

CLAIMS

WHAT IS CLAIMED IS:

- ~~1. A container for detecting an immune response to a pathogen by a subject, wherein said container comprises an antigen of said pathogen and an anticoagulation agent, and wherein said antigen comprises a specific T cell recognized epitope of said pathogen.~~
- ~~2. The container of claim 1, wherein said antigen is lyophilized.~~
- ~~3. The container of claim 1, wherein said antigen is in a solution.~~
- ~~4. The container of any one of the preceding claims, further comprising a sample obtained from said subject.~~
- ~~5. The container of claim 4, wherein said sample comprises PBMCs, CD8 T cells, NK cells, CD4 T cells, B cells, or any combination thereof.~~
- ~~6. The container of claim 4 wherein said sample comprises a body fluid.~~
- ~~7. The container of claim 6, wherein said sample comprises saliva.~~
- ~~8. The container of claim 6, wherein said sample comprises whole blood.~~
- ~~9. The container of any one of the preceding claims, wherein said subject is a human.~~
- ~~10. The container of claim 9 for use in detecting an immune response to said pathogen for a subject that exhibits symptoms of a condition associated with exposure to said pathogen.~~
- ~~11. The container of claim 9 for use in detecting an immune response to said pathogen for a subject that exhibits symptoms of a condition associated with exposure to said pathogen.~~
- ~~12. The container of claim 9 for use in detecting an immune response to said pathogen for a subject suspected to have been exposed to said pathogen.~~
- ~~13. The container of any one of the preceding claims for use in incubating whole blood for at least 30 minutes.~~
- ~~14. The container of any one of the preceding claims for use in incubating whole blood for at least 6 hours.~~
- ~~15. The container of any one of the preceding claims for use in incubating whole blood for at least 24 hours.~~
- ~~16. The container of any one of the preceding claims, wherein said antigen is stable for at least 30 days.~~

- ~~17. The container of claim 16, wherein said antigen is stable for at least 6 months.~~
- ~~18. The container of claim 17, wherein said antigen is stable for at least a year.~~
- ~~19. The container of any one of the preceding claims, wherein said anticoagulation agent is heparin, citrate, unfractionated heparin, bivalirudin, or hirudin.~~
- ~~20. The container of claim 19, wherein said anticoagulation agent is lithium heparin.~~
- ~~21. The container of any one of the preceding claims, wherein said pathogen is a mammalian pathogen.~~
- ~~22. The container of claim 21 wherein said pathogen is a human pathogen.~~
- ~~23. The container of claim 21 wherein said pathogen is an animal pathogen.~~
- ~~24. The container of any one of the preceding claims, wherein said pathogen is a viral pathogen and said antigen is from a disease causing virus.~~
- ~~25. The container of claim 24, wherein said pathogen is a mammalian viral pathogen.~~
- ~~26. The container of claim 24, wherein said pathogen is a human or animal viral pathogen.~~
- ~~27. The container of any one of claims 24-26, wherein said antigen is from a DNA virus.~~
- ~~28. The container of any one of claims 24-26, wherein said antigen is from a RNA virus.~~
- ~~29. The container of claim 28, wherein said antigen is from a retrovirus.~~
- ~~30. The container of claim 28, wherein said antigen is from a Coronavirus, a Rhabdovirus, a paramyxovirus, an influenza virus, a respiratory syncytial virus, a cytomegalovirus, a Hepatitis virus or a orthomyxovirus.~~
- ~~31. The container of claim 30, wherein said antigen is from a SARS-CoV2 virus.~~
- ~~32. The container of claim 31, wherein said antigen comprises a polypeptide comprising a sequence with at least 80% identity to SEQ ID NO: 1.~~
- ~~33. The container of claim 31, wherein said antigen comprises a polypeptide comprising a sequence with at least 85% identity to SEQ ID NO: 1.~~
- ~~34. The container of claim 31, wherein said antigen comprises a polypeptide comprising a sequence with at least 90% identity to SEQ ID NO: 1.~~
- ~~35. The container of any one of claims 31-34, wherein said antigen comprises a polypeptide comprising a sequence with at least 80% identity to SEQ ID NO: 2.~~

