CLAIMS

1. A method of diagnosing or monitoring *Mycobacterium avium* subspecies *paratuberculosis* (MAP) infection, which method comprises detecting the presence of the polypeptide (MAP P900) encoded by the positive strand of IS*900*, or a fragment thereof, in a sample from a subject, in which MAP P900, or a fragment thereof, is detected using an antibody, or an antigen-binding fragment thereof, that binds to MAP P900, in which the antibody, or an antigen-binding fragment thereof, binds the region of MAP P900 defined by amino acids 24 to 71 or 329 to 386 of SEQ ID NO: 1.

2. A method according to Claim 1, in which the antibody, or an antigen-binding fragment thereof, binds the region of MAP P900 defined by:

- 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;
- amino acids 26 to 71 of SEQ ID NO: 1, in which serine 37 is phosphorylated;
- amino acids or 345 to 359 or 350 to 359 of SEQ ID NO: 1, in which serine 357 is phosphorylated and/or serine 358 is phosphorylated.

3. A method according to Claim 1 or 2, in which two or more of the antibodies, or antigen-binding fragments thereof, are used to detect the MAP P900 polypeptide, or the fragment thereof.

4. A method according to Claim 3, in which the method uses:

(a) - at least one antibody that binds to the region of MAP P900 defined by 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, in which 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, in which serine 357 and/or serine 358 is optionally phosphorylated; and/or

(b) - 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, optionally in which 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 YLSALVSIRTDPSSR or VSIRTDPSSR, 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.

5. A method according to Claim 4, in which the method comprises determining whether the region of MAP P900 between amino acids 273 to 406 of SEQ ID NO: 1, or a fragment thereof, has been cleaved from membrane-bound MAP P900, and optionally whether the cleaved region of MAP P900, or the fragment thereof, has spread from microbial cells and/or host cells containing membrane bound MAP P900.

6. A method according to any preceding claim, in which 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 MVINDDAQRLL[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 YLSALVSIRTDPSSR or NLKRPRRYDRRLLRA or to the amino acid sequence YLSALVSIRTDPSSR in which one of more of the serine residues is phosphorylated.

7. A method according to any preceding claim, in which the sample is a sample of a body fluid, such as blood, semen, amniotic fluid, cerebrospinal fluid, synovial fluid or breast milk

or a tissue sample, such as skin, gastrointestinal tract, thyroid, lymph node, brain or genitourinary tract.

8. A method according to any preceding claim, in which 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.

9. A method according to any of Claims 1 to 7, in which the subject is an animal.

10. An antibody, or antigen-binding fragment thereof, which specifically binds to:

(i) the region of MAP P900 defined by amino acids 26 to 71 of SEQ ID NO: 1;

(ii) 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; or

(iii) the amino acid sequence YLSALVSIRTDPSSR or NLKRPRRYDRRLLRA 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.

11. An antibody, or antigen-binding fragment thereof, according to Claim 10, in which the antibody binds to the amino acid sequence:

(i) MVINDDAQRLLSQR, AAVTTLADGGEVTWAID or MVINDDAQRLL[pS]QR; or

(ii) MVINDDAQRLLSQR or YLSALVSIRTDPSSR, when one or more of the serine residues is phosphorylated.

12. An antibody according to Claim 10 or 11 which is:

(i) a monoclonal, chimeric, recombinant, synthetic or single domain antibody; and/or

(ii) a labelled antibody.

13. Use of an antibody, or antigen-binding fragment thereof, that binds to MAP P900, in which the antibody, or antigen-binding fragment thereof, binds the region of MAP P900 defined by amino acids 24 to 71 or 329 to 386 of SEQ ID NO: 1 in an *ex vivo* method of diagnosing or monitoring MAP infection.

14. Use of an antibody, or antigen-binding fragment thereof, that binds to MAP P900, in which the antibody, or antigen-binding fragment thereof, binds the region of MAP P900 defined by amino acids 24 to 71 or 329 to 386 of SEQ ID NO: 1 in an *ex vivo* method of monitoring treatment of MAP infection, or 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.

