

without delays. Thus, to improve the problem of emerging antimicrobial resistance and to improve care, physicians in primary care settings require ultra-rapid tests to identify the pathogen and determine its antimicrobial susceptibility. What is needed, therefore, are devices and methods for rapidly and accurately diagnosing infection and antimicrobial resistance or susceptibility.

SUMMARY

[0006] Provided herein are devices and methods can rapidly identify a cell, including a cancer cell, or microorganism, including a pathogen, quantify their load, and diagnose their susceptibility or resistance to drugs, such as antibiotics. In some examples the devices can enable phenotypic detection and metabolic profiling of drug susceptibility or drug resistance using individual microorganisms or cells which can originate from various sample types, including clinical or environmental samples. These sample types can include, but are not limited to, blood, cerebral spinal fluid (CSF), saliva and urine and can also include environmental samples, such as from water or a hospital surface. In some examples, the devices enable incubation of cells with drugs, such as antibiotics, and then rapidly extract and quantify nucleic acids or other molecules in a contamination-free platform. The devices can use digital single- molecule measurements in microfluidics devices, which provide ultra-sensitive measurements that improve detection limits while providing quantitative data, important for differentiating pathogens from contaminants and enabling earlier differentiation between drug-resistant and susceptible organisms or cells. In some examples, these devices can differentiate the state of individual microorganisms or cells from a clinical sample, and understand the timing of their individual responses to drugs, such as for example antibiotics, providing ultra-fast drug- susceptibility measurements.

[0007] In some examples, the data associated with the sample comprises measurements from greater than one spatially-isolated compartment each of the compartments comprising a portion of the sample.

[0008] In some examples, the sample undergoes a nucleic acid amplification. In some examples, the nucleic acid amplification reaction is a loop mediated amplification (LAMP) reaction. In some examples, the nucleic acid amplification reaction is a PCR reaction. In some examples, the method is performed at about or at a temperature range of 55-65°C. In some examples, at least a portion of the sample is partitioned into an array comprising at least 2 or more. In some examples, the array is a SlipChip. In some examples, the nucleic acid that is amplified is RNA.

[0009] In one aspect, there is provided a method, comprising: providing a first sample comprising a target nucleic acid from a first portion of a population of bacteria and a second sample comprising a target nucleic acid from a second portion of said population of bacteria, wherein the first portion has been treated with an antibiotic, and wherein the second portion has not been treated with the antibiotic; distributing said first sample among a plurality of first analysis regions; distributing said second sample among a plurality of second analysis regions; contacting each of the first and second analysis regions with a reagent for performing a digital amplification reaction to detect the presence or absence of a threshold number of target nucleic acids in each of the first and second analysis regions; and detecting the presence or absence of the threshold number of target nucleic acids in each of the first and second analysis regions, wherein the distribution of each sample among the analysis regions is effected such that the threshold number of target nucleic acids is detected in some of the first and second analysis regions and is not detected in some of the first and second analysis regions after said digital amplification reaction. Also provided herein are methods for detecting and quantifying target molecules from a sample, comprising: providing a first sample comprising a target molecule from a first portion of a population of bacteria and a second sample comprising a target molecule from a second portion of said population of bacteria, wherein the first portion has been treated with an antibiotic, and wherein the second portion has not been treated with the antibiotic; distributing said first sample among a plurality of first analysis regions; distributing said second sample among a plurality of second analysis regions; contacting each of the first and second analysis regions with a reagent for performing a reaction to detect the presence or absence of a threshold number of target molecules in each of the first and second analysis regions; and detecting the presence or absence of the threshold number of target molecules in each of the first and second analysis regions, wherein the distribution of each sample among the analysis regions is effected such that the threshold number of target molecules is detected in some of the first and second analysis regions and is not detected in some of the first and second analysis regions after said reaction.

[0010] In some examples, the threshold number is greater than zero, one, two, three, four, or five. In some examples, the distribution of each sample among the analysis regions is effected such that at least some of the analysis regions do not have the target molecule, and at least some of the analysis regions have only one target molecule. In some examples, the distribution of each sample among the analysis regions is effected such that at least one of the analysis regions contains only one target molecule. In some examples, the first sample comprises said reagent, and wherein contacting the plurality of first analysis regions with the reagent comprises said step of distributing said first sample among said plurality of first analysis regions. In some examples, the second sample comprises said reagent, and wherein contacting the plurality of second analysis regions with the reagent comprises said step of distributing said second sample among said plurality of second analysis regions.