- ~~36. The container of claim 35, wherein said antigen further comprises a polypeptide comprising a sequence with at least 85% identity to SEQ ID NO: 2.~~
- ~~37. The container of claim 36, wherein said antigen further comprises a polypeptide comprising a sequence with at least 90% identity to SEQ ID NO: 2.~~
- ~~38. The container of any one of claims 31-37, wherein said antigen comprises a peptide comprising 5-14 consecutive amino acids of SEQ ID NO: 1.~~
- ~~39. The container of any one of claims 31-38, wherein said antigen comprises a peptide comprising 5-14 consecutive amino acids of SEQ ID NO: 2.~~
- ~~40. The container of any one of claims 31-37, wherein said antigen comprises a peptide comprising at least 15 consecutive amino acids of SEQ ID NO: 1.~~
- ~~41. The container of any one of claims 31-38, wherein said antigen comprises a peptide comprising at least 15 consecutive amino acids of SEQ ID NO: 2.~~
- ~~42. The container of any one of the preceding claims, wherein said antigen comprises at least two specific T cell recognized epitopes of said virus.~~
- ~~43. The container of any one of the preceding claims, wherein said T cell recognition is by a CD4+ T cell.~~
- ~~44. The container of any one of claims 1-42, wherein said T cell recognition is by a CD8+ T cell.~~
- ~~45. The container of any one of the preceding claims, wherein said container further comprises a second antigen comprising a second specific T cell recognized epitope of said pathogen.~~
- ~~46. The container of any one of claims 1-45, wherein said container further comprises a second antigen of a second pathogen, and wherein said second antigen comprises a specific T cell recognized epitope of said second pathogen.~~
- ~~47. The container of claim 46, wherein said second pathogen is a human pathogen.~~
- ~~48. The container of claim 47, wherein said pathogen is a viral pathogen and second said antigen is from a disease causing virus.~~
- ~~49. The container of claim 48, wherein said second pathogen is a mammalian viral pathogen.~~
- ~~50. The container of claim 49, wherein said second pathogen is a human or animal viral pathogen.~~
- ~~51. The container of any one of the preceding claims, wherein said container further comprises one or more of a sugar, an amino acid, and a vitamin.~~

- ~~52. The container of claim 51 wherein said sugar is a glucose.~~
- ~~53. The container of any one of the preceding claims further comprising an aluminum adjuvant.~~
- ~~54. The container of claim 53, wherein said aluminum adjuvant and said antigen are at a ratio of 1:1 to 3:1 by weight, wherein weight of said aluminum adjuvant is as measured by weight of aluminum ion.~~
- ~~55. A container for detecting an immune response to a SARS-Cov2 virus in a subject, wherein said container comprises:~~
- ~~(i) lithium heparin,~~
 - ~~(ii) glucose,~~
 - ~~(iii) an aluminum adjuvant,~~
 - ~~and~~
 - ~~(iv) a SARS-Cov2 antigen that comprises the sequence of SEQ ID NO: 2,~~
- ~~wherein said aluminum adjuvant and said SARS-Cov2 antigen are at a ratio of 1:1 to 3:1 by weight, wherein weight of said aluminum adjuvant is as measured by weight of aluminum ion.~~
- ~~56. A kit for detecting an immune response to a virus in a subject comprising the container of any one of the preceding claims and a second container comprising an anticoagulation agent and not said antigen.~~
- ~~57. The kit of claim 56, further comprising a detection agent for detecting an immune cell response to said antigen, wherein said detection agent quantifies an indicator of said immune cell response.~~
- ~~58. The kit of claim 57, wherein said indicator comprises an amount of an immune response analyte.~~
- ~~59. The kit of claim 58, wherein said indicator comprises a ratio of two or more immune response analytes.~~
- ~~60. The kit of claim 58 or 59, wherein said immune response analyte or said two or more immune response analytes are immunoproteins.~~
- ~~61. The kit of claim 60 wherein said immune response analyte or said two or more immune response analytes are nucleic acids encoding said immunoproteins.~~
- ~~62. The kit of claim 57, wherein said indicator is an immune cell proliferation indicator.~~
- ~~63. The kit of claim 62, wherein said immune response analyte is an IFN-gamma protein or a nucleic acid encoding an IFN-gamma protein.~~

