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. Use of a therapeutic drug in the manufacture of a medicament for treating a secondary infection in a subject with a first infection, wherein

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

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

3. The use 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 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 of claim 2, wherein the first infection is a COVID-19 infection.

5. The method of claim 1 or the use of claim 2, wherein the first infection is a viral lung infection.

6. The method of claim 1 or the use of claim 2, wherein the secondary infection is a bacterial or fungal infection.

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

8. The method of claim 1 or the use of claim 2, wherein the first and secondary infections are respiratory infections caused by different microbes.

9. The method of claim 1 or the use of claim 2, wherein the first and second infections are pneumonia caused by different microbes.

10. The method of claim 1 or the use of claim 2, wherein the at least two microbes comprise at least two respiratory pathogens.

11. The method or use of 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.*

12. The method of claim 1 or the use of claim 2, wherein the amount of total mcfNA is an aggregated amount of each type of mcfNA in the sample.

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

14. The method of claim 1 or the use of claim 2, wherein the mcfNA is mcfDNA.

15. The method of claim 1 or the use of claim 2, wherein the amount of mcfNA in the sample is measured by:

(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.

16. The method or use of claim 15, wherein levels of biomarkers of innate immunity or epithelial or endothelial injury are to be further measured in the plasma sample of the subject.

17. The method of claim 1 or the use of claim 2, 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.

18. The use of claim 17, 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.

19. The method of claim 1, further comprising

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

20. The use of claim 2, wherein

(a) 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

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