[0011] In some examples, reaction comprises nucleic acid amplification. In some examples, the nucleic acid amplification is essentially isothermal. In some examples, the nucleic acid amplification is a polymerase chain reaction. In some examples, the nucleic acid amplification is a nucleic acid sequence based amplification (NASBA), self-sustained sequence replication (3SR), loop mediated isothermal amplification (LAMP), strand displacement amplification (SDA), whole genome amplification, multiple displacement amplification, strand displacement amplification, helicase dependent amplification, nicking enzyme amplification reaction, recombinant polymerase amplification, reverse transcription PCR, or ligation mediated PCR.

[0012] In some examples, said analysis regions comprise wells. In some examples, the target molecule is a nucleic acid. In some examples, the reagent is a nucleic acid amplification reagent. In some examples, the method of detecting and quantifying target molecules from a sample further comprises exposing each of the analysis regions to conditions effective for amplification of the nucleic acid.

[0013] In some examples, the target molecule comprises a segment of a bacterial chromosome or plasmid. In some examples, the target molecule is less than 50 kDa, less than 100 kDa, less than 200 kDa, or less than 400 kDa from the origin of replication. In some examples, the target molecule comprises a sequence of a gene on said bacterial chromosome or plasmid. In some examples, the target molecule comprises mRNA. In some examples, the target molecule is amplified by an amplification reaction, said amplification reaction comprising primers complementary to a sequence of the target molecule. In some examples, the mRNA encodes *recA* or *lexA*.

[0014] In some examples, the reagent is disposed in a plurality of reagent regions. In some examples, contacting is effected by placing the plurality of reagent regions in fluid communication with the first or second plurality of analysis regions. In some examples, contacting comprises effecting relative motion between a substrate comprising the reagent regions with a substrate comprising the first and second plurality of analysis regions. In some examples, the method of detecting and quantifying target molecules from a sample further comprises analyzing the detection of the presence or the absence of the threshold number of target molecules in each plurality of analysis regions to determine a resistance or susceptibility to an antibiotic in the population of bacteria.

[0015] In some examples, the target molecule has been removed from the bacteria in the first sample, and wherein the time between initial exposure of the bacteria to antibiotic and the removal of the target molecules is less than the mean doubling time of the bacteria during a growth phase. In some examples, the detection of the presence or absence of the threshold number of target molecules in each of the first and second analysis regions is performed less than 3 hours, 2 hours, or one hour after the end of exposure of the first sample to the antibiotic. In some examples, the detection of the presence or absence of the threshold number of target molecules in each of the first and second analysis regions is performed less than 45 minutes, 30 minutes, 15 minutes, or 10 minutes after the end of exposure of the first sample to the antibiotic. In some examples, the detection of the presence or absence of the threshold number of target molecules in each of the first and second analysis regions is performed less than 3 hours, less than 2 hours, or less than 1 hour after the end of exposure of the first sample to the antibiotic. In

some examples, the detection of the presence or absence of the threshold number of target molecules in each of the first and second analysis regions is performed less than 45 minutes, less than 30 minutes, less than 15 minutes, or less than 10 minutes after the end of exposure of the first sample to the antibiotic.

[0016] In some examples, the bacteria from said first sample have been treated with antibiotic for a period of no more than 2 hours, no more than 1 hour, no more than 45 minutes, no more than 30 minutes, no more than 15 minutes, or no more than 10 minutes. In some examples, the time from the first exposure of the bacteria from the first sample to an antibiotic to the detection step is less than 3 hours, less than 2 hours, less than 1 hour after, less than 45 minutes, or less than 30 minutes.

[0017] In some examples, the first plurality of analysis regions comprises at least 10, 20, 30, 40, or 50 analysis regions. In some examples, the second plurality of analysis regions comprises at least 10, 20, 30, 40, or 50 analysis regions. In some examples, the distribution of the first and second sample into the first and second plurality of analysis regions is performed in parallel. In some examples, the first sample or second sample comprises less than 10,000, 5,000, 1,000, 500, 200, 100, 50, 20, or 10 target molecules.

