

BRIEF DESCRIPTION OF THE DRAWINGS

[0006] Figure 1 identifies labeled polynucleotide sequences, or molecular beacons, that are capable of binding to amplicon produced from each primer set.

SUMMARY

[0007] The present disclosure encompasses, in some examples, a composition comprising a set of polynucleotides selected from the group consisting of Set-1 through Set-81. 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. In a preferred implementation, the label is a fluorophore, which preferably is covalently attached to a terminus of the polynucleotide. In a particularly preferred example, the probe or polynucleotide is a molecular beacon comprising a fluorophore, a quencher, and a polynucleotide. In one example, the fluorophore is FAM and the quencher is BHQ1. In an alternate implementation, the fluorophore is ATTO 565 or Alexa 594 and the quencher is BHQ1 or BHQ2.

[0008] In some implementations, composition comprises a labeled polynucleotide comprising a sequence selected from the group consisting of nucleotides 5-34 of SEQ ID NO: 97, nucleotides 5-31 of SEQ ID NO: 98, nucleotides 3-31 of SEQ ID NO: 99, nucleotides 5-30 of SEQ ID NO: 100, nucleotides 6-29 of SEQ ID NO: 101, nucleotides 6-30 of SEQ ID NO: 102, nucleotides 5-26 of SEQ ID NO: 103, nucleotides 4-27 of SEQ ID NO: 104, nucleotides 7-30 of SEQ ID NO: 105, nucleotides 5-27 of SEQ ID NO: 106, nucleotides 6-29 of SEQ ID NO: 107, nucleotides 6-29 of SEQ ID NO: 108, nucleotides 6-29 of SEQ ID NO: 109, nucleotides 6-30 of SEQ ID NO: 110, nucleotides 8-31 of SEQ ID NO: 111, nucleotides 6-29 of SEQ ID NO: 112, nucleotides 8-31 of SEQ ID NO: 113, nucleotides 5-29 of SEQ ID NO: 114, nucleotides 5-29 of SEQ ID NO: 115, nucleotides 4-28 of SEQ ID NO: 116, and nucleotides 5-30 of SEQ ID NO: 117, nucleotides 8-28 of SEQ ID NO: 118, nucleotides 8-30 of SEQ ID NO: 119, nucleotides 8-29 of SEQ ID NO: 120, nucleotides 4-33 of SEQ ID NO: 121, nucleotides 7-34 of SEQ ID NO: 122, nucleotides 9-34 of SEQ ID NO: 123, nucleotides 8-34 of SEQ ID NO: 124, nucleotides 6-28 of SEQ ID NO: 125, nucleotides 7-28 of SEQ ID NO: 126, nucleotides 3-27 of SEQ ID NO: 127, nucleotides 7-32 of SEQ ID NO: 128, nucleotides 4-27 of SEQ ID NO: 129, nucleotides 6-27 of SEQ ID NO: 130, and nucleotides 8-29 of SEQ ID NO: 131. In further implementations, the labeled polynucleotide can comprise a sequence selected from the group consisting of SEQ ID NOs: 97 through 131. In certain

implementations, the sequence of the labeled polynucleotide is selected from the group consisting of SEQ ID NOs: 97 through 131. In other examples, the sequence of the labeled polynucleotide is selected from the group consisting of SEQ ID NOs: 97 through 131.

