

Claims ~~WHAT IS CLAIMED IS:~~

1. A method for detecting the presence of beta-hemolytic pathogens in a sample comprising:

(a) adding a sample suspected of containing a beta-hemolytic pathogen to a growth medium containing sterically-stabilized liposomes comprising an encapsulated fluorophore to form a test assay mixture;

(b) incubating the test assay mixture for a period of time sufficient for the beta-hemolytic pathogen, if present, to form a population of test cells and to cause lysis of sterically-stabilized liposomes to release the fluorophore into the assay mixture for assimilation into the population of test cells;

(c) detecting the fluorescence of the population of test cells; and

(d) comparing the amount of fluorescence of the population of test cells with the amount of fluorescence of a population of control cells,

wherein the sterically-stabilized liposomes are prepared from a mixture of

(1) a membrane forming phospholipid selected from the group consisting of (a) a saturated phosphatidylcholine, preferably hydrogenated egg yolk phosphatidylcholine (HEPC), and (b) a synthetic phosphatidylcholine with saturated fatty acid tails selected from the group consisting of dimyristoyl-phosphatidylcholine, dipalmitoyl-phosphatidylcholine, and distearoyl-phosphatidylcholine;

(2) cholesterol; and

(3) a conjugate of polyethylene glycol (PEG), wherein the PEG has a molecular weight from 1,000 to 5,000, preferably 2,000, and wherein the PEG is conjugated to a molecule selected from the group consisting of distearoyl phosphatidylethanolamine (DSPE), dipalmitoyl phosphatidylethanolamine (DPPE), and dimyristoyl phosphatidylethanolamine (DMPE);

wherein the molar ratio of (1):(2) is in the range of 2:1 to 1:1 and (3) is present at 5% (mol/mol).

2. The method of claim 1, wherein alpha- and gamma-hemolytic pathogens do not significantly lyse the sterically-stabilized liposomes.

3. ~~The method of claim 1 or 2, wherein the sterically-stabilized liposomes are liposomes stabilized with PEG, preferably PEG2000.~~

34. The method of claim 1 or 23, wherein the sterically stabilized liposomes are prepared from a mixture of (1) a saturated phosphatidylcholine, preferably hydrogenated egg yolk phosphatidylcholine (HEPC), (2) cholesterol and (3) distearoyl phosphatidylethanolamine (DSPE)-PEG2000 in a molar ratio of (1):(2):(3) of 50:45:5.
45. The method of any one of claims 1-34, wherein the growth medium is a solid medium, preferably wherein the solid medium comprises brain heart infusion (BHI), fetal bovine serum (FBS), the sterically-stabilized liposomes and agarose.
- ~~6.~~ ~~The method of claim 5, wherein the population of test cells is one or more test colonies and the population of control cells is one or more control colonies.~~
- ~~7.~~ ~~The method of claim 5 or 6, wherein the solid medium comprises brain heart infusion (BHI), fetal bovine serum (FBS), the sterically stabilized liposomes and agarose.~~
5. The method of claim 4, wherein the population of test cells is one or more test colonies and the population of control cells is one or more control colonies.
68. The method of ~~any one of~~ claims 4 or 5-7, wherein the fluorophore is Hoechst 33342 (H33342).
79. The method of any one of claims 45-68, wherein the fluorescence is visualized and quantified, preferably further comprising quantitating a textural component of colony fluorescence for the one or more test colonies and the one or more control colonies and comparing the textural components.
- ~~10.~~ ~~The method of any one of claims 5-9, further comprising quantitating a textural component of colony fluorescence for the one or more test colonies and the one or more control colonies and comparing the textural components.~~
844. The method of any one of claims 1-4, wherein the growth medium is a liquid medium, preferably wherein the incubation is in wells of a well plate.

- ~~12. The method of claim 11, wherein the incubation is in wells of a well plate.~~
- ~~913. The method of claim ~~811~~ or ~~12~~, wherein the growth medium comprises brain heart infusion (BHI) and the sterically-stabilized liposomes, preferably wherein the growth medium further comprises fetal bovine serum (FBS), more preferably wherein the growth medium further comprises an antioxidant.~~
- ~~14. The method of claim 13, wherein the growth medium further comprises fetal bovine serum (FBS).~~
- ~~15. The method of claim 13 or 15, wherein growth medium further comprises an antioxidant~~
- ~~1016. The method of ~~any one of~~ claims ~~8~~ or ~~911-15~~, wherein the fluorophore is sulforhodamine B (SRB).~~
- ~~1117. The method of any one of claims ~~811-1016~~, wherein the population of control cells is a population of alpha- or gamma-hemolytic cells, preferably wherein the alpha-hemolytic cells are *S. pneumoniae* cells.~~
- ~~18. The method of claim 17, wherein the alpha-hemolytic cells are *S. pneumoniae* cells.~~
- ~~1219. The method of any one of claims ~~8-11-18~~, wherein the fluorescence is visualized and quantified, preferably the method further comprises determining the time-to-detection (TTD) of the population of test cells.~~
- ~~20. The method of any one of claims 11-19, which further comprises determining the time-to-detection (TTD) of the population of test cells.~~
- ~~1321. The method of any one of claims 1-~~1220~~, wherein images of the fluorescence are captured, stored and analyzed for the detection of the fluorescence, preferably wherein the images are stored on a computer medium or in cloud storage, more preferably wherein the images are analyzed locally or remotely.~~