[0018] Provided herein is a method of determining a resistance or susceptibility to an antibiotic in a population of bacteria, comprising: providing a first sample comprising a target molecule from a first portion of a population of bacteria and a second sample comprising a target molecule from a second portion of said population of bacteria, wherein the first portion has been treated with an antibiotic, and wherein the second portion has not been treated with the antibiotic; distributing said first sample among a plurality of first analysis regions; distributing said second sample among a plurality of second analysis regions; contacting each of the first and second analysis regions with a reagent for performing a reaction to detect the presence or absence of a threshold number of target molecules in each of the first and second analysis regions; detecting the presence or absence of the threshold number of target molecules in each of the first and second analysis regions, wherein the distribution of each sample among the analysis regions is effected such that the threshold number of target molecules is detected in some of the first and second analysis regions and is not detected in some of the first and second analysis regions after said reaction; and analyzing the results of the detection to determine a resistance or susceptibility to said antibiotic in the population of bacteria.

[0019] Provided herein is a method of determining a resistance or susceptibility to an antibiotic in a population of bacteria, comprising: distributing a population of bacteria into a plurality of clonal isolation regions, the distribution being effected such that at least some of the

clonal isolation regions are statistically estimated to each contain a single isolated bacterium; expanding each of the single isolated bacteria to generate a plurality of clonal populations; distributing each of said plurality of clonal populations into at least one treatment region from a plurality of treatment regions and into at least one control region from a plurality of control regions; contacting the first plurality of treatment regions with an antibiotic, the plurality of control regions not contacted with the antibiotic; for each of the first plurality of treatment regions and each of the plurality of control regions, distributing one or more target molecules from each clonal population into a distinct plurality of analysis regions; contacting each of the analysis regions with a reagent for performing a reaction to detect the presence or absence of a threshold number of target molecules in each of the first and second analysis regions; and detecting the presence or absence of the threshold number of target molecules in each of the first and second analysis regions, wherein the distribution of each sample among the analysis regions is effected such that the threshold number of target molecules is detected in some of the distinct plurality of analysis regions and is not detected in some of the distinct plurality of analysis regions after said reaction.

[0020] In some examples, the method of determining a resistance or susceptibility to an antibiotic in a population of bacteria further comprises analyzing the results of the detection to determine a resistance or susceptibility to said antibiotic in at least some of said plurality of clonal populations.

[0021] In one aspect, provided herein are methods, comprising: providing a first sample comprising a target nucleic acid from a first portion of a population of cells and a second sample comprising a target nucleic acid from a second portion of said population of cells, wherein the first portion has been treated with a drug, and wherein the second portion has not been treated with the drug; distributing said first sample among a plurality of first analysis regions; distributing said second sample among a plurality of second analysis regions; contacting each of the first and second analysis regions with a reagent for performing a digital amplification reaction to detect the presence or absence of a threshold number of target nucleic acids in each of the first and second analysis regions; and detecting the presence or absence of the threshold number of target nucleic acids in each of the first and second analysis regions, wherein the distribution of each sample among the analysis regions is effected such that the threshold number of target nucleic acids is detected in some of the first and second analysis regions and is not detected in some of the first and second analysis regions after said digital amplification reaction.

[0022] Provided herein are kits comprising: a container comprising a plurality of analysis regions, a plurality of reagent regions comprising a component of a nucleic acid amplification,

wherein the container comprises a first layer and a second layer configured to move relative to the other between a first position, where the plurality of analysis regions and the plurality of reagent regions are isolated from each other, and a second position, wherein at least some of the plurality of analysis regions is in fluid communication with at least some of the plurality of reagent regions; and instructions for use for determining a resistance or susceptibility to an antibiotic in a population of bacteria.

[0023] In some examples, the component of a nucleic acid amplification comprises primers for amplifying *recA* or *lexA* mRNA. In some examples, the component of a nucleic acid amplification comprises primers for amplifying a nucleic acid target on a bacterial chromosome or plasmid less than 50kDa, less than 100 kDa, less than 200 kDa, or less than 400 kDa from the origin of replication. In some examples, the container comprises at least 10, 20, 30, 40, or 50 analysis regions. In some examples, the container comprises at least 10, 20, 30, 40, or 50 reagent regions.