[0009] In some examples, the set of polynucleotides is selected from the group consisting of Set-12, Sets 14-27, Set-39, Sets 41-54, Set-66, and Sets 68-81, and the composition comprises a labeled polynucleotide comprising a sequence selected from the group consisting of nucleotides 5-26 of SEQ ID NO: 103, nucleotides 4-27 of SEQ ID NO: 104, nucleotides 7-30 of SEQ ID NO: 105, nucleotides 6-29 of SEQ ID NO: 107, nucleotides 6-29 of SEQ ID NO: 108, nucleotides 6-29 of SEQ ID NO: 109, nucleotides 8-31 of SEQ ID NO: 111, nucleotides 5-29 of SEQ ID NO: 114, nucleotides 5-29 of SEQ ID NO: 115, nucleotides 4-28 of SEQ ID NO: 116, nucleotides 5-30 of SEQ ID NO: 117, nucleotides 8-28 of SEQ ID NO: 118, nucleotides 8-30 of SEQ ID NO: 119, nucleotides 9-34 of SEQ ID NO: 123, nucleotides 8-34 of SEQ ID NO: 124, nucleotides 7-32 of SEQ ID NO: 128, and nucleotides 4-27 of SEQ ID NO: 129. In some implementations, the labeled polynucleotide comprises a sequence selected from the group consisting of SEQ ID NO: 103, SEQ ID NO: 104, SEQ ID NO: 105, SEQ ID NO: 107, SEQ ID NO: 108, SEQ ID NO: 109, SEQ ID NO: 111, SEQ ID NO: 114, SEQ ID NO: 115, SEQ ID NO: 116, SEQ ID NO: 117, SEQ ID NO: 118, SEQ ID NO: 119, SEQ ID NO: 123, SEQ ID NO: 124, SEQ ID NO: 128, and SEQ ID NO: 129. In some implementations, the sequence of the labeled polynucleotide is SEQ ID NO: 103, SEQ ID NO: 104, SEQ ID NO: 105, SEQ ID NO: 107, SEQ ID NO: 108, SEQ ID NO: 109, SEQ ID NO: 111, SEQ ID NO: 114, SEQ ID NO: 115, SEQ ID NO: 116, SEQ ID NO: 117, SEQ ID NO: 118, SEQ ID NO: 119, SEQ ID NO: 123, SEQ ID NO: 124, SEQ ID NO: 128, and SEQ ID NO: 129. In a preferred implementation, the sequence of the labeled polynucleotide is SEQ ID NO: 115, and the set of polynucleotides is selected from the group consisting of Set-20, Set-24, Set-47, Set-51, Set-74 and Set-78. Even more preferably, the set of polynucleotides is Set-20 or Set-24.

[0010] In yet another example, the set of polynucleotides is selected from the group consisting of Sets 12-27, Sets 39-54, and Sets 66-81, and the composition comprises a labeled polynucleotide comprising a sequence selected from the group consisting of nucleotides 5-34 of SEQ ID NO: 97, nucleotides 5-31 of SEQ ID NO: 98, nucleotides 3-31 of SEQ ID NO: 99, nucleotides 5-30 of SEQ ID NO: 100, nucleotides 6-29 of SEQ ID NO: 101, nucleotides 5-27 of SEQ ID NO: 106, nucleotides 6-30 of SEQ ID NO: 110, nucleotides 6-29 of SEQ ID NO: 112, nucleotides 8-29 of SEQ ID NO: 120, nucleotides 4-33 of SEQ ID NO: 121, and nucleotides 7-34 of SEQ ID NO: 122. More particularly, the labeled polynucleotide can

comprise a sequence selected from the group consisting of SEQ ID NO: 97, SEQ ID NO: 98, SEQ ID NO: 99, SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 106, SEQ ID NO: 110, SEQ ID NO: 112, SEQ ID NO: 120, SEQ ID NO: 121, and SEQ ID NO: 122. In certain implementations, the sequence of the labeled polynucleotide is selected from the group consisting of SEQ ID NO: 97, SEQ ID NO: 98, SEQ ID NO: 99, SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 106, SEQ ID NO: 110, SEQ ID NO: 112, SEQ ID NO: 120, SEQ ID NO: 121, and SEQ ID NO: 122.

[0011] In one implementation, the set of polynucleotides is selected from the group consisting of Set-12, Sets 14-18, Set-20, Set-24, Set-27, Set-39, Sets 41-45, Set-47, Set-51, Set-54, Set-66, Sets 68-72, Set 74, Set-78, and Set-81, and the composition comprises a labeled polynucleotide comprising a sequence selected from the group consisting of nucleotides 6-28 of SEQ ID NO: 125 and nucleotides 7-28 of SEQ ID NO: 126. In certain implementations, the labeled polynucleotide comprises a sequence selected from the group consisting of SEQ ID NO: 125 and SEQ ID NO: 126. In some examples, the sequence of the labeled polynucleotide is SEQ ID NO: 125 or SEQ ID NO: 126.

[0012] In another implementation, the set of polynucleotides is selected from the group consisting of Sets 14-27, Sets 41-54, and Sets 68-81, and the composition comprises a labeled polynucleotide comprising a sequence selected from the group consisting of nucleotides 8-31 of SEQ ID NO: 113 and nucleotides 3-27 of SEQ ID NO: 127. In some examples, the labeled polynucleotide comprises a sequence selected from the group consisting of SEQ ID NO: 113 and SEQ ID NO: 127. In other examples, the sequence of the labeled polynucleotide is SEQ ID NO: 113 or SEQ ID NO: 127.

