

CLAIMS:

1. A method for determining whether one or more specified microorganisms are present within a biological sample that potentially comprises the microorganisms comprising:

(a) immobilizing the biological sample on and/or within a solid substrate at a first location;

(b) transferring the immobilized biological sample to at least a second location and performing an extraction step on the solid substrate so as to extract any microorganism DNA immobilized on and/or within the solid substrate;

(c) performing a nucleic acid amplification step on microorganism DNA extracted in step (b), wherein the amplification step is directed towards amplification of at least one highly conserved sequence, from one or more specific microorganisms, and wherein amplified sequences are designated as target sequences;

(d) combining the target sequences with a plurality of primer sequences comprised within a diagnostic multiplexing panel (DMP), wherein each primer sequence facilitates genotyping of the target sequence;

(e) performing a primer extension reaction on the combination of target sequences and the DMP present in (d), thereby producing a DMP reaction product; and

(f) analysing the reaction product so as to determine genotype of any target sequences that are present and correlating the genotype of the target sequences in the reaction product with the identification of specified microorganisms present in the biological sample.

2. The method of claim 1, wherein the first location is remote from the second location.

3. The method of claims 1 and 2, wherein microorganisms are pathogenic organisms.

4. The method of any previous claim, wherein the biological sample comprises at least one of the group consisting of: urine; saliva; blood; sputum; semen; faeces; a nasal swab; tears; a vaginal swab; a rectal swab; a cervical smear; a tissue biopsy; and a urethral swab.

5. The method of any previous claim, wherein the biological sample is immobilized on and/or within a solid substrate at the first location, and an extraction step is performed on the solid substrate so as to extract any microorganism DNA immobilized on and/or within the solid substrate at the second location.

6. The method of claim 5, wherein the solid substrate comprises an absorbent fibrous material impregnated with one or more reagents that act to immobilize and inactivate any microorganisms present in the biological sample.

7. The method of claim 6, wherein the solid substrate comprises a material selected from a cellulose-based paper; a microfibrinous membrane; a glass-fibre material; a polymeric fibre material; a woven fabric; and a non-woven fabric.

8. The method of claims 6 and 7, wherein the solid substrate comprises Whatman FTA® or Whatman FTA® Elute reagent.

9. The method of claims 6 and 7, wherein the solid substrate includes Whatman FTA® Elute paper.

10. The method of any previous claim, wherein the specified microorganisms are selected from one or more of the group consisting of: bacteria; fungi; viruses; and protozoa.

5 11. The method of claim 10, wherein the specified microorganisms include one or more infectious pathogenic microorganisms.

12. The method of claim 11, wherein the one or more pathogenic microorganisms include human pathogens that are the causative agents in one or more of the diseases selected from the group consisting of: sexually transmitted infection; food poisoning; tuberculosis; virally induced cancer; encephalitis; malaria; hepatitis; meningitis; leishmaniasis; African trypanosomiasis; pneumonia; plague; SARS; MRSA; rabies; anthrax; Rift valley fever; tularemia; shigella; botulism; yellow fever; Q fever; ebola; dengue fever; West Nile fever; dysentery; influenza; measles; and typhus.

13. The method of any of claims 10 to 12, wherein the bacteria are selected from the group consisting of *Mycoplasma* spp.; *Chlamydia* spp.; *Ureaplasma* spp.; *Neisseria* spp.; *Gardnerella* spp.; *Trichomonas* spp.; and *Treponema* spp.

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14. The method of any of claims 10 to 13, wherein the yeast includes *Candida albicans*.

15. The method of any of claims 10 to 14, wherein the viruses are selected from the group consisting of: cytomegalovirus (CMV); hepatitis A virus (HAV); hepatitis B virus (HBV); hepatitis C virus (HCV), hepatitis E virus (HEV), hepatitis G and GB virus (GBV-C); human immunodeficiency viruses (HIV); human papilloma viruses (HPV); herpes simplex viruses (HSV); Molluscum contagiosum virus (MCV); influenza virus; Epstein-Barr virus (EBV) and varicella-zoster virus (VZV).

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16. The method of any previous claim, wherein the DMP is directed towards identification of alleles from a combination of microorganisms potentially present

in the biological sample, wherein the combination includes bacteria, viruses and fungi.