[0024] In some examples, the antibiotic is selected from the group consisting of: aminoglycosides, cephalosporins, tetracyclines, sulfonamides, macrolides, vancomycin, and β -lactams. In certain of these examples, the instructions indicate that a decrease in nucleic acid target detection in a portion of the population of bacteria treated with said antibiotic as compared to a portion of the population of bacteria not treated with said antibiotic indicates that the population of bacteria is susceptible to said antibiotic.

[0025] In some examples, the antibiotic is selected from the group consisting of: HPUra, hydroxyurea, trimethoprim, ciprofloxacin, and MMC. In certain of these examples, the instructions indicate that an increase in nucleic acid target detection in a portion of the population of bacteria treated with said antibiotic as compared to a portion of the population of bacteria not treated with said antibiotic indicates that the population of bacteria is susceptible to said antibiotic.

[0026] Provided herein is a device for processing a sample, comprising an incubation module, a sample preparation module, and a digital quantification module, wherein said device is configurable to place each module in fluid communication with the other modules; wherein said incubation module comprises an incubation chamber configured to incubate an organism with a drug, wherein said sample preparation module is configured to extract a target analyte from said organism; and wherein said digital quantification module comprises a plurality of reaction areas configured to perform digital detection of the presence or absence of said target analytes in said reaction area.

WHAT IS CLAIMED IS:

1. A method, comprising:
 - providing a first sample comprising a target nucleic acid from a first portion of a population of bacteria and a second sample comprising a target nucleic acid from a second portion of said population of bacteria, wherein the first portion has been treated with an antibiotic, and wherein the second portion has not been treated with the antibiotic;
 - distributing said first sample among a plurality of first analysis regions;
 - distributing said second sample among a plurality of second analysis regions;
 - contacting each of the first and second analysis regions with a reagent for performing a digital amplification reaction to detect the presence or absence of a threshold number of target nucleic acids in each of the first and second analysis regions; and
 - detecting the presence or absence of the threshold number of target nucleic acids in each of the first and second analysis regions, wherein the distribution of each sample among the analysis regions is effected such that the threshold number of target nucleic acids is detected in some of the first and second analysis regions and is not detected in some of the first and second analysis regions after said digital amplification reaction.
2. The method of claim 1, wherein the threshold number is greater than zero, one, two, three, four, or five.
3. The method of claim 1, wherein the distribution of each sample among the analysis regions is effected such that at least some of the analysis regions do not have the target nucleic acid, and at least some of the analysis regions have only one target nucleic acid.
4. The method of claim 1, wherein the distribution of each sample among the analysis regions is effected such that at least one of the analysis regions contains only one target nucleic acid.
5. The method of claim 1, wherein the first sample comprises said reagent, and wherein contacting the plurality of first analysis regions with the reagent comprises said step of distributing said first sample among said plurality of first analysis regions.
6. The method of claim 1, wherein the second sample comprises said reagent, and wherein contacting the plurality of second analysis regions with the reagent comprises said step of distributing said second sample among said plurality of second analysis regions.
7. The method of claim 1, wherein said reaction comprises nucleic acid amplification.

8. The method of claim 7, wherein said nucleic acid amplification is essentially isothermal.
9. The method of claim 7, wherein said nucleic acid amplification is a polymerase chain reaction.
10. The method of claim 7, wherein said nucleic acid amplification is selected from the group consisting of: nucleic acid sequence based amplification (NASBA), self-sustained sequence replication (3SR), loop mediated isothermal amplification (LAMP), strand displacement amplification (SDA), whole genome amplification, multiple displacement amplification, strand displacement amplification, helicase dependent amplification, nicking enzyme amplification reaction, recombinant polymerase amplification, reverse transcription PCR and ligation mediated PCR.
11. The method of claim 1, wherein said analysis regions comprise wells.
12. The method of claim 1, wherein the reagent is a nucleic acid amplification reagent.
13. The method of claim 12, further comprising exposing each of the analysis regions to conditions effective for amplification of the nucleic acid.
14. The method of claim 1, wherein the target nucleic acid comprises a segment of a bacterial chromosome or plasmid.
15. The method of claim 14, wherein the target nucleic acid is less than 50 kb, less than 100 kb, less than 200 kb, or less than 400 kb from the origin of replication.
16. The method of claim 14, wherein the target nucleic acid comprises a sequence of a gene on said bacterial chromosome or plasmid.
17. The method of claim 1, wherein the target nucleic acid comprises mRNA.
18. The method of claim 1, wherein the target nucleic acid is amplified by an amplification reaction, said amplification reaction comprising primers complementary to a sequence of the target nucleic acid.
19. The method of claim 17, wherein the mRNA encodes recA or lexA.
20. The method of claim 1, wherein the reagent is disposed in a plurality of reagent regions.
21. The method of claim 17, wherein contacting is effected by placing the plurality of reagent regions in fluid communication with the first or second plurality of analysis regions.
22. The method of claim 21, wherein contacting comprises effecting relative motion between a substrate comprising the reagent regions with a substrate comprising the first and second plurality of analysis regions.
23. The method of claim 1, further comprising analyzing the detection of the presence or the absence of the threshold number of target nucleic acids in each plurality of analysis

