CLAIMS

What is Claimed is:

1. A method of detecting a secondary infection in a subject with a first infection, comprising

   (a) preparing a plasma sample from blood obtained from the subject with the first infection, wherein the plasma sample comprises microbial cell-free nucleic acids (mcfNA) from at least two different microbes;

   (b) producing a sequencing library comprising mcfNA attached to adapters;

   (c) measuring an amount of total mcfNA in the plasma sample by performing next generation sequencing on the sequencing library comprising the mcfNA attached to adapters, wherein the total mcfNA comprises mcfNA from at least two different microbes;

   (d) comparing the amount of total mcfNA comprising mcfNA from at least two different microbes to a threshold amount of total mcfNA; and

   (e) detecting a secondary infection that is different from the first infection when the amount of total mcfNA comprising mcfNA from at least two different microbes exceeds the threshold amount of total mcfNA.

2. ~~A method of~~Use of a therapeutic drug in the manufacture of a medicament for treating a secondary infection in a subject with a first infection, wherein ~~comprising~~

   (a) the secondary infection is detected in ~~collecting~~ a blood sample collected from the subject with the first infection~~;~~

   ~~(b) detecting a secondary infection~~ when an amount of total microbial cell-free nucleic acids (mcfNA) comprising mcfNA from at least two microbes in the blood sample exceeds a threshold amount of total mcfNA, wherein the amount of total mcfNA is calculated by next generation sequencing; and

   ~~(c)~~(b) ~~administering a~~ the therapeutic drug is to be administered to the subject with the first infection in order to treat the secondary infection.

3. The use~~method~~ of claim 2 wherein detection of the secondary infection in a blood sample collected from the subject and administration of the therapeutic drug is to be repeated ~~further comprising:~~

   ~~(d) repeating (a), (b), and (c)~~ until the amount of total mcfNA in the blood decreases to a value at or below the threshold amount of total mcfNA.

4. The method of claim 1 or the use ~~any one~~ of claim~~s~~ 2~~1~~-3, wherein the first infection is a COVID-19 infection.

5. The method of claim 1 or the use ~~any one~~ of claim~~s~~ 2~~1~~-4, wherein the first infection is a viral lung infection.

~~6. The method of any one of claims 1-5, wherein the first infection is COVID-19 pneumonia.~~

~~7.~~6. The method of claim 1 or the use ~~any one~~ of claim~~s~~ 2~~1-6~~, wherein the secondary infection is a bacterial or fungal infection.

~~8.~~7. The method of claim 1 or the use ~~any one~~ of ~~the preceding~~ claim~~s~~ 2, ~~further comprising determining~~ wherein a presence of at least one bacterium, fungus, or parasite is to be further determined in the subject.

~~9.~~8. The method of claim 1 or the use ~~any one~~ of ~~the preceding~~ claim~~s~~ 2, wherein the first and secondary infections are respiratory infections caused by different microbes.

~~10.~~9. The method of claim 1 or the use ~~any one~~ of ~~the preceding~~ claim~~s~~ 2, wherein the first and second infections are pneumonia caused by different microbes.

~~11.~~10. The method of claim 1 or the use ~~any one~~ of claim~~s~~ ~~1-10~~2, wherein the at least two microbes comprise at least two respiratory pathogens.

~~12.~~11. The method or use of ~~any one of claims 1-11~~claim 10, wherein the at least two microbes are at least two microbes from the group consisting of S. *aureus*, *P. aeruginosa* and *K Pneumoniae*.

~~13. The method of any one of claims 1-12, wherein the first infection is culture-positive pneumonia.~~

~~14. The method of any one of claims 1-12, wherein the first infection is culture-negative pneumonia.~~

~~15. The method of any one of claims 1-14, wherein the at least two microbes comprise *Candida*.~~

~~16.~~12. The method of claim 1 or the use ~~any one~~ of claim~~s~~ 1-~~15~~2, wherein the amount of total mcfNA is an aggregated amount of each type of mcfNA in the sample.

~~17. The method of any one of claims 1-15, wherein the amount of total mcfNA is an aggregated amount of total bacterial mcfNA in the sample.~~

~~18. The method of any one of claims 1-15, wherein the amount of total mcfNA is an aggregated amount of total mcfNA from respiratory pathogens in the sample.~~

~~19.~~13. The method of claim 1 or the use ~~any one~~ of claim~~s~~ ~~1-18~~2, wherein the threshold amount of total mcfNA is an amount of mcfNA measured in plasma of a healthy or un-infected subject.

