

CLAIMS

1. A method of diagnosing or monitoring *Mycobacterium avium* subspecies
paratuberculosis (MAP) infection, which method comprises detecting the presence of the
5 polypeptide (MAP P900) encoded by the positive strand of IS900, or a fragment thereof, in
a sample from a subject, wherein MAP P900, or a fragment thereof, is detected using an
antibody, or an antigen-binding fragment thereof, that binds to MAP P900.
2. A method according to claim 1, wherein the antibody, or an antigen-binding
10 fragment thereof, binds the region of MAP P900 defined by amino acids 24 to 71 or 329 to
386 of SEQ ID NO: 1.
3. A method according to claim 1 or 2, wherein the antibody, or an antigen-binding
fragment thereof, binds the region of MAP P900 defined by:
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 - amino acids 26 to 71 of SEQ ID NO: 1;
 - amino acids or 329 to 385 of SEQ ID NO: 1;
 - amino acids 26 to 39 of SEQ ID NO: 1;
 - amino acids or 52 to 68 of SEQ ID NO: 1;
 - amino acids or 345 to 359 or 350 to 359 of SEQ ID NO: 1;
 - 20 - amino acids 26 to 71 of SEQ ID NO: 1, wherein serine 37 is
phosphorylated;
 - amino acids or 345 to 359 or 350 to 359 of SEQ ID NO: 1, wherein serine
357 is phosphorylated and/or serine 358 is phosphorylated.
- 25 4. A method according to any one of claims 1 to 3, wherein two or more of said
antibodies, or antigen-binding fragments thereof, are used to detect MAP P900.
5. A method according to claim 4, wherein the method uses:
 - at least one antibody that binds to the region of MAP P900 defined by
30 amino acids 26 to 71 of SEQ ID NO: 1 and at least one further antibody that binds to the
region of MAP P900 defined by amino acids or 329 to 385 of SEQ ID NO: 1;

- at least one antibody that binds to the region of MAP P900 defined by amino acids 26 to 39 of SEQ ID NO: 1 and at least one further antibody that binds to the region of MAP P900 defined by amino acids or 52 to 68 of SEQ ID NO: 1;
 - at least one antibody that binds to the region of MAP P900 defined by amino acids 52 to 68 of SEQ ID NO: 1 and at least one further antibody that binds to the region of MAP P900 defined by amino acids or 345 to 359 or 350 to 359 of SEQ ID NO: 1;
 - at least one antibody that binds to the region of MAP P900 defined by amino acids 26 to 71 of SEQ ID NO: 1, wherein serine 37 is optionally phosphorylated and at least one further antibody that binds to the region of MAP P900 defined by amino acids or 345 to 359 or 350 to 359 of SEQ ID NO: 1, wherein serine 357 and/or serine 358 is optionally phosphorylated.
6. A method according to claim 5, wherein the method comprises determining whether the region of MAP P900 between amino acids 273 to 406 of SEQ ID NO: 1, or a fragment of either thereof, has been cleaved from membrane-bound MAP P900.
7. A method according to claim 6, wherein the method comprises determining whether the cleaved region of MAP P900, or fragment thereof, has spread from microbial cells and/or host cells containing membrane bound MAP P900.
8. A method according to any one of claims 2 to 6, wherein the antibody that binds to the region of MAP P900 defined by amino acids 24 to 71 binds to the amino acid sequence MVINDDAQRLLSQR, AAVTTLADGGEVTWAID or MVINDDAQRL[pS]QR and/or the antibody that binds to the region of MAP P900 defined by amino acids 329 to 386 of SEQ ID NO: 1 binds to the amino acid sequence YLSALVSIRTPSSR or NLKRPRRYDRLLRA or to the amino acid sequence YLSALVSIRTPSSR in which one of more of the serine residues is phosphorylated.
9. A method according to any one of claims 4 to 8, wherein the method uses at least one antibody that specifically binds to a non-phosphorylated MAP P900 sequence and at least one further antibody that specifically binds to phosphorylated MAP P900 sequence.

10. A method according to claim 9, wherein the non-phosphorylated MAP P900 sequence is MVINDDAQRLLSQR, and the phosphorylated MAP P900 sequence is MVINDDAQRLL[pS]QR, or the non-phosphorylated MAP P900 sequence is YLSALVSIRTDPSR or VSIRTDPSR, and the phosphorylated MAP P900 sequence is YLSALVSIRTDPS[pS]R, YLSALVSIRTDP[pS]SR, VSIRTDP[pS][pS]R, VSIRTDP[pS]SR or VSIRTDP[pS][pS]R.
11. A method according to any one of the preceding claims, wherein the sample is a sample of a body fluid or a tissue sample.
12. A method according to claim 11, wherein body fluid is blood, semen, amniotic fluid, cerebrospinal fluid, synovial fluid or breast milk, or the tissue is skin sample, gastrointestinal tract, thyroid, lymph node, brain or genitourinary tract.
13. A method according to any one of the preceding claims, wherein the subject has Crohn's disease, psoriasis, thyroiditis, Parkinson's disease, type 1 diabetes, arthritis, ankylosing spondylitis, irritable bowel syndrome, ulcerative colitis, inflammatory bowel disease, Alzheimer's disease, multiple sclerosis, sarcoidosis, idiopathic pulmonary fibrosis and/or chronic fatigue syndrome.
14. A method according to any one of claims 1 to 12, wherein the subject is an animal.
15. An antibody, or antigen-binding fragment thereof, which specifically binds to the region of MAP P900 defined by amino acids 26 to 71 of SEQ ID NO: 1.
16. An antibody, or antigen-binding fragment thereof, according to claim 15, wherein the antibody binds to the amino acid sequence MVINDDAQRLLSQR, AAVTTLADGGEVTWAID or MVINDDAQRLL[pS]QR.
17. An antibody, or antigen-binding fragment thereof, which specifically binds to a phosphorylated sequence within the region of MAP P900 defined by amino acids 26 to 71 or 345 to 371 of SEQ ID NO: 1.