17. The method of claim 16, wherein the microorganisms are all pathogens.

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18. The method any of claims 16 or 17, wherein the DMP is directed towards identification of microorganisms that are associated with a particular type of disease.

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19. The method of claim 18, wherein the type of disease is selected from the group consisting of: sexually transmitted infection; food poisoning; tuberculosis; virally induced cancer; encephalitis; malaria; hepatitis; meningitis; pneumonia; plague; and influenza.

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20. The method of claim 19, wherein the disease is sexually transmitted infection and the DMP comprises primer sequences that hybridise with one or more target sequences obtained from microorganisms selected from the group consisting of: *Mycoplasma genitalum*; *Mycoplasma hominis*; *Chlamydia trachomatis*; *Ureaplasma urealyticum*; *Neisseria gonorrhoea*; *Gardnerella vaginalis*; *Trichomonas vaginalis*; *Treponema pallidum*; CMV; HAV; HBV; HCV; HEV, GBV-C, HIV-1; HIV-2; HPV; HSV-1; HSV-2; MCV; VZV; EBV; and *Candida albicans*.

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21. The method of any previous claim wherein the highly conserved polymorphic allele comprises all or a part of a microorganism gene selected from: a bacterial 16S rRNA; a bacterial 32S rRNA; a yeast 16S rRNA; a yeast 18S rRNA; and a viral polymerase.

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22. The method of any previous claim, wherein the highly conserved sequence comprises a polymorphic allele selected from the group consisting of: a single nucleotide polymorphism (SNP); an insertion; a deletion; an inversion; and a substitution.

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23. The method of any previous claim, wherein if one or more specified microorganisms is present in the biological sample the primer extension reaction produces a DMP reaction product comprising at least one extended primer sequence of a known predetermined molecular weight.

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24. The method of claim 23, wherein if two or more specified microorganisms are present in the biological sample the primer extension reaction produces a DMP reaction product comprising at least two extended primer sequences each of a known predetermined molecular weight that is different to the other.

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25. The method of any of claims 23 and 24, wherein DMP reaction product(s) are analysed using a technique that resolves extended primer sequences according to their molecular weight.

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26. The method of claim 25, wherein DMP reaction product(s) are analysed using a technique selected from: MALDI-TOF mass spectrometry; and/or capillary electrophoresis.

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27. The method of any of claims 1 to 22, wherein the primer extension reaction comprises a primer labelling reagent such that if one or more specified microorganisms is present in the biological sample the primer extension reaction incorporates the labelling reagent into the primer extension product, thereby producing a DMP reaction product comprising the labelling reagent.

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28. The method of claim 27, wherein the primer labelling reagent comprises a label selected from: a radiolabel; a fluorescent label; and an antigen.

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29. The method of claims 27 and 23, wherein DMP reaction product(s) are analysed using a technique that identifies presence of an incorporated labelling reagent in the primer extension product.

30. The method of claim 29, wherein the analysis technique is selected from SNPstream® and/or SNPlex®.

31. The method of any previous claim, wherein the plurality of primer sequences comprised within the DMP are immobilized on a solid support.

5 32. The method of claim 31, wherein the solid support is selected from one of: glass; and silicon.

33. The method of any previous claim, wherein the nucleic amplification step comprises a plurality of amplification primers that are directed towards
10 amplification of a plurality of highly conserved sequences from one or more specified microorganisms.

34. The method of claim 33, wherein the plurality of amplification primers comprise one or more primer pairs selected from the group consisting of SEQ
15 ID NOS: 1/ 2; 3/ 4; 5/6; 7/8; 9/10; 11/12; 13/ 14; 15/ 16; 17/18; 19/20; 21/22; 23/24; 25/26; 27/28; and 29/30.

35. The method of claim 33, wherein the plurality of amplification primers comprise one or more primer pairs selected from the group consisting of SEQ
20 ID NOS: 46/47; 48/49; 50/51; 52/53; 54/55; 56/57; 58/59; 60/61; 62/63; 64/65; 66/67; 68/69; 70/71; 72/73; and 74/75.

36. The method of any previous claim, wherein the DMP comprises one or more primer sequences selected from SEQ ID NOS: 31-45.
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37. The method of any previous claim, wherein the DMP comprises one or more primer sequences selected from SEQ ID NOS: 76-90.