~~20. The method of any one of claims 1-19, wherein the amount of total mcfNA is measured by metagenomic next generation sequencing.~~

~~21. .~~14. The method of claim 1 or the use ~~any one~~ of claim~~s~~ 2~~1-20~~, wherein the mcfNA is mcfDNA.

~~22. The method of any one of claims 1-21, wherein the plasma or blood sample is spiked with a known concentration of synthetic normalization controls.~~

~~23. The method of any one of claim 1-22, wherein the mcfNA is extracted from the plasma of the subject.~~

~~24. The method of claim 23, wherein a DNA sequencing library is constructed from the extracted mcfNA, and sequence reads are produced from the sequencing library.~~

~~25.~~15. The method of claim 1 or the use ~~any one~~ of claim~~s 1-24~~2, wherein the amount of mcfNA in the sample is measured by~~measuring the amount of mcfNA in the sample comprises~~:

(a) aligning the sequence reads with a microorganism database, wherein the microorganism library comprises more than 10,000 genomic reference sequences;

(b) retaining reliable reads comprising alignments with high percent identity and high query coverage;

(c) assigning relative abundances to each taxon based on the number of reliable reads and their alignments;

(d) computing statistical significance values for each estimate of taxon abundance; and

(e) using taxon abundance to determine mcfNA concentration; and/or

(f) using abundance of spiked synthetic normalization controls to calculate the molecules per microliter **(MPM)** value of mcfNA in the sample.

~~26.~~16. The method or use of ~~any one of claims 1-~~claim ~~25~~15, ~~further comprising measuring~~ wherein levels of biomarkers of innate immunity or epithelial or endothelial injury are to be further measured in the plasma sample of the subject.

~~27. The method of claim 26, wherein the biomarkers are selected from the group consisting of IL-6, IL-8, IL-10, RAGE, TNFRl, angiopoietin-2, procalcitonin, fractalkine, pentraxin-3, and ST2.~~

~~28. The method of claim 27, wherein the biomarkers comprise IL-8 or ST2.~~

~~29. The method of claim 27, wherein the biomarkers comprise procalcitonin or pentraxin-3.~~

~~30.~~17. The method of claim 1 or the use~~any one~~ of claim~~s 26-29~~2, ~~further comprising comparing~~wherein the amount of mcfNA in the patient is to be further compared with the biomarker levels using an algorithm to yield a test score.

~~31.~~18. The use~~method~~ of claim ~~30~~17, ~~further comprising administering~~ wherein a therapeutic drug is to be further administered to the patient based on the test score, wherein the therapeutic drug is optionally an antimicrobial drug, an antibiotic drug, or an antifungal drug.

~~32. The method of any one of claims 1-31, wherein the amount is measured in molecules per microliter of plasma **(MPM).**~~

~~33. The method of any one of claims 1-32, wherein the threshold amount of total mcfNA is greater than 400~~

MPM for all types ofmcfNA in the sample.

34. The method of claim 33, wherein the threshold amount of total mcfNA is greater than 600 **MPM** for total mcfNA in the sample when the total mcfNA is determined by aligning sequence reads to a genomic database comprising sequences from at least 100 different microbes.

35. The method of any one of claims 1-32, wherein the threshold amount of total mcfNA is greater than 4000 **MPM** for mcfNA from respiratory pathogens in the sample.

36. The method of any one of claims 1-32, wherein the threshold amount of total mcfNA is greater than 4000 **MPM** when the total mcfNA is determined by aligning sequence reads to a genomic database comprising sequences from at least 100 different microbes.

37. The method of any one of claims 1-35, wherein the subject in (a) has received an empiric antibiotic.

38.19. A The method of detecting an inflammatory response in a patientclaim 1, further comprising

(a) preparing a plasma sample from blood obtained from the patient, wherein the plasma sample comprises microbial cell-free nucleic acids (mcfNA);

(b) producing a sequencing library comprising mcfNA attached to adapters;

(c) measuring an amount of total mcfNA in the plasma sample, wherein the total mcfNA comprises mcfNA from at least two different microbes;

(d) comparing the amount of the total mcfNA to a threshold amount ofmcfNA; and

(e) (a) detecting an inflammatory response when the amount of total mcfNA exceeds the threshold amount of total mcfNA.

39.20. A The usemethod of treating an inflammatory response in a patient of claim 2, comprisingwherein

(a) collecting a blood sample from the patient;

(b) (a) detecting an inflammatory response is detected in the patient when an amount of total mcfNA in the blood sample comprises mcfNA from at least two different microbes and exceeds a threshold amount of total mcfNA; and

(c)(b) administering an anti-inflammatory drug is to be administered to the patient to treat the inflammatory response.