15. Use of an antibody, or antigen-binding fragment thereof, that binds to MAP P900, in which the antibody, or antigen-binding fragment thereof, binds the region of MAP P900 defined by amino acids 24 to 71 or 329 to 386 of SEQ ID NO: 1 to:

(i) detect MAP in a food product, such as milk or other dairy product or a meat product;

(ii) detect MAP in an environmental sample, such as surface water, river water, water-treatment plant water, domestic water, or an aerosol, soil or sediment; or

(iii) capture a peptide for Mass Spectrometry (MS) characterisation.

16. Use according to Claim 13, 14 or 15, in which the antibody, or an antigen-binding fragment thereof, binds the region of MAP P900 defined by:

- 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;
- amino acids 26 to 71 of SEQ ID NO: 1, in which serine 37 is phosphorylated;
- amino acids or 345 to 359 or 350 to 359 of SEQ ID NO: 1, in which serine 357 is phosphorylated and/or serine 358 is phosphorylated.

17. Use according to any of Claims 13 to 16, in which 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 MVINDDAQRLL[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 YLSALVSIRTDPSSR or NLKRPRRYDRRLLRA or to the amino acid sequence YLSALVSIRTDPSSR in which one of more of the serine residues is phosphorylated.

18. An antibody, or antigen-binding fragment thereof, that binds to MAP P900, in which the antibody, or antigen-binding fragment thereof, binds the region of MAP P900 defined by amino acids 24 to 71 or 329 to 386 of SEQ ID NO: 1 for use in therapeutic or diagnostic method carried out on the human or animal body.

19. Use of an antibody, or antigen-binding fragment thereof, that binds to MAP P900, in which the antibody, or antigen-binding fragment thereof, binds the region of MAP P900 defined by amino acids 24 to 71 or 329 to 386 of SEQ ID NO: 1 in the manufacture of a medicament for treating or preventing MAP infection.

20. An antibody, or the antigen-binding fragment thereof, for use according to Claim 18, or the use of the antibody, or the antigen-binding fragment thereof, according to Claim 19, in which the antibody, or an antigen-binding fragment thereof, binds the region of MAP P900 defined by:

- 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;
- amino acids 26 to 71 of SEQ ID NO: 1, in which serine 37 is phosphorylated;
- amino acids or 345 to 359 or 350 to 359 of SEQ ID NO: 1, in which serine 357 is phosphorylated and/or serine 358 is phosphorylated.

CLAIMS

1. A method of diagnosing or monitoring *Mycobacterium avium* subspecies *paratuberculosis* (MAP) infection, which method comprises detecting the presence of the polypeptide (MAP P900) encoded by the positive strand of IS*900*, or an extracellular fragment of MAP P900, in a sample from a subject, in which MAP P900, or an extracellular fragment of MAP P900, is detected using an antibody, or an antigen-binding fragment thereof, that binds to MAP P900, in which the antibody, or an antigen-binding fragment thereof, binds the region of MAP P900 defined by amino acids 24 to 71 or 329 to 386 of SEQ ID NO: 1.

2. A method according to Claim 1, in which the antibody, or an antigen-binding fragment thereof, binds the region of MAP P900 defined by:

- 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;
- amino acids 26 to 71 of SEQ ID NO: 1, in which serine 37 is phosphorylated;
- amino acids or 345 to 359 or 350 to 359 of SEQ ID NO: 1, in which serine 357 is phosphorylated and/or serine 358 is phosphorylated.

3. A method according to Claim 1 or 2, in which two or more of the antibodies, or antigen-binding fragments thereof, are used to detect the MAP P900 polypeptide, or the fragment thereof.

4. A method according to Claim 3, in which the method uses:

(a) - at least one antibody that binds to the region of MAP P900 defined by 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, in which 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, in which serine 357 and/or serine 358 is optionally phosphorylated; and/or

(b) - 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, optionally in which 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 YLSALVSIRTDPSSR or VSIRTDPSSR, 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.

5. A method according to Claim 4, in which the method comprises determining whether the region of MAP P900 between amino acids 273 to 406 of SEQ ID NO: 1, or a fragment thereof, has been cleaved from membrane-bound MAP P900, and optionally whether the cleaved region of MAP P900, or the fragment thereof, has spread from microbial cells and/or host cells containing membrane bound MAP P900.