38. The method of any previous claims, wherein one or more control
30 competitor sequences are combined with the target sequences prior to the nucleic acid amplification step of part (c), wherein each competitor sequence is identical to a corresponding target sequence except that the competitor comprises a sequence variation at a specified position compared to the corresponding target sequence.

39. The method of claim 38, wherein the sequence variation comprises an artificially introduced SNP.

5 40. The method of any previous claims, wherein one or more control sequences are combined with the target sequences prior to the nucleic acid amplification step of part (c), and wherein the one or more control sequences comprise a sequence of DNA selected from: a species unrelated to that of the biological sample; a species unrelated to the microorganism(s) being tested for;
10 and a synthetic DNA sequence, and wherein the nucleic acid amplification step and the DMP comprise corresponding primer sequences that specifically hybridise with each of the one or more control sequences.

15 41. The method of any previous claim, wherein the biological sample is obtained from a human.

42. The method of any of claims 1 to 40, wherein the biological sample is obtained from one of the group consisting of: a non-human animal; a plant; and a foodstuff.

20 43. A diagnostic multiplexing panel (DMP), suitable for use in genotyping pathogenic microorganisms known to cause at least one infectious disease that may be present within a biological sample, the DMP comprising a plurality of primer sequences directed at identification of at least two or more highly
25 conserved sequences of at least one microorganism known to cause an infectious disease, when used in a primer extension reaction.

44. The DMP of claim 43, wherein the infectious disease is selected from one or more of the group consisting of: sexually transmitted infection; food
30 poisoning; tuberculosis; virally induced cancer; encephalitis; malaria; hepatitis; meningitis; pneumonia; plague; and influenza.

45. The DMP of claims 43 and 44, wherein the DMP comprises primer sequences that that are suitable for genotyping microorganisms selected from

the group consisting of: *Mycoplasma genitalum*; *Mycoplasma hominis*; *Chlamydia trachomatis*; *Ureaplasma urealyticum*; *Neisseria gonorrhoea*; *Gardnerella vaginalis*; *Trichomonas vaginalis*; *Treponema pallidum*; CMV; HAV; HBV; HCV; HEV, GBV-C, HIV-1; HIV-2; HPV; HSV-1; HSV-2; MCV; VZV; EBV; and *Candida albicans*.

46. The DMP of any of claims 43 to 45 wherein the highly conserved sequence comprises all or a part of a microorganism gene selected from: a bacterial 16S rRNA; a bacterial 32S rRNA; a yeast 16S rRNA; a yeast 18S rRNA; and a viral polymerase.

47. The DMP of any of claims 43 to 46, wherein the highly conserved sequence comprises a polymorphic allele selected from the group consisting of: a single nucleotide polymorphism (SNP); an insertion; a deletion; an inversion; and a substitution.

48. The DMP of any of claims 43 to 47, wherein the plurality of primer sequences are designed such that the primer extension reaction produces a DMP reaction product comprising at least one extended primer sequence of a known predetermined relative molecular weight.

49. The DMP of any of claims 43 to 48, wherein the plurality of primer sequences are designed such that if two or more microorganisms are present in the biological sample the primer extension reaction produces a DMP reaction product comprising at least two extended primer sequences each of a known predetermined relative molecular weight that is different to that of the other(s).

50. The DMP of any of claims 48 and 49, wherein DMP reaction product(s) are analysed using a technique that resolves extended primer sequences according to their relative molecular weight.

51. The DMP of any of claims 48 to 50, wherein each primer sequence comprised within the DMP is of a different relative molecular mass.

52. The DMP of any of claims 48 to 51, wherein each primer sequence comprised within the DMP is of a different length relative to other primer sequences in the DMP.

5 53. The DMP of any of claims 48 to 52, wherein DMP reaction product(s) are analysed using a technique selected from one of: MALDI-TOF mass spectrometry; and/or capillary electrophoresis.

10 54. The DMP of any of claims 43 to 53, wherein the DMP comprises one or more primer sequences selected from SEQ ID NOS: 31-45.

55. The DMP of any of claims 43 to 53, wherein the DMP comprises one or more primer sequences selected from SEQ ID NOS: 76-90.