regions to determine a resistance or susceptibility to an antibiotic in the population of bacteria.

24. The method of claim 1, wherein the target nucleic acid has been removed from the bacteria in the first sample, and wherein the time between initial exposure of the bacteria to antibiotic and the removal of the target nucleic acids is less than the mean doubling time of the bacteria during a growth phase.
25. The method of claim 1, wherein said detection of the presence or absence of the threshold number of target nucleic acids in each of the first and second analysis regions is performed less than 3 hours, 2 hours, or one hour after the end of exposure of the first sample to the antibiotic.
26. The method of claim 25, wherein said detection of the presence or absence of the threshold number of target nucleic acids in each of the first and second analysis regions is performed less than 45 minutes, 30 minutes, 15 minutes, or 10 minutes after the end of exposure of the first sample to the antibiotic.
27. The method of claim 1, wherein said detection of the presence or absence of the threshold number of target nucleic acids in each of the first and second analysis regions is performed less than 3 hours, less than 2 hours, or less than 1 hour after the end of exposure of the first sample to the antibiotic.
28. The method of claim 27, wherein said detection of the presence or absence of the threshold number of target nucleic acids in each of the first and second analysis regions is performed less than 45 minutes, less than 30 minutes, less than 15 minutes, or less than 10 minutes after the end of exposure of the first sample to the antibiotic.
29. The method of claim 1, wherein said bacteria from said first sample have been treated with antibiotic for a period of no more than 2 hours, no more than 1 hour, no more than 45 minutes, no more than 30 minutes, no more than 15 minutes, or no more than 10 minutes.
30. The method of claim 1, wherein the time from the first exposure of the bacteria from the first sample to an antibiotic to the detection step is less than 3 hours, less than 2 hours, less than 1 hour after, less than 45 minutes, or less than 30 minutes.
31. The method of claim 1, wherein the first plurality of analysis regions comprises at least 10, 20, 30, 40, or 50 analysis regions.
32. The method of claim 1, wherein the second plurality of analysis regions comprises at least 10, 20, 30, 40, or 50 analysis regions.
33. The method of claim 1, wherein the distribution of the first and second sample into the

first and second plurality of analysis regions is performed in parallel.

34. The method of claim 1, wherein the first sample or second sample comprises less than 10,000, 5,000, 1,000, 500, 200, 100, 50, 20, or 10 target nucleic acids.
35. A method, comprising:
 - providing a first sample comprising a target nucleic acid from a first portion of a population of cells and a second sample comprising a target nucleic acid from a second portion of said population of cells, wherein the first portion has been treated with a drug, and wherein the second portion has not been treated with the drug;
 - distributing said first sample among a plurality of first analysis regions;
 - distributing said second sample among a plurality of second analysis regions;
 - contacting each of the first and second analysis regions with a reagent for performing a digital amplification reaction to detect the presence or absence of a threshold number of target nucleic acids in each of the first and second analysis regions; and
 - detecting the presence or absence of the threshold number of target nucleic acids in each of the first and second analysis regions, wherein the distribution of each sample among the analysis regions is effected such that the threshold number of target nucleic acids is detected in some of the first and second analysis regions and is not detected in some of the first and second analysis regions after said digital amplification reaction.