with reduced cost, spatial fingerprint, but still requires approximately 90 minutes to generate a diagnostic result.

[0009] What is needed, therefore, are new assays compatible with point of care devices that offer high sensitivity, significantly reduced time to answer, reduced equipment cost, and the potential for sample in answer out utilization.

SUMMARY

[0010] In some examples, provided herein is a composition comprising a set of polynucleotides selected from the group consisting of Set-1 through Set-27. In a first aspect, the present disclosure refers to a composition comprising a sequence-specific primer set selected from the group consisting of Set-15, Set-1, Set-2, Set-3, Set-4, Set-5, Set-6, Set-7, Set-8, Set-9, Set-10, Set-11, Set-12, Set-13, and Set-14. In some examples, the composition further comprises a probe. In some examples, the probe comprises a label. In some examples, the probe is a labeled polynucleotide.

[0011] In some examples, the probe is a labeled polynucleotide having a sequence selected from the group consisting of SEQ ID NO: 73-76 and the set of polynucleotides is selected from Sets 12-15.

[0012] In some examples, the label is a fluorophore. In some examples, the fluorophore is covalently attached to a terminus of the polynucleotide. In some examples, the probe is a molecular beacon comprising a quencher. In some examples, the fluorophore is FAM and the quencher is BHQ1. In other examples, the fluorophore is ATTO 565 or Alexa 594 and the quencher is BHQ1 or BHQ2.

[0013] Also provided herein is a molecular beacon comprising a fluorophore, a quencher and a polynucleotide, wherein the polynucleotide is selected from the group consisting of SEQ ID NOs: 73-76 and the set of polynucleotides is selected from Sets 12-15. In some examples, the fluorophore is FAM and the quencher is BHQ1. In other examples, the fluorophore is ATTO 565 or Alexa 594 and the quencher is BHQ1 or BHQ2.

[0014] Also provided herein is a method of detecting *Neisseria gonorrhoeae* in a test sample, the method comprising: (a) extracting nucleic acid from the test sample; (b) amplifying a target sequence by reacting the nucleic acid extracted in step (a) with a reaction mixture comprising a strand displacement DNA polymerase and a sequence-specific primer set, wherein said sequence-specific primer set is selected from the group consisting of Set-1 through Set-27; and (c) detecting the presence or absence of an amplified product of step (b); wherein the presence of said amplification product is indicative of the presence of *Neisseria gonorrhoeae* in the test sample. In a second aspect, the present disclosure refers to a method of detecting *Neisseria gonorrhoeae* in a test sample, the method comprising: extracting nucleic acid from the test sample; amplifying a target sequence by reacting the nucleic acid extracted in step (a) with a reaction mixture comprising a strand displacement DNA polymerase and a sequence-specific primer set, wherein said sequence-specific primer set is selected from the group consisting of Set-15, Set-1, Set-2, Set-3, Set-4, Set-5, Set-6, Set-7, Set-8, Set-9, Set-10, Set-11, Set-12, Set-13, and Set-14; and detecting the presence or absence of an amplified product of step (b), wherein the presence of said amplification product is indicative of the presence of *Neisseria Gonorrhoeae* in the test sample.

[0015] In some examples of the method of detecting *Neisseria gonorrhoeae* in a test sample, the amplification in step (b) of the target sequence is performed at between about 60° C and 67° C for less than 30 minutes. In some examples, the amplification step is performed for less than 15 minutes. In some examples, the amplification step is performed for less than ten minutes.

[0016] In some examples of the method of detecting *Neisseria gonorrhoeae* in a test sample, detecting the presence or absence of the amplification product comprises hybridizing the amplified product with a probe comprising a polynucleotide attached to a label.

[0017] In some examples of the method of detecting *Neisseria gonorrhoeae* in a test sample, the polynucleotide comprises a sequence selected from the group consisting of SEQ ID NO: 73-76 and the sequence-specific primer set is selected from Sets 12-15.