~~64. The kit of claim 63, further comprising one or more primers that hybridize with said nucleic acid encoding said IFN-gamma protein.~~

~~65. The kit of any one of claims 56-64 further comprising a lysis agent.~~

~~66. The kit of any one of claims 56-65 further comprising instructions for quantifying said immune-response analytes.~~

~~67. The kit of any one of claims 56-66 further comprising a set of primers that hybridize with a nucleic acid of said pathogen.~~

~~68. The kit of claim 67, wherein said set of primers hybridize with a nucleic acid encoding a SARS-CoV-2 protein.~~

~~69. A kit for detecting an immune response to a SARS-CoV-2 virus by a subject, wherein said kit comprises:~~

~~a) a first container comprising (i) lithium heparin (ii) glucose, (iii) an aluminum adjuvant, and (iv) a SARS-CoV-2 antigen that comprises the peptide sequence of SEQ ID NO: 2,~~

~~b) a second container comprising lithium heparin, glucose, and not said SARS-CoV-2 antigen, wherein said first and second containers are steril glass or polycarbonate containers, and~~

~~c) a set of primers that comprise a sequence selected from Table 2.~~

~~wherein said aluminum adjuvant and said SARS-CoV-2 antigen are at a ratio of 1:1 to 3:1 by weight, wherein weight of said aluminum adjuvant is as measured by weight of aluminum ion.~~

~~70.~~1. A method for detecting an immune response to a pathogen by a subject, comprising:

i) incubating a blood sample from said subject in a container comprising an antigen and an anticoagulation agent, wherein said antigen comprises a specific T cell recognized epitope of said pathogen, and

ii) incubating a second blood sample from said subject in a second container comprising said anticoagulation agent in (i) and not said antigen,

iii) quantifying an amount of an immune-response analyte in said container and said second container, wherein said immune-response analyte is a nucleic acid, wherein said quantifying comprises amplifying the nucleic acid and performing nanopore sequencing,

iv) comparing said amount of said immune-response analyte in said container and said second container to determine whether the subject has an immune response to said pathogen.

2. The method of claim 1, further comprising isolating total RNA from said container.

3. The method of claim 1 or 2, wherein said ~~quantifying~~amplifying in (iii) comprises ~~using mass spectrometry~~loop-mediated isothermal proliferation (LAMP).
- ~~71. The method of any one of claims 1 to determine said amount of said immune response analyte.~~
- ~~72. The method of claim 70 or 71, wherein said immune response analyte is a protein.~~
- ~~73. The method of claim 72, further comprising isolating said immune response analyte.~~
- ~~74. The method of claim 73, further comprising isolating a nucleic acid encoding said immune response analyte, encodes IFN-gamma or IL-2.~~
- ~~75. The method of claim 71, further comprising isolating total RNA from any one of claims 1 to 4, wherein said ~~container~~incubating in step (i) and step (ii) is done for less than or equal to 4 hours.~~
6. The method of any one of claims ~~72-75~~1 to 5, wherein said determining is done within 96 hours of said incubating.
7. The method of any one of claims 1 to 6, wherein said determining is done with a specificity of at least 95%.
- ~~76. The method of any one of claims 1 to 7, wherein said ~~quantifying in (iii)~~ comprises ~~Western blot, mass spectrometry assay, UV spectrometry assay, aptamer based assay, or colorimetric assay~~determining is done with a sensitivity of at least 80%.~~
- ~~77. The method of claim 74 any one of claims 1 to 8, wherein said ~~quantifying in (iii)~~ comprises ~~amplifying~~ subject has a latent infection of said ~~nucleic acid encoding said immune response marker~~pathogen.~~
- ~~78. The method of claim 77, wherein said ~~quantifying in (iii)~~ comprises determining the amount of said nucleic acid encoding said immune response marker with ~~loop-mediated isothermal proliferation (LAMP) assay, RNA seq, or quantitative RT-PCR~~.~~
- ~~79. The method of claim 78, wherein said ~~quantifying in (ii)~~ further comprises analysis using a nanopore technology.~~
10. The method of any one of claims 1 to 9, wherein said pathogen is SARS-CoV-2.
11. The method of any one of claims ~~70-79~~1 to 10, wherein said antigen comprises a peptide comprising at least 15 consecutive amino acids of SEQ ID NO: 1 or SEQ ID NO: 2.
- ~~80. 12. The method of any one of claims 1 to 11, wherein said container further comprises a second antigen comprising a specific T cell recognized epitope of a second pathogen, wherein said second container does not comprise said second antigen, and wherein said comparing in (iv) comprises comparing said amount of said immune-response analyte in said container and said second container to determine whether the subject has an immune response to said second pathogen.~~