15 56. The DMP of any of claims 43 to 55, wherein the plurality of primer sequences comprised within the DMP are immobilized on a solid support.

57. The DMP of claim 56, wherein the solid support is selected from one of: glass; and silicon.

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58. A microorganism testing kit suitable for personal use by a user located in a first location, the kit comprising a testing surface located within a sealable chamber, the testing surface further comprising a solid substrate that is capable of immobilizing a biological sample either within it or upon its surface, and
25 wherein once a biological sample is deposited upon the testing surface, the chamber is sealed around the testing surface such that the testing kit can be despatched to a second location for analysis to determine whether one or more microorganisms are present in the biological sample.

30 59. The testing kit of claim 58, wherein at the second location the testing kit is dissembled and an extraction step is performed on the solid substrate so as to extract any microorganism DNA immobilized upon and/or within the solid substrate.

60. The testing kit of claims 58 and 59, wherein the solid substrate comprises an absorbent fibrous material impregnated with one or more reagents that act to immobilize and inactivate any microorganisms present in the biological sample.

61. The testing kit of claim 60, wherein the solid substrate comprises a material selected from a cellulose-based paper; a microfibrinous membrane; a glass-fibre material; a polymeric fibre material; a woven fabric; and a non-woven fabric.

62. The testing kit of claims 60 and 61, wherein the solid substrate comprises Whatman FTA[®] or Whatman FTA[®] elute reagent.

63. The testing kit of claims 60 and 61, wherein the solid substrate includes Whatman FTA[®] Elute paper.

64. The testing kit of any of claims 58 to 63, wherein the biological sample comprises at least one of the group consisting of: urine; saliva; blood; sputum; semen; faeces; a nasal swab; tears; a vaginal swab; a rectal swab; a cervical smear; a tissue biopsy; and a urethral swab.

65. The testing kit of any of claims 58 to 64, wherein the one or more microorganisms are pathogenic and are a causative agent of a disease selected from the group consisting of: sexually transmitted infection; food poisoning; tuberculosis; virally induced cancer; encephalitis; malaria; hepatitis; meningitis; pneumonia; plague; and influenza.

66. The testing kit of any of claims 58 to 65, wherein the second location is remote from the first location.

67. The testing kit of any of claims 58 to 66, wherein the means of despatch is via a regular postal service.

68. A method of treating an animal that is suspected of carrying one or more infectious microorganisms, comprising obtaining a biological sample from the animal, testing the biological sample according to the method of any of claims 1 to 42, thereby diagnosing whether the animal is infected with one or more infectious microorganisms, and administering treatment to the animal, which treatment is configured appropriately in light of the information regarding the type(s) of infectious microorganisms found to be present in the biological sample.

69. The method of claim 68, wherein the animal is a human.

70. The method of claims 68 or 69, wherein the treatment is further configured appropriately according to information regarding the anti-biotic resistance status of one or more of the infectious microorganisms found to be present in the biological sample.

Claims

1. A method for determining whether two or more species of specified microorganisms are present within a biological sample that potentially comprises the microorganisms comprising:

(a) immobilizing the biological sample on and/or within a solid substrate at a first location;

(b) transferring the immobilized biological sample to at least a second location and performing an extraction step on the solid substrate so as to extract any microorganism DNA immobilized on and/or within the solid substrate;

(c) performing a nucleic acid amplification step on microorganism DNA extracted in step (b), wherein the amplification step comprises a plurality of amplification primers that are directed towards amplification of a plurality of highly conserved sequences from the two or more specific microorganisms, and wherein amplified sequences are designated as target sequences;

(d) combining the target sequences with a plurality of primer sequences comprised within a diagnostic multiplexing panel (DMP), wherein each primer sequence facilitates genotyping of the target sequence;

(e) performing a primer extension reaction on the combination of target sequences and the DMP present in (d), thereby producing a DMP reaction product; and

(f) analysing the reaction product so as to determine genotype of any target sequences that are present and correlating the genotype of the target sequences in the reaction product with the identification of specified microorganisms present in the biological sample;

wherein the specified microorganisms are selected from one or more of the group consisting of: *Mycoplasma* spp., *Chlamydia* spp., *Ureaplasma* spp., *Neisseria* spp., *Gardnerella* spp., *Trichomonas* spp., and *Treponema* spp., and *Candida albicans*;

and wherein the DMP comprises one or more primer sequences selected from SEQ ID NOs: 31-45, and/or one or more primer sequences selected from SEQ ID NOs: 76-90. more primer sequences selected from SEQ ID NOs: 76-90.

