CLAIMS

1. A method for quantifying total live bacteria in a sample comprising:

obtaining a sample comprising live bacteria;

contacting the live bacteria with at least one D-amino acid probe under conditions sufficient for bacterial cell wall synthesis, wherein:

the live bacteria covalently incorporate the at least one D-amino acid probe, and the D-amino acid probe comprises a clickable bioorthogonal handle, to provide bacteria comprising the clickable bioorthogonal handle;

contacting the bacteria comprising the clickable bioorthogonal handle with a fluorescent label comprising a clickable bioorthogonal reactive group, wherein the clickable bioorthogonal reactive group forms a covalent bond with the clickable bioorthogonal handle, to thereby provide labeled bacteria; and

detecting and quantifying the labeled bacteria,

to thereby quantify the total live bacteria in the sample.

2. The method of claim 1, wherein the sample comprises a mixed population of bacteria, optionally comprising both gram-negative and gram-positive bacteria.

3. The method of claim 1 or claim 2, wherein the D-amino acid probe comprises a single amino acid or a dipeptide.

4. The method of any one of claims 1-3, wherein the clickable bioorthogonal reactive group of the fluorescent label comprises an azide, and the clickable bioorthogonal handle of the D-amino acid probe comprises an alkyne or cycloalkyne group; or wherein the clickable bioorthogonal reactive group of the fluorescent label comprises an alkyne or cycloalkyne group, and the clickable bioorthogonal handle of the D-amino acid probe comprises an azide.

5. The method of any one of claims 1-4, wherein the D-amino acid probe comprises D-propargylglycine (EDA), optionally wherein the at least one D-amino acid probe comprises a combination of EDA and EDA-DA.

6. The method of claim 5, wherein the D-amino acid probe comprises a combination of EDA and EDA-DA used at a ratio of from about 1:2, to about 2:1, optionally wherein the combination

of EDA and EDA-DA is used at a ratio of about 1:1.

7. The method of any one of claims 1-6, wherein the contacting the live bacteria with at least one D-amino acid probe comprises incubating the live bacteria with the at least one D-amino acid probe for between about 10 and about 120 minutes, or for between about 20 and about 90 minutes.

8. The method of any one of claims 1-7, wherein the sample comprising live bacteria is supplemented with a cell metabolism booster, optionally where the cell metabolism booster comprises at least one of glucose and sodium pyruvate.

9. The method of any one of claims 1-8, wherein the sample comprising live bacteria is supplemented with a small molecule that controls cell doubling, optionally wherein the small molecule comprises DL-serine hydroxamate or chloramphenicol.

10. The method of any one of claims 1-9, further comprising removing the unincorporated D-amino acid probe from the sample and/or further comprising removing the unreacted fluorescent label from the sample.

11. The method of any one of claims 1-10, wherein the method further comprises a fixation step after the live bacteria covalently incorporate the at least one D-amino acid probe, and before contacting the bacteria comprising the clickable bioorthogonal handle with a fluorescent label, wherein the fixation step disrupts the outer membrane of Gram-negative bacteria and/or reduces non-specific staining of the sample components by the fluorescent label.

12. The method of claim 11, wherein the fixation step comprises the addition of an organic solvent, optionally where the organic solvent is ethanol.

13. The method of any one of claims 1-12, wherein the clickable bioorthogonal handle of the D-amino acid probe reacts with the clickable bioorthogonal reactive group of the fluorescent label to form a 1,2,3-triazole.

14. The method of any one of claims 1-13, wherein the reaction between the D-amino acid and the fluorescent label is a copper catalyzed click chemistry reaction.

15. The method of any one of claims 1-13, wherein the reaction between the D-amino acid and the fluorescent label is a copper-free reaction, optionally wherein the reaction between the D-amino acid and the fluorescent label is a copper-free strain promoted click chemistry reaction.

16. The method of any one of claims 1-15, further comprising flowing the labeled bacteria including the at least one D-amino acid probe toward an imaging region of an assay device, the imaging region including a fluorescence detector.

17. The method of any one of claims 1-16, wherein quantifying the total live bacteria in the sample is performed using microfluidic device such as a ferrofluid-based microfluidic device.

18. A quantification system configured to quantify live bacteria from a sample according to the method of any one of claims 1-17, comprising:

a ferrofluidic assay device configured to receive a microfluidic cartridge containing a sample;

the microfluidic cartridge comprising a plurality of microfluidic channels, each microfluidic channel comprising an imaging window;

an imager configured to image each window of the cartridge either separately or together;

a controller configured to control at least one of the ferrofluidic assay device, the microfluidic cartridge containing sample mixed with ferrofluid, and the imager; and

assay processing components comprising at least one of reagents, and controls;

wherein the system is configured to at least one of moving or otherwise locating the labeled bacteria to one or more of the windows where they can be any and all of imaged and quantified.

19. The quantification system of claim 18, wherein the imager comprises a fluorescent microscope with a digital camera.

20. The quantification system of claim 18 or claim 19, wherein upon generation of a magnetic field, the ferrofluid drives suspended bacteria to the top and center of the cartridge by the action of ferrofluid and magnetic force.