40. The method of claims 38 or 39, wherein the subject has pneumonia.

41. The method of 40, wherein the pneumonia is culture-positive pneumonia.

42. The method of claim 40, wherein the pneumonia is culture-negative pneumonia.

43. The method of any one of claims 38-42, wherein the mcfNA is mcfDNA.

44. The method of any one of claims 38-43, wherein threshold amount of mcfNA is greater than 100,000 molecules per microliter of plasma **(MPM).**

45. The method of any one of claims 38-44, wherein threshold amount of mcfNA is greater than 100,000 molecules per microliter of plasma **(MPM)** for mcfNA from known respiratory pathogens.

46. The method of any one of claims 38-45, further comprising measuring levels of biomarkers of innate immunity or epithelial or endothelial injury in the plasma sample of the patient.

47. The method of claim 46, wherein the biomarkers are selected from the group consisting of IL-6, IL-8, IL-10, RAGE, TNFRl, angiopoietin-2, procalcitonin, fractalkine, pentraxin-3, and ST2.

48. The method of claim 46, wherein the biomarker is IL-8 or ST2.

49. The method of claim 46, wherein the biomarker is procalcitonin or pentraxin-3.

50. The method of any one of claims 38-49, further comprising comparing the amount ofmcfNA in the subject with the biomarker levels using an algorithm to yield a test score.

51. The method of claim 50 further comprising administering a therapeutic drug to the subject based on the test score.

52. A method of detecting a bacterial infection in a patient with a COVID-19 infection, comprising

(a) preparing a plasma sample from blood obtained from the patient with the COVID-19 infection, wherein the plasma sample comprises microbial cell-free nucleic acids (mcfNA);

(b) producing a sequencing library comprising the mcfNA attached to the adapters;

(c) conducting next generation sequencing on the sequencing library to produce sequence reads corresponding to the mcfNA;

(d) aligning the sequence reads to sequences from a database comprising at least 1000 bacterial reference sequences;

(e) determining an amount ofmcfNA from at least one bacterium based on the aligning o the sequence reads; and

(f) identifying a bacterial infection in the patient based on the amount of mcNA from the at least one bacterium.

53. A method of diagnosing and treating a bacterial infection in a patient with a COVID-19 infection, compnsmg

(a) collecting a blood sample from the patient with the COVID-19 infection;

(b) detecting the bacterial infection when an amount of bacterial mcfNA in the blood sample exceeds a threshold amount of mcfNA; and

(c) administering a therapeutic drug to the patient to treat the bacterial infection.

54. The method of any one of claims 52-53, wherein the patient has COVID-19 pneumonia.

55. The method of any one of claims 52-54, wherein the bacterial infection is a respiratory infection.

56. The method of any one of claims 52-55, wherein the mcfNA is bacterial mcfNA from S. *aureus, P.. aeruginosa* or *K Pneumoniae.*

57. The method of any one of claims 52-56, wherein the patient has culture-positive pneumonia.

58. The method of any one of claims 52-56, wherein the patient has culture-negative pneumonia.

59. The method of any one of claims 52-58, wherein the threshold amount ofmcfNA is the amount of mcfNA measured in plasma of a healthy or uninfected subject.

60. The method of any one of claims 52-59, wherein the amount ofmcfNA is measured by metagenomic next generation sequencing.

61. The method of any one of claims 52-60, wherein the mcfNA is mcfDNA.

62. The method of any one of claims 52-61, wherein the plasma is spiked with a known concentration of synthetic normalization controls.

63. A nucleic acid sequencing system for detecting secondary infection in a subject with a first infection compnsmg:

(a) a next-generation sequencing device comprising a flow cell and a computer processor that outputs data comprising sequence reads collected from measurements conducted in the flow cell; and

(b) a computing device that comprises quantitation of total microbial cell-free nucleic acids (mcfNA) logic that (i) detects mcfNA from at least two different microbes by aligning the sequence reads to microbial reference sequence reads;

(ii) calculates total mcfNA as a function of molecules per microliter of plasma, wherein the total mcfNA is an aggregate value of mcfNA from the at least two different microbes; and

(iii) comprises an event generator to generate an event indicative a secondary infection when the total mcfNA exceeds a threshold value.

64. The nucleic acid sequencing system of claim 63, wherein the quantitation of total microbial cell-free nucleic acids (mcfNA) logic comprises logic that excludes sequence reads from the analysis if they align to human reference sequences.

65. The nucleic acid sequencing system of claim 63 or 64, wherein the quantitation of total microbial cell-free nucleic acids (mcfNA) logic comprises logic that excludes sequence reads from the analysis if they align to a synthetic nucleic acid refence.

66. The nucleic acid sequencing system of any one of claims 63-65, wherein the mcfNA is

microbial cell-free DNA.