6. A method according to any preceding claim, in which 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 MVINDDAQRLL[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 YLSALVSIRTDPSSR or NLKRPRRYDRRLLRA or to the amino acid sequence YLSALVSIRTDPSSR in which one of more of the serine residues is phosphorylated.

7. A method according to any preceding claim, in which the sample is a sample of a body fluid, such as blood, semen, amniotic fluid, cerebrospinal fluid, synovial fluid or breast milk

or a tissue sample, such as skin, gastrointestinal tract, thyroid, lymph node, brain or genitourinary tract.

8. A method according to any preceding claim, in which 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.

9. A method according to any of Claims 1 to 7, in which the subject is an animal.

10. An antibody, or antigen-binding fragment thereof, which specifically binds to the amino acid sequence YLSALVSIRTDPSSR, NLKRPRRYDRRLLRA, YLSALVSIRTDPS[pS]SR, MVINDDAQRLLSQR, AAVTTLADGGEVTWAID or MVINDDAQRLL[pS]QR.

11. An antibody according to Claim 10 which is a monoclonal, chimeric, recombinant, synthetic or single domain antibody.

12. An antibody according to Claim 10 or 11 which is a labelled antibody.

13. Use of an antibody, or antigen-binding fragment thereof, that binds to MAP P900, in which the antibody, or antigen-binding fragment thereof, binds the region of MAP P900 defined by amino acids 24 to 71 or 329 to 386 of SEQ ID NO: 1 in an *ex vivo* method of diagnosing or monitoring MAP infection.

14. Use of an antibody, or antigen-binding fragment thereof, that binds to MAP P900, in which the antibody, or antigen-binding fragment thereof, binds the region of MAP P900 defined by amino acids 24 to 71 or 329 to 386 of SEQ ID NO: 1 in an *ex vivo* method of monitoring treatment of MAP infection, or 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.

15. Use of an antibody, or antigen-binding fragment thereof, that binds to MAP P900, in which the antibody, or antigen-binding fragment thereof, binds the region of MAP P900 defined by amino acids 24 to 71 or 329 to 386 of SEQ ID NO: 1 to:

(i) detect MAP in a food product, such as milk or other dairy product or a meat product;

(ii) detect MAP in an environmental sample, such as surface water, river water, water-treatment plant water, domestic water, or an aerosol, soil or sediment; or

(iii) capture a peptide for Mass Spectrometry (MS) characterisation.

16. Use according to Claim 13, 14 or 15, in which the antibody, or an antigen-binding fragment thereof, binds the region of MAP P900 defined by:

- 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;
- amino acids 26 to 71 of SEQ ID NO: 1, in which serine 37 is phosphorylated;
- amino acids or 345 to 359 or 350 to 359 of SEQ ID NO: 1, in which serine 357 is phosphorylated and/or serine 358 is phosphorylated.

17. Use according to any of Claims 13 to 16, in which 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 MVINDDAQRLL[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 YLSALVSIRTDPSSR or NLKRPRRYDRRLLRA or to the amino acid sequence YLSALVSIRTDPSSR in which one of more of the serine residues is phosphorylated.

18. An antibody, or antigen-binding fragment thereof, that binds to MAP P900, in which the antibody, or antigen-binding fragment thereof, binds the region of MAP P900 defined by amino acids 24 to 71 or 329 to 386 of SEQ ID NO: 1 for use in therapeutic method carried out on the human or animal body.

19. Use of an antibody, or antigen-binding fragment thereof, that binds to MAP P900, in which the antibody, or antigen-binding fragment thereof, binds the region of MAP P900

defined by amino acids 24 to 71 or 329 to 386 of SEQ ID NO: 1 in the manufacture of a medicament for treating MAP infection.

20. An antibody, or the antigen-binding fragment thereof, for use according to Claim 18, or the use of the antibody, or the antigen-binding fragment thereof, according to Claim 19, in which the antibody, or an antigen-binding fragment thereof, binds the region of MAP P900 defined by:

- 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;
- amino acids 26 to 71 of SEQ ID NO: 1, in which serine 37 is phosphorylated;
- amino acids or 345 to 359 or 350 to 359 of SEQ ID NO: 1, in which serine 357 is phosphorylated and/or serine 358 is phosphorylated.