18. An antibody, or antigen-binding fragment thereof, according to claim 17, wherein the antibody binds to the sequence MVINDDAQRLLSQR or YLSALVSIRTPSSR, when one or more of the serine residues is phosphorylated.
- 5 19. An antibody, or antigen-binding fragment thereof, that specifically binds to the amino acid sequence YLSALVSIRTPSSR or NLKRPRRYDRLLRA or a phosphorylated form of either thereof, within the region of MAP P900 defined by amino acids 329 to 360 of SEQ ID NO: 1.
- 10 20. An antibody according to any one of claims 15 to 19 which is a monoclonal, chimeric, recombinant, synthetic or single domain antibody.
21. An antibody according to any one of claims 15 to 20 which is a labelled antibody.
- 15 22. Use of an antibody, or antigen-binding fragment thereof, as defined in any one of the preceding claims in an *ex vivo* method of diagnosing or monitoring MAP infection.
23. Use of an antibody, or antigen-binding fragment thereof, as defined in any one of claims 1 to 21 in an *ex vivo* method of monitoring treatment of MAP infection, or
- 20 treatment of a subject having Crohn's disease, Ulcerative Colitis, Psoriasis, Thyroiditis, Sarcoidosis, Parkinson's disease, Multiple Sclerosis, Type 1 Diabetes, arthritis, ankylosing spondylitis and irritable bowel syndrome, inflammatory bowel disease, Alzheimer's disease, multiple sclerosis, idiopathic pulmonary fibrosis and/or chronic fatigue syndrome.
- 25 24. Use of an antibody, or antigen-binding fragment thereof, as defined in any one of claims 1 to 21 to detect MAP in a food product.
25. Use according to claim 24 wherein the food product is milk or other dairy product or a meat product.
- 30 26. Use of an antibody, or antigen-binding fragment thereof as defined in any one of claims 1 to 21 in a method for detecting MAP in an environmental sample.

27. Use according to claim 26 wherein the environmental sample is a sample of surface water, river water, water-treatment plant water, domestic water, or an aerosol, soil or sediment.
- 5 28. Use of an antibody, or antigen-binding fragment thereof, as defined in any one of claims 1 to 21 to capture a peptide for Mass Spectrometry (MS) characterisation.
29. A peptide fragment of MAP P900 of up to 40 amino acids comprising one or more of the following sequences: MVINDDAQRLLSQR, VTTLADGGEVTWAIID,
- 10 NLKRPRRYDRLLRA, VSIRTDPSR and YLSALVSIRTDPSR, wherein the peptide is optionally phosphorylated.
30. A peptide according to claim 29, wherein the peptide consists of up to 25 amino acids of the MAP P900 amino acid sequence.
- 15 31. A peptide according to claim 29 or 30, wherein the peptide is phosphorylated and comprises one of the following sequences: MVINDDAQRL[pS]QR or YLSALVSIRTDPS[pS]R, YLSALVSIRTD[pS]SR or YLSALVSIRTD[pS][pS]R.
- 20 32. A peptide according to any one of claims 29 to 31, wherein the peptide is modified at the N-terminus and/or at the C-terminus and/or is conjugated or coupled to a carrier molecule.
33. A method of generating an antibody that specifically binds to MAP P900, which
- 25 method comprises administering a peptide according to any one of claims 29 to 31 to a laboratory animal to generate an immune response and isolating antibodies from the animal.
34. A method according to claim 33, which further comprises screening the isolated
- 30 antibodies for specific binding to MAP P900 by determining whether the antibodies bind to a peptide according to any one of claims 29 to 31 and whether the antibodies bind to homologous peptides from related organisms, wherein a peptide is specific for MAP P900 when it binds to a peptide according to any one of claims 29 to 31 but does not bind to the homologous peptides from related organisms.

35. An antibody, or antigen-binding fragment thereof, as defined in any one of claims 1 to 21 for use in therapeutic or diagnostic method carried out on the human or animal body.
- 5 36. An antibody, or antigen-binding fragment thereof, as defined in any one of claims 1 to 21 for use in a method of treating or preventing MAP infection.