISV is either a VHH, sequence optimized VHH, humanized VHH or camelized VH or another ISV which is other than a VHH, humanized VHH or camelized VH that is a VH domain or that comprises a VH domain.

19. Method for predicting according to claim 17 or 18, in which the ISV is a VHH, sequence optimized VHH, humanized VHH or camelized VH.

20. Method for reducing the tendency of an ISV having the sequence VTVSS at its C-terminal end to give rise to protein interference by pre-existing proteins binding to the C-terminal end of the ISV in an anti-drug-antibody assay, wherein said method comprises modifying said ISV by adding 1 to 5 amino acid residues, each independently chosen from any naturally occurring amino acids, to this C-terminal end, such that the tendency to give rise to protein interference is substantially reduced as compared to the ISV having the sequence VTVSS at its C-terminal end.

21. Method according to claim 20, which comprises adding 1, 2, 3, 4 or 5 amino acid residues to the C-terminal end of the ISV.

22. Method according to claim 20 or 21, wherein said amino acid residues are independently chosen from alanine, glycine, valine, leucine or isoleucine.

23. Method according to any of claims 20 or 21, which further comprises introducing one or more amino acid substitutions, additions or deletions in the C-terminal region of the ISV, which C-terminal region comprises the C-terminal sequence VTVSS (SEQ ID NO:33) and the amino acid residues at positions 11, 13, 14, 15, 82, 82a, 82b, 83, 84, 107 and 108.

24. Method according to claim 23, which comprises introducing one or more amino acid substitutions in the C-terminal region of the ISV.

25. Method according to claim 20, wherein the reduction of the tendency to give rise to protein interference is determined using a method according to any of claims 1 or 2.

26. A method of use of a C-terminal extension for reducing the tendency of an ISV to give rise to protein interference, wherein said C-terminal extension is added to the C-terminal sequence VTVSS (SEQ ID NO:33) of the ISV and comprises 1 to 5 amino acid residues independently chosen from any naturally occurring amino acids.

27. A method of use according to claim 26, wherein the C-terminal extension comprises 1, 2, 3, 4 or 5 amino acid residues.

28. A method of use according to claim 26 or 27, wherein said amino acid residues are independently chosen from alanine, glycine, valine, leucine or isoleucine.

29. A method of use according to any of claims 26 or 27 , wherein said C-terminal extension has the formula (X)n, in which:
    a) n = 1, 2 or 3 in which each X = Ala or Gly; or
    b) n = 1, 2 or 3 in which each X = Ala; or
    c) n = 1, 2 or 3 in which each X = Gly; or
    d) n = 2 or 3 in which at least one X = Ala or Gly ; or
    e) n = 2 or 3 in which all but one X = Ala or Gly,

30. A method of use according to claim 29, wherein said C-terminal extension has the formula (X)n, in which:
    a) X is Ala and n is 1, 2 or 3; or
    b) X is Gly and n is 2 or 3.

31. A method of use according to claim 29, wherein said C-terminal extension has the formula (X)n, in which X is Ala and n is 1.