[0013] In yet another example, the set of polynucleotides is selected from the group consisting of Sets 12-20, Sets 22-27, Sets 39-47, Sets 49-54, Sets 66-74, and Sets 76-81, and the composition comprises a labeled polynucleotide comprising nucleotides 6-27 of SEQ ID NO: 130. In some implementations, the labeled polynucleotide comprises SEQ ID NO: 130. In other examples, the sequence of the labeled polynucleotide is SEQ ID NO: 130.

[0014] In one implementation, the set of polynucleotides is selected from the group consisting of Set-13, Set-40 and Set-67, and the composition comprises a labeled polynucleotide comprising a sequence selected from the group consisting of nucleotides 6-30 of SEQ ID NO: 102 and nucleotides 8-29 of SEQ ID NO: 131. In such an example, the labeled polynucleotide can comprise a sequence selected from the group consisting of SEQ ID NO:

102 and SEQ ID NO: 131. In another example, the sequence of the labeled polynucleotide is SEQ ID NO: 102 or SEQ ID NO: 131.

[0014a] In one aspect, the present disclosure refers to a composition comprising a set of primers selected from the group consisting of: (a) a primer set comprising a forward outer primer comprising SEQ ID NO: 87, a backward outer primer comprising SEQ ID NO: 82, a forward inner primer comprising SEQ ID NO: 94, a backward inner primer comprising SEQ ID NO: 84, a forward loop primer comprising SEQ ID NO: 76, and a backward loop primer comprising SEQ ID NO: 86, (b) a primer set comprising a forward outer primer comprising SEQ ID NO: 87, a backward outer primer comprising SEQ ID NO: 82, a forward inner primer comprising SEQ ID NO: 94, and a backward inner primer comprising SEQ ID NO: 84, and (c) a primer set comprising a forward inner primer comprising SEQ ID NO: 94 and a backward inner primer comprising SEQ ID NO: 84.

[0015] Another example of the disclosure provides molecular beacons comprising a fluorophore, a quencher, and a polynucleotide, wherein the polynucleotide comprises a sequence selected from the group consisting of nucleotides 5-34 of SEQ ID NO: 97, nucleotides 5-31 of SEQ ID NO: 98, nucleotides 3-31 of SEQ ID NO: 99, nucleotides 5-30 of SEQ ID NO: 100, nucleotides 6-29 of SEQ ID NO: 101, nucleotides 6-30 of SEQ ID NO: 102, nucleotides 5-26 of SEQ ID NO: 103, nucleotides 4-27 of SEQ ID NO: 104, nucleotides 7-30 of SEQ ID NO: 105, nucleotides 5-27 of SEQ ID NO: 106, nucleotides 6-29 of SEQ ID NO: 107, nucleotides 6-29 of SEQ ID NO: 108, nucleotides 6-29 of SEQ ID NO: 109, nucleotides 6-30 of SEQ ID NO: 110, nucleotides 8-31 of SEQ ID NO: 111, nucleotides 6-29 of SEQ ID NO: 112, nucleotides 8-31 of SEQ ID NO: 113, nucleotides 5-29 of SEQ ID NO: 114, nucleotides 5-29 of SEQ ID NO: 115, nucleotides 4-28 of SEQ ID NO: 116, nucleotides 5-30 of SEQ ID NO: 117, nucleotides 8-28 of SEQ ID NO: 118, nucleotides 8-30 of SEQ ID NO: 119, and nucleotides 8-29 of SEQ ID NO: 120.

[0016] Yet another example of the disclosure provides methods of detecting *Chlamydia trachomatis* 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-81, 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 *Chlamydia trachomatis* in the test sample. In one aspect, the present disclosure refers to a method of detecting *Chlamydia trachomatis* in a test sample, the method comprising: (a) 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: i. a primer set comprising a forward outer primer comprising SEQ ID NO: 87, a backward outer primer comprising SEQ ID NO: 82, a forward inner primer comprising SEQ ID NO: 94, a backward inner primer comprising SEQ ID NO: 84, a forward loop primer comprising SEQ ID NO: 76, and a backward loop primer comprising SEQ ID NO: 86, ii. a primer set comprising a forward outer primer comprising SEQ ID NO: 87, a backward outer primer comprising SEQ ID NO: 82, a forward inner primer comprising SEQ ID NO: 94, and a backward inner primer comprising SEQ ID NO: 84, and iii. a primer set comprising a forward inner primer comprising SEQ ID NO: 94 and a backward inner primer comprising SEQ ID NO: 84; 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 *Chlamydia trachomatis* in the test sample. In one example, the amplification in step (b) of the target sequence is performed at between about 60° C and about 67°C for less than 30 minutes. Preferably, the amplification step is performed for less than fifteen minutes, less than ten minutes or less than six minutes. In some implementations, the reaction mixture further comprises a reverse transcriptase. In some implementations, *Chlamydia trachomatis* is present in the test sample at a concentration of ≤ 100 IFU/mL, ≤ 50 IFU/mL, ≤ 5 IFU/mL, or even ≤ 2 IFU/mL. In one implementation, *Chlamydia trachomatis* is present in the test sample at a concentration of ≤ 5 IFU/ml and the amplification step is performed for less than 15 minutes. In another implementation, *Chlamydia trachomatis* is present in the test sample at a concentration of ≤ 10 IFU/ml and the amplification step is performed for less than six minutes.