~~81.~~13. The method of any one of claims ~~70-80~~1 to 12, further comprising v) quantifying an amount of a second immune-response analyte in said container and said second container, and vi) comparing said amount of said second immune-response analyte in said container and said second container to determine whether said subject has an immune response to said pathogen.

~~82.~~14. The method of claim ~~81~~13, wherein said comparing in vi) comprises comparing a ratio of said immune-response analyte to said second immune-response analyte in said container and said second container to determine whether said subject has an immune response to said pathogen.

~~83.~~15. The method of any one of claims ~~70-82~~1 to 11, further comprising ~~vii~~v) detecting said pathogen ~~or said second pathogen~~ in said sample, wherein said detecting comprises amplifying a nucleic acid of said pathogen ~~or said second pathogen~~.

~~84. The method of claim 83, wherein said amplifying comprises LAMP assay, RNA-Seq, or RT-PCR.~~

~~85. The method of claim 84, wherein said amplifying is with a set of primers, wherein said set of primers hybridize with said nucleic acid.~~

~~86. The method of claim 85, wherein said set of primers hybridize with a nucleic acid encoding a SARS-CoV-2 protein.~~

~~87.~~16. A method for detecting an immune response to a SARS-Cov2 virus by a subject, comprising:
 i) incubating a whole blood sample from said subject in a container comprising lithium heparin, glucose, an aluminum adjuvant, and a specific SARS-Cov2 antigen for at least 24 hours,
 ii) incubating a second whole blood sample from said subject in a second container comprising lithium heparin, glucose, an aluminum adjuvant, and not a SARS-Cov2 antigen, and
 iii) quantifying an amount of an IFN-gamma mRNA in said container and said second container to determine whether said subject has an immune response to said SARS-Cov2 virus,
 wherein said aluminum adjuvant and said SARS-Cov2 antigen are at a ratio of 1:1 to 3:1 by weight, wherein weight of said aluminum adjuvant is as measured by weight of aluminum ion, wherein said SARS-Cov2 antigen comprises the peptide sequence of SEQ ID NO: 2~~7~~1.

~~17. and~~The method of claim 16, wherein said quantifying in (iii) comprises amplifying said IFN-gamma mRNA ~~with~~using primers selected from Table 2.

18. The method of claim 17, wherein said amplifying comprises loop-mediated isothermal proliferation (LAMP) ~~assay using primers selected from Table 2.~~.

19. The method of any one of claims 16 to 18, wherein said quantifying in (iii) further comprises nanopore sequencing.
20. The method of any one of claims 16 to 19, wherein said determining in (iii) is done within 96 hours of said incubating and with a specificity of at least 95% and a sensitivity of at least 80%.