[0006] As such, assays that generally require a minimum amount of growth or a minimum growth rate may not detect target cells that do not exhibit the required growth characteristics during the assay.

[0007] Related patent applications include: PCT/US2014/026536, filed on March 13, 2014, which is hereby incorporated by reference, in its entirety, for all purposes.

SUMMARY

[0008] Disclosed herein is a cellular detection method that operates independent of cell growth. While existing cellular detection methodologies benefit from cell growth, the methods disclosed herein demonstrate embodiments that are independent of cell growth.

[0009] The methods disclosed herein are generally independent of growth – which can be an important feature for detecting cells at a metabolic state that does not support adequate growth (e.g., cells encountered in clinical samples) and for strains of cells with lower than expected growth rates.

[0010] Disclosed herein are various methods, systems, and compositions for the growth independent detection of cells.

[0010a] In one aspect, there is provided growth-independent method for detecting a microorganism of interest in a sample, comprising:

- contacting the sample with a plurality of non-replicative transduction particles (NRTPs) such that the plurality of NRTPs transduces one or more microorganisms of interest in the sample, wherein the plurality of NRTPs comprises a reporter nucleic acid sequence, and wherein the growth rate of the one or more microorganisms of interest is less than logarithmic phase;
- providing conditions for activation of the reporter nucleic acid sequence; and detecting a signal produced by the reporter nucleic acid sequence, wherein the presence of the signal indicates the presence of the one or more microorganisms of interest, and wherein the absence of the signal indicates the absence of the one or more microorganisms of interest.

[0010b] In one aspect, there is provided a sample or cell culture comprising a plurality of non-replicative transduction particles (NRTPs) and one or more microorganisms of interest, optionally wherein the plurality of NRTPs comprises a reporter nucleic acid sequence, and wherein the growth rate of the one or more microorganisms of interest is less than logarithmic phase.

[0011] For example, a method disclosed herein can include a growth-independent method for detecting a microorganism of interest in a sample, comprising: contacting the sample with a

[0012] plurality of non-replicative transduction particles (NRTPs) such that the plurality of NRTPs transduces one or more microorganisms of interest in the sample, wherein the plurality of NRTPs comprises a reporter nucleic acid sequence, and wherein the growth rate of the one or more microorganisms of interest is less than logarithmic phase; providing conditions for activation of the reporter nucleic acid sequence; and detecting a signal produced by the reporter nucleic acid sequence, wherein the presence of the signal indicates the presence of the one or more microorganisms of interest, and wherein the absence of the signal indicates the absence of the one or more microorganisms of interest.

[0013] As a further example, a composition disclosed herein can include a sample or a cell culture comprising a plurality of non-replicative transduction particles (NRTPs) and one or more microorganisms of interest, optionally wherein the plurality of NRTPs comprises a reporter nucleic acid sequence, and wherein the growth rate of the one or more microorganisms of interest is less than logarithmic phase.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

[0014] These and other features, aspects, and advantages of the present invention will become better understood with regard to the following description, and accompanying drawings, where:

CLAIMS

What is claimed:

1. A growth-independent method for detecting a microorganism of interest in a sample, comprising:
   - contacting the sample with a plurality of non-replicative transduction particles (NRTPs) such that the plurality of NRTPs transduces one or more microorganisms of interest in the sample, wherein the plurality of NRTPs comprises a reporter nucleic acid sequence, and wherein the growth rate of the one or more microorganisms of interest is less than logarithmic phase;
   - providing conditions for activation of the reporter nucleic acid sequence; and
   - detecting a signal produced by the reporter nucleic acid sequence, wherein the presence of the signal indicates the presence of the one or more microorganisms of interest, and wherein the absence of the signal indicates the absence of the one or more microorganisms of interest.
2. The method of claim 1, wherein the growth rate of the one or more microorganisms of interest is less than 0.1 divisions per hour, and wherein the one or more microorganisms of interest is Methicillin Resistant *Staphylococcus aureus* (MRSA).
3. The method of any one claims 1 or 2, further comprising providing an antimicrobial agent to the sample and detecting for the signal produced by the reporter nucleic acid sequence to determine whether the one or more microorganisms of interest is susceptible or non-susceptible to the antimicrobial agent.
4. The method of any one of claims 1 to 3, wherein the one or more microorganism of interest is in stationary phase; or wherein the one or more microorganism of interest is undergoing no growth.
5. The method of any one of claims 1 to 4, wherein the growth rate of the one or more microorganisms of interest is negative or homeostatic.
6. The method of any one of claims 1 to 5, wherein the one or more microorganism of interest comprises a Methicillin Resistant *Staphylococcus aureus* (MRSA) cell, *Staphylococcus aureus*, *Staphylococcus spp.*, Enterobacteriaceae, *Enterococcus spp. Streptococcus spp.*, *Acinetobacter spp.*, *Pseudomonas spp.*, *Stenotrophomonas spp.*, or *Mycobacterium spp.*.
7. The method of any one of claims 1 to 6, wherein the sample is a clinical sample.