2. The method of claim 1, wherein the first location is remote from the second location.
3. The method of claim 1 or claim, 2, wherein the biological sample comprises at least one of the group consisting of: urine; saliva; blood; sputum; semen; faeces; a nasal swab; tears; a vaginal swab; a rectal swab; a cervical smear; a tissue biopsy; and a urethral swab.
4. The method of any previous claim, wherein the solid substrate comprises an absorbent fibrous material impregnated with one or more reagents that act to immobilize and inactivate any microorganisms present in the biological sample.
5. The method of claim, 4, wherein the solid substrate comprises a material selected from a cellulose-based paper; a microfibrinous membrane; a glass-fibre material; a polymeric fibre material; a woven fabric; and a non-woven fabric; and/or wherein the solid substrate comprises Whatman FTA® or Whatman FTA® Elute reagent or Whatman FTA® Elute paper.
6. The method of any previous claim, wherein the DMP is directed towards identification of alleles from a combination of microorganisms potentially present in the biological sample, wherein the combination includes bacteria, fungi and viruses.
7. The method of claim, 6, wherein the viruses are selected from the group consisting of: cytomegalovirus (CMV); hepatitis A virus (HAV); hepatitis B virus (HBV); hepatitis C virus (HCV), hepatitis E virus (HEV), hepatitis G and GB virus (GBV-C); human immunodeficiency viruses (HIV); human papilloma viruses (HPV); herpes simplex viruses (HSV); Molluscum contagiosum virus (MCV); influenza virus; Epstein-Barr virus (EBV) and varicella-zoster virus (VZV).
8. The method any of any previous claim, wherein the microorganisms are all pathogens and the DMP is directed towards identification of microorganisms that are associated with sexually transmitted infection.

9. The method of claim 8, wherein the DMP comprises primer sequences that hybridise with one or more target sequences obtained from microorganisms selected from the group consisting of: *Mycoplasma genitalum*; *Mycoplasma hominis*; *Chlamydia trachomatis*; *Ureaplasma urealyticum*; *Neisseria gonorrhoea*; *Gardnerella vaginalis*; *Trichomonas vaginalis*; *Treponema pallidum*; and *Candida albicans*.
10. The method of any previous claim wherein the highly conserved polymorphic allele comprises all or a part of a microorganism gene selected from: a bacterial 16S rRNA; a bacterial 32S rRNA; a yeast 16S rRNA; a yeast 18S rRNA; and a viral polymerase.
11. The method of any previous claim, wherein if two or more specified microorganisms are present in the biological sample the primer extension reaction produces a DMP reaction product comprising at least two extended primer sequences each of a known predetermined molecular weight that is different to the other.
12. The method of claim 11, wherein DMP reaction product(s) are analysed using a technique that resolves extended primer sequences according to their molecular weight.
13. The method of any of claims 1 to 10, wherein the primer extension reaction comprises a primer labelling reagent such that if a specified microorganisms is present in the biological sample the primer extension reaction incorporates the labelling reagent into the primer extension product, thereby producing a DMP reaction product comprising the labelling reagent, and wherein DMP reaction product(s) are analysed using a technique that identifies presence of an incorporated labelling reagent in the primer extension product.
14. The method of any previous claim, wherein the plurality of primer sequences comprised within the DMP are immobilized on a solid support.
15. The method of any previous claim, wherein the plurality of amplification primers comprise one or more primer pairs selected from the group consisting of SEQ ID NOs: 1/2, 3/4, 5/6, 7/8, 9/10, 11/12, 13/14, 15/16, 17/18, 19/20, 21/22, 23/24, 25/26, 27/28 and 29/30; and/or one or more primer pairs selected from the group consisting of SEQ ID NOs: 46/47; 48/49; 50/51; 52/53; 54/55; 56/57; 58/59; 60/61; 62/63; 64/65; 66/67; 68/69; 70/71; 72/73; and 74/75.