~~22. The method of claim 21, wherein the images are stored on a computer medium~~

~~23. The method of claim 21, wherein the images are stored in cloud storage.~~

~~24. The method of any one of claims 21-23, wherein the images are analyzed locally.~~

~~25. The method of any one of claims 21-23, wherein the images are analyzed remotely.~~

~~1426.~~ The method of any one of claims 1-~~1325~~, wherein the presence of a beta-hemolytic pathogen in the sample is indicated if the fluorescence of the population of test cells is significantly greater than the fluorescence of the population of control cells.

~~1527.~~ A kit for detecting the presence of a beta-hemolytic pathogen in a sample, the kit comprising sterically-stabilized liposomes and a fluorophore, wherein the sterically-stabilized liposomes are lysed by a beta-hemolytic pathogen and are not lysed by an alpha-hemolytic pathogen or by a gamma-hemolytic pathogen,

wherein the sterically-stabilized liposomes are prepared from a mixture of

(1) a membrane forming phospholipid selected from the group consisting of (a) a saturated phosphatidylcholine, preferably hydrogenated egg yolk phosphatidylcholine (HEPC), and (b) a synthetic phosphatidylcholine with saturated fatty acid tails selected from the group consisting of dimyristoyl-phosphatidylcholine, dipalmitoyl-phosphatidylcholine, and distearoyl-phosphatidylcholine;

(2) cholesterol; and

(3) a conjugate of polyethylene glycol (PEG), wherein the PEG has a molecular weight from 1,000 to 5,000, preferably 2,000, and wherein the PEG is conjugated to a molecule selected from the group consisting of distearoyl phosphatidylethanolamine (DSPE), dipalmitoyl phosphatidylethanolamine (DPPE), and dimyristoyl phosphatidylethanolamine (DMPE);

wherein the molar ratio of (1):(2) is in the range of 2:1 to 1:1 and (3) is present at 5% (mol/mol).

16. The kit of claim 15, wherein the sterically stabilized liposomes are prepared from a mixture of (1) a saturated phosphatidylcholine, preferably hydrogenated egg yolk

phosphatidylcholine (HEPC), (2) cholesterol, and (3) DSPE-PEG2000 in a molar ratio of (1):(2):(3) of 50:45:5.

1728. The kit of claim 15 or 1627, wherein the fluorophore is encapsulated in the liposomes, or wherein the fluorophore is separate from the liposomes and encapsulated into the liposomes prior to use.

~~29. The kit of claim 27, wherein the fluorophore is separate from the liposomes and encapsulated into the liposomes prior to use.~~

1830. The kit of any one of claims 15, 16 or 1727-29, wherein the kit further comprises a growth medium, preferably wherein the growth medium is a solid growth medium or a liquid growth medium.

~~31. The kit of claim 30, wherein the growth medium is a solid growth medium.~~

~~32. The kit of claim 30, wherein the growth medium is a liquid growth medium~~

1933. The kit of any one of claims 1527-1832, wherein the kit also comprises a control pathogen.

2034. Use of sterically-stabilized liposomes comprising an encapsulated fluorophore for the detection of a beta-hemolytic pathogen in a sample, wherein the sterically-stabilized liposomes are lysed by a beta-hemolytic pathogen and are not lysed by an alpha-hemolytic pathogen or by a gamma-hemolytic pathogen,

wherein the sterically-stabilized liposomes are prepared from a mixture of

(1) a membrane forming phospholipid selected from the group consisting of (a) a saturated phosphatidylcholine, preferably hydrogenated egg yolk phosphatidylcholine (HEPC), and (b) a synthetic phosphatidylcholine with saturated fatty acid tails selected from the group consisting of dimyristoyl-phosphatidylcholine, dipalmitoyl-phosphatidylcholine, and distearoyl-phosphatidylcholine;

(2) cholesterol; and

(3) a conjugate of polyethylene glycol (PEG), wherein the PEG has a molecular weight from 1,000 to 5,000, preferably 2,000, and wherein the PEG is conjugated to a molecule selected from the group consisting of distearoyl phosphatidylethanolamine (DSPE), dipalmitoyl phosphatidylethanolamine (DPPE), dimyristoyl phosphatidylethanolamine (DMPE);

wherein the molar ratio of (1):(2) is in the range of 2:1 to 1:1 and (3) is present at 5% (mol/mol).

21. The use of sterically-stabilized liposomes according to claim 20, wherein the sterically-stabilized liposomes are prepared from a mixture of (1) a saturated phosphatidylcholine, preferably hydrogenated egg yolk phosphatidylcholine (HEPC), (2) cholesterol and (3) distearoyl phosphatidylethanolamine (DSPE)-PEG2000 in a molar ratio of (1):(2):(3) of 50:45:5.

~~2235.~~ Use of the kit of any one of claims ~~15-1927-33~~ for the use of a kit described herein for the detection of a beta-hemolytic pathogen in a sample.