8. The method of any one of claims 1 to 7, wherein the providing step comprises contacting the sample with a fatty aldehyde bacterial luciferase substrate reagent, optionally wherein the reagent is tridecanal.
9. The method of claim 3, wherein the antimicrobial agent is cefoxitin, a β-lactam, an extended-spectrum β-lactam, an Aminoglycoside, an Ansamycin, a Carbacephem, Carbapenems, any generation of Cephalosporin, a Glycopeptide, a Lincosamide, a Lipopeptide, a Macrolide, a Monobactam, a Nitrofuran, an Oxazolidonone, a Penicillin, a Polypeptide, a Quinolone, a Fluoroquinolone, a Streptogramin, a Sulfonamide, a Tetracycline, a Rifampicin, a mycobacterial antibiotic, Chloramphenicol, and/or Mupirocin.
10. The method of any one of claims 1 to 9, wherein the reporter nucleic acid sequence is a luciferase gene, optionally wherein the luciferase gene is *luxAB*, *luc*, *ruc*, or *nluc*.
11. The method of any one of claims 1 to 10, wherein the reporter nucleic acid sequence is operatively linked to a constitutive promoter, optionally wherein the constitutive promoter is a *S. aureus clpB* promoter.
12. The method of any one of claims 1 to 11, wherein the reporter nucleic acid sequence is selected from the group consisting of genes encoding enzymes mediating luminescence reactions (luxA, luxB, luxAB, luc, ruc, nluc), genes encoding enzymes mediating colorimetric reactions (lacZ, HRP), genes encoding fluorescent proteins (GFP, eGFP, YFP, RFP, CFP, BFP, mCherry, near-infrared fluorescent proteins), nucleic acid molecules encoding affinity peptides (His-tag, 3X-FLAG), and genes encoding selectable markers (ampC, tet(M), CAT, erm).
13. The method of any one of claims 1 to 12, wherein the reporter nucleic acid sequence is operatively linked to a constitutive promoter.
14. The method of any one of claims 1 to 13, further comprising providing an antibiotic to the sample at a pre-determined concentration and detecting a presence or absence of the

signal to determine whether the one or more microorganisms of interest is resistant or sensitive to the antibiotic.

15. The method of any one of claims 1 to 15, further comprising providing varying pre-determined concentrations of antibiotic to the sample and detecting the amount of the signal to determine the minimum inhibitory concentration of the one or more microorganisms of interest to the antibiotic.
16. A sample or cell culture comprising a plurality of non-replicative transduction particles (NRTPs) and one or more microorganisms of interest, optionally wherein the plurality of NRTPs comprises a reporter nucleic acid sequence, and wherein the growth rate of the one or more microorganisms of interest is less than logarithmic phase.
17. The sample or cell culture of claim 16, further comprising an antimicrobial agent.
18. The sample or cell culture of any one of claims 16 or 17, wherein the one or more microorganism of interest is in stationary phase; or wherein the one or more microorganism of interest is undergoing no growth.
19. The sample or cell culture of any one of claims 16 to 18, wherein the one or more microorganisms of interest comprises a Methicillin Resistant *Staphylococcus aureus* (MRSA) cell, *Staphylococcus aureus*, *Staphylococcus spp.*, Enterobacteriaceae, *Enterococcus spp. Streptococcus spp.*, *Acinetobacter spp.*, *Pseudomonas spp.*, *Stenotrophomonas spp.*, or *Mycobacterium spp.*.
20. The sample or cell culture of any one of claims 16 to 19, further comprising a fatty aldehyde bacterial luciferase substrate reagent, optionally wherein the reagent is tridecanal.
21. The sample or cell culture of claim 18, wherein the antimicrobial agent is cefoxitin, a β-lactam, an extended-spectrum β-lactam, an Aminoglycoside, an Ansamycin, a Carbacephem, Carbapenems, any generation of Cephalosporin, a Glycopeptide, a Lincosamide, a Lipopeptide, a Macrolide, a Monobactam, a Nitrofuran, an Oxazolidonone, a Penicillin, a Polypeptide, a Quinolone, a Fluoroquinolone, a Streptogramin, a Sulfonamide, a Tetracycline, a Rifampicin, a mycobacterial antibiotic, Chloramphenicol, and/or Mupirocin.
22. The sample or cell culture of any one of claims 16 to 21, wherein the reporter nucleic acid sequence is a luciferase gene, optionally wherein the luciferase gene is *luxAB*, *luc*, *ruc*, or *nluc*.

23. The sample or cell culture of any one of claims 16 to 22, wherein the reporter nucleic acid sequence is operatively linked to a constitutive promoter, optionally wherein the constitutive promoter is a *S. aureus clpB* promoter.
24. The sample or cell culture of any one of claims 16 to 23, wherein the reporter nucleic acid sequence is selected from the group consisting of genes encoding enzymes mediating luminescence reactions (luxA, luxB, luxAB, luc, ruc, nluc), genes encoding enzymes mediating colorimetric reactions (lacZ, HRP), genes encoding fluorescent proteins (GFP, eGFP, YFP, RFP, CFP, BFP, mCherry, near-infrared fluorescent proteins), nucleic acid molecules encoding affinity peptides (His-tag, 3X-FLAG), and genes encoding selectable markers (ampC, tet(M), CAT, erm).
25. The sample or cell culture of any one of claims 16 to 24, wherein the reporter nucleic acid sequence is operatively linked to a constitutive promoter.