[0018] In some examples of the method of detecting *Neisseria gonorrhoeae* in a test sample, the probe is a molecular beacon. In some examples, the reaction mixture further comprises a reverse transcriptase. In some examples, *Neisseria gonorrhoeae* is present in the test sample at a concentration of ≤ 100 CFU/mL. In some examples, *Neisseria gonorrhoeae* is present in the test sample at a concentration of ≤ 10 CFU/mL.

[0019] Also provided herein, according to some examples of the disclosure, is a kit comprising a composition comprising a set of polynucleotides selected from the group consisting of Set-1 through Set-27 and amplification reagents. In a third aspect, the present disclosure refers to a kit comprising a composition of the first aspect. In some examples, the amplification reagents comprise a strand displacement polymerase. In some examples, the kit further comprises a probe.

[0020] Also provided herein is a method of detecting *Neisseria gonorrhoeae* in a test sample, the method comprising: (a) extracting nucleic acid from the test sample; (b) amplifying a target sequence by reacting the nucleic acid extracted in step (a) for less than twenty minutes with a reaction mixture comprising a strand displacement DNA polymerase and a sequence-specific LAMP primer set; and (c) detecting the presence or absence of an amplified product of step (b); wherein the presence of said amplification product is indicative of the presence of *Neisseria gonorrhoeae* in the test sample.

[0021] In some examples of the method, the nucleic acid is reacted with the reaction mixture for less than fifteen minutes. In some examples of the method, the target sequence is located in the cytochrome C peroxidase (*ccpA*) gene of *Neisseria gonorrhoeae*.

[0022] In some examples of the method, *Neisseria gonorrhoeae* is present in the test sample at a concentration of ≤ 100 CFU/mL. In some examples of the method, *Neisseria gonorrhoeae* is present in the test sample at a concentration of ≤ 10 CFU/mL.

[0023] In some examples of the method, the test sample comprises one or more other microorganisms in addition to *Neisseria gonorrhoeae*, and wherein the target sequence from *Neisseria gonorrhoeae* is preferentially amplified over a polynucleotide sequence from the one or more other microorganisms.

[0024] In some examples, the disclosure provides a nucleic acid sequence at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%%, at least 99.1%, at least 99.2%, at least 99.3%, at least 99.4%, at least 99.5%, at least 99.6%, at least 99.7%, at least 99.8% or at least 99.9% identical to SEQ ID NOs 1-76 and methods of using those nucleic acid sequences to detect *Neisseria gonorrhoeae* in a test sample.

DETAILED DESCRIPTION

[0025] Detecting low concentrations of species (down to a few molecules or microorganisms in a sample) is a challenge in medicine. The present invention relates to the selective detection of *Neisseria gonorrhoeae*. In particular, based on new detection strategies utilizing nucleic acid amplification, particularly RT-LAMP, and molecular beacon detection, *N. gonorrhoeae* infections can be diagnosed using the methods and reagents described herein. Using RNA (either ribosomal RNA (rRNA) or messenger RNA) as the target regions provides multiple copies of the target per *N. gonorrhoeae* genome. Accordingly, this facilitates the detection of *N. gonorrhoeae* in samples utilizing the approaches described herein relative to techniques that target genomic DNA, even when present in multiple copies per genome. In addition, the molecular beacon detection reagents described herein provide additional specificity, failing to bind, in most cases, to off target amplified DNA, thereby minimizing the occurrence of, e.g., false positives. This specificity is illustrated in, *inter alia*, Example 3 (Tables 4 and 5) provided below. Many other features of the invention are also described herein.

[0026] As used herein, "nucleic acid" includes both DNA and RNA, including DNA and RNA containing non-standard nucleotides. A "nucleic acid" contains at least one polynucleotide (a "nucleic acid strand"). A "nucleic acid" may be single-stranded or double-stranded. The term "nucleic acid" refers to nucleotides and nucleosides which make up, for example, deoxyribonucleic acid (DNA) macromolecules and ribonucleic acid (RNA)

We claim:

1. A composition comprising a sequence-specific primer set selected from the group consisting of Set-15, Set-1, Set-2, Set-3, Set-4, Set-5, Set-6, Set-7, Set-8, Set-9, Set-10, Set-11, Set-12, Set-13, and Set-14.