[0017] In certain examples, detecting the presence or absence of the amplification product comprises hybridizing the amplified product with a probe comprising a polynucleotide attached to a label. In a preferred implementation, the label is a fluorophore, which preferably is covalently attached to a terminus of the polynucleotide. In a particularly preferred example, the probe or polynucleotide is a molecular beacon comprising a fluorophore, a quencher, and a polynucleotide. In one example, the fluorophore is FAM and the quencher is BHQ1. In an alternate implementation, the fluorophore is ATTO 565 or Alexa 594 and the quencher is BHQ1 or BHQ2. This method can be practiced using any combination of primer set and labeled polynucleotide, e.g. molecular beacon, described herein. Figure 1 identifies labeled polynucleotide sequences, or molecular beacons, that are capable of binding to the amplicon resulting from each primer set, and therefore are useful in detecting the presence of *Chlamydia trachomatis* in a test sample.

[0018] In one aspect, the present disclosure refers to a kit comprising a composition as disclosed herein. Yet another example of the disclosure provides kits comprising the compositions comprising a set of polynucleotides selected from the group consisting of Set-1 through Set-81. In some examples, the kit further comprises a strand displacement polymerase and, optionally, a reverse transcriptase. In certain examples, the kit comprises a molecular beacon comprising a fluorophore, a quencher, and a polynucleotide, wherein the polynucleotide comprises a sequence selected from the group consisting of nucleotides 5-34 of SEQ ID NO: 97, nucleotides 5-31 of SEQ ID NO: 98, nucleotides 3-31 of SEQ ID NO: 99, nucleotides 5-30 of SEQ ID NO: 100, nucleotides 6-29 of SEQ ID NO: 101, nucleotides 6-30 of SEQ ID NO: 102, nucleotides 5-26 of SEQ ID NO: 103, nucleotides 4-27 of SEQ ID NO: 104, nucleotides 7-30 of SEQ ID NO: 105, nucleotides 5-27 of SEQ ID NO: 106, nucleotides 6-29 of SEQ ID NO: 107, nucleotides 6-29 of SEQ ID NO: 108, nucleotides 6-29 of SEQ ID NO: 109, nucleotides 6-30 of SEQ ID NO: 110, nucleotides 8-31 of SEQ ID NO: 111, nucleotides 6-29 of SEQ ID NO: 112, nucleotides 8-31 of SEQ ID NO: 113, nucleotides 5-29 of SEQ ID NO: 114, nucleotides 5-29 of SEQ ID NO: 115, nucleotides 4-28 of SEQ ID NO: 116, nucleotides 5-30 of SEQ ID NO: 117, nucleotides 8-28 of SEQ ID NO: 118, nucleotides 8-30 of SEQ ID NO: 119, nucleotides 8-29 of SEQ ID NO: 120, nucleotides 4-33 of SEQ ID NO: 121, nucleotides 7-34 of SEQ ID NO: 122, nucleotides 9-34 of SEQ ID NO: 123, nucleotides 8-34 of SEQ ID NO: 124, nucleotides 6-28 of SEQ ID NO: 125, nucleotides 7-28 of SEQ ID NO: 126, nucleotides 3-27 of SEQ ID NO: 127, nucleotides 7-32 of SEQ ID NO: 128, nucleotides 4-27 of SEQ ID NO: 129, nucleotides 6-27 of SEQ ID NO: 130, and nucleotides 8-29 of SEQ ID NO: 131. The polynucleotide sequence of the molecular beacon

can comprise a sequence selected from the group consisting of SEQ ID NO: 97 through SEQ ID NO: 131. In some examples, the polynucleotide sequence of the molecular beacon consists of a sequence selected from the group consisting of SEQ ID NO: 97 through SEQ ID NO: 131. In one example, the polynucleotide sequence of the molecular beacon consists of SEQ ID NO: 115 and the set of polynucleotides is Set-20 or Set-24.