67. The nucleic acid sequencing system of any one of claims 63-66, wherein the threshold value is at least 600MPM.

68. The nucleic acid sequencing system of any one of claims 63-67, wherein the threshold value is at least 4000 **MPM.**

69. A method of detecting a localized respiratory infection in a subject comprising:

(a) obtaining or providing a plasma sample from the subject, wherein the subject is not bacteremic

(b) performing next generation sequencing or metagenomic sequencing on cell-free nucleic acids from the plasma sample and producing sequence reads; and

(c) aligning the sequence reads with sequences of respiratory pathogens in order to detect the presence and quantity of at least one respiratory pathogen, wherein the at least one respiratory pathogen is associated with the localized respiratory infection.

70. The method of claim 69, wherein the cell-free nucleic acids are cell-free DNA.

71. The method of claim 69 or 70, wherein the sequence reads aligned with the sequences of respiratory pathogens correspond to microbial cell-free DNA.

72. The method of any one of claims 69-71, wherein the respiratory infection is pneumonia.

73. The method of claim 72, wherein the respiratory infection is bacterial pneumonia.

74. The method of any one of claims 69-73, wherein the at least one respiratory pathogen is at least one bacterium associated with a respiratory infection.

75. The method of claim 74, wherein the respiratory infection is a bacterial respiratory infection.

76. The method of any one of claims 69-71, wherein the at least one respiratory pathogen is *S. aureus*, *P.. aeruginosa* or *K Pneumoniae*.

77. The method of any one of claims 69-76, further comprising adding synthetic nucleic acids to the plasma sample.

78. The method of claim 77, further comprising performing next generation sequencing on the synthetic nucleic acids.

79. The method of any one of claims 69-78, further comprising attaching adapters to the cell-free nucleic acids in order to produce cell-free nucleic acids attached to the adapters.

80. The method of claim 79, wherein the adapters are ligated to the cell-free nucleic acids.

81. The method of 79, wherein the adapters are attached to the cell-free nucleic acids by a primer extension reaction.

82. The method of claim 79, wherein the adapters comprise a sequence unique to the subject.

83. The method of claim 82, further comprising combining the cell-free nucleic acids attached

to the adapters with cell-free nucleic acids obtained from a different subject.

84. The method of claim 83, wherein the cell-free nucleic acids obtained from a different subject are attached to adapters that comprise a sequence unique to the different subject.

85. A method of detecting secondary infection in a subject exhibiting pneumonia, said method comprising

(a) obtaining a plasma sample from said subject, (b) evaluating the amount of microbial cell-free nucleic acids in said sample; (c) comparing said amount of microbial cell free nucleic acids to a threshold level; and (d) detecting a secondary infection if said amount of microbial cell-free nucleic acids exceeds said threshold level.

86. The method of 85, wherein said subject has COVID-19.

87. The method of claim 85, wherein said secondary infection is bacterial or fungal.

88. The method of claim 85, further comprising determining the presence and quantity of at least one

89. A method of identifying a secondary infection at a site oflocalization in a subject with a viral infection, comprising a) obtaining a plasma sample from said subject, (b) evaluating the amount of microbial cell-free nucleic acids in said sample; (c) comparing said amount of microbial cell free nucleic acids to a threshold level; and (d) detecting an infection at a site oflocalization in said subject if said amount of microbial cell free nucleic acids exceeds said threshold level.

90. The method of claim 89, wherein said site oflocalization is the lungs.

91. A non-invasive method of detecting a respiratory infection in a subject exhibiting a pneumonia, said method comprising a) obtaining a plasma sample from said subject, (b) evaluating the amount of microbial cell-free nucleic acids in said sample; (c) comparing said amount of microbial cell-free nucleic acids to a threshold level; and (d) detecting a respiratory infection if said amount of microbial cell free nucleic acids exceeds said threshold level.

92. The method of claim 91, wherein said subject has Covid-19 and is at risk for pneumonia.

93. A method for treating a patient suspected of having a secondary infection, the method comprising: determining whether the patient will benefit from anti-microbial therapy by:

determining in a sample from the patient a microbial cell-free nucleic acid level value (amount) and determining in a sample from the patient the level of a set of biomarkers, wherein the set of biomarker genes comprising biomarkers of innate immunity (e.g., IL-8 and ST2) and/or bacterial infections (e.g., procalcitonin and pentraxin-3); comparing the expression level values determined in step (1) with the biomarker levels of step (2) to yield a test score.

~~94.~~ ~~The method of claim 93, further comprising administering a treatment regimen comprising an anti- microbial therapy to the patient based on the test score.~~