2. The composition of claim 1, wherein the sequence-specific primer set is selected from the group consisting of: Set-15, Set-5, Set-11, Set-13, and Set-14.

3. The method of claim 2, wherein the sequence-specific primer set is Set-15.

4. The composition of claim 1, further comprising a probe.

5. The composition of claim 4, wherein the probe comprises a label.

6. The composition of claim 5, wherein the probe is a labeled polynucleotide.

7. The composition of claim 6, wherein the labeled polynucleotide comprises a sequence selected from the group consisting of: nucleotides 3-21 of SEQ ID NO: 75, nucleotides 6-22 of SEQ ID NO: 73, nucleotides 5-24- of SEQ ID NO: 74, and nucleotides 3-24 of SEQ ID NO: 76.

8. The composition of claim 6, wherein the labeled polynucleotide comprises a sequence selected from the group consisting of SEQ ID NO: 75, SEQ ID NO: 73, SEQ ID NO: 74, and SEQ ID NO: 76.

9. The composition of claim 6, wherein the label is a fluorophore.

10. The composition of claim 9, wherein the fluorophore is covalently attached to a terminus of the polynucleotide.

11. The composition of claim 4, wherein the probe is a molecular beacon comprising a fluorophore, a quencher, and a polynucleotide.

12. The composition of claim 11, wherein the molecular beacon comprises a sequence selected from the group consisting of SEQ ID NO: 75, SEQ ID NO: 73, SEQ ID NO: 74, and SEQ ID NO: 76.

13. The composition of claim 12, wherein the polynucleotide sequence consists of SEQ ID NO: 75.

14. A method of detecting *Neisseria gonorrhoeae* in a test sample, the method comprising:

extracting nucleic acid from the test sample;

amplifying a target sequence by reacting the nucleic acid extracted in step (a) with a reaction mixture comprising a strand displacement DNA polymerase and a sequence-specific primer set, wherein said sequence-specific primer set is selected from the group consisting of Set-15, Set-1, Set-2, Set-3, Set-4, Set-5, Set-6, Set-7, Set-8, Set-9, Set-10, Set-11, Set-12, Set-13, and Set-14; and

detecting the presence or absence of an amplified product of step (b), wherein the presence of said amplification product is indicative of the presence of *Neisseria Gonorrhoeae* in the test sample.

15. The method of claim 14, wherein the amplification step is performed for less than 15 minutes.

16. The method of claim 14, wherein the amplification step is performed for less than ten minutes.

17. The method of claim 14, wherein the reaction mixture further comprises a reverse transcriptase.

18. The method of claim 14, wherein detecting the presence or absence of the amplification product comprises hybridizing the amplified product with a probe comprising a polynucleotide attached to a label.

19. The method of claim 18, wherein the labeled polynucleotide comprises a sequence selected from the group consisting of: nucleotides 3-21 of SEQ ID NO: 75, nucleotides 6-22

of SEQ ID NO: 73, nucleotides 5-24- of SEQ ID NO: 74, and nucleotides 3-24 of SEQ ID NO: 76.

20. The method of claim 19, wherein the labeled polynucleotide comprises a sequence selected from the group consisting of: SEQ ID NO: 75, SEQ ID NO: 73, SEQ ID NO: 74, and SEQ ID NO: 76.

21. The method of claim 14, wherein the sequence-specific primer set is selected from the group consisting of: Set-15, Set-5, Set-11, Set-13, and Set-14.

22. The method of claim 21, wherein the sequence-specific primer set is Set-15.

23. The method of claim 14, wherein *Neisseria Gonorrhoeae* is present in the test sample at a concentration of ≤ 100 CFU/mL.

24. The method of claim 23, wherein *Neisseria Gonorrhoeae* is present in a test sample at a concentration of ≤ 10 CFU/mL.

25. The method of claim 24, wherein *Neisseria Gonorrhoeae* is present in the test sample at a concentration of ≤ 10 CFU/mL and the amplification reaction is performed for less than 15 minutes.

26. A kit comprising a composition according to claim 1.

27. The kit of claim 26, further comprising a strand displacement polymerase.

28. The kit of claim 27, further comprising a reverse transcriptase.