[0019] Yet another example of the disclosure provides methods of detecting *Chlamydia trachomatis* 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 ten 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 *Chlamydia trachomatis* in the test sample. In one aspect, the present disclosure refers to a method of detecting *Chlamydia trachomatis* 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 ten minutes with a reaction mixture comprising a strand displacement DNA polymerase and a sequence-specific LAMP primer set comprising a forward outer primer comprising SEQ ID NO: 87, a backward outer primer comprising SEQ ID NO: 82, a forward inner primer comprising SEQ ID NO: 94, a backward inner primer comprising SEQ ID NO: 84, a forward loop primer comprising SEQ ID NO: 76, and a backward loop primer comprising SEQ ID NO: 86; 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 *Chlamydia trachomatis* in the test sample. In some implementations, *Chlamydia trachomatis* is present in the test sample at a concentration of ≤ 100 IFU/mL, ≤ 50 IFU/mL, ≤ 5 IFU/mL, or even ≤ 2 IFU/mL. In certain implementations, the amplifying step comprises 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-81. In such implementations, detecting the presence or absence of the amplification product can comprise hybridizing the amplified product with a molecular beacon comprising a polynucleotide sequence selected from the group consisting of SEQ ID NO: 97 through SEQ ID NO: 131. In some implementations, detecting the presence or absence of the amplification product comprises hybridizing the amplified product with a molecular beacon comprising a polynucleotide sequence selected from the group consisting of SEQ ID NO: 99 through SEQ ID NO: 120.

[0020] In some examples of the methods described herein, the test sample comprises one or more other microorganisms in addition to *Chlamydia trachomatis*, and wherein the target sequence from *Chlamydia trachomatis* is preferentially amplified over a polynucleotide sequence from the one or more other microorganisms.

[0021] 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 any one of SEQ ID NOs 1-96 and methods of using those nucleic acid sequences to detect *Chlamydia trachomatis* in a test sample. 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 any one of the group consisting of nucleotides 3-31 of SEQ ID NO: 99, nucleotides 5-30 of SEQ ID NO: 100, nucleotides 6-29 of SEQ ID NO: 101, nucleotides 6-30 of SEQ ID NO: 102, nucleotides 5-26 of SEQ ID NO: 103, nucleotides 4-27 of SEQ ID NO: 104, nucleotides 7-30 of SEQ ID NO: 105, nucleotides 5-27 of SEQ ID NO: 106, nucleotides 6-29 of SEQ ID NO: 107, nucleotides 6-29 of SEQ ID NO: 108, nucleotides 6-29 of SEQ ID NO: 109, nucleotides 6-30 of SEQ ID NO: 110, nucleotides 8-31 of SEQ ID NO: 111, nucleotides 6-29 of SEQ ID NO: 112, nucleotides 8-31 of SEQ ID NO: 113, nucleotides 5-29 of SEQ ID NO: 114, nucleotides 5-29 of SEQ ID NO: 115, nucleotides 4-28 of SEQ ID NO: 116, and nucleotides 5-30 of SEQ ID NO: 117 and methods of using those nucleic acid sequences to detect *Chlamydia trachomatis* in a test sample.

DETAILED DESCRIPTION

[0022] 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 *Chlamydia trachomatis*. In particular, based on new detection strategies utilizing nucleic acid amplification, particularly RT-LAMP, and molecular beacon detection, *Chlamydia* 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 *C. trachomatis* genome. Accordingly, this facilitates the detection of *C. trachomatis* 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 4 provided below. Many other features of the invention are also described herein.

[0023] 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) macromolecules.

WE CLAIM:

1. A composition comprising a set of primers selected from the group consisting of:
 - (a) a primer set comprising a forward outer primer comprising SEQ ID NO: 87, a backward outer primer comprising SEQ ID NO: 82, a forward inner primer comprising SEQ ID NO: 94, a backward inner primer comprising SEQ ID NO: 84, a forward loop primer comprising SEQ ID NO: 76, and a backward loop primer comprising SEQ ID NO: 86,
 - (b) a primer set comprising a forward outer primer comprising SEQ ID NO: 87, a backward outer primer comprising SEQ ID NO: 82, a forward inner primer comprising SEQ ID NO: 94, and a backward inner primer comprising SEQ ID NO: 84, and
 - (c) a primer set comprising a forward inner primer comprising SEQ ID NO: 94 and a backward inner primer comprising SEQ ID NO: 84.
2. The composition of claim 1, further comprising a probe.
3. The composition of claim 2, wherein the probe is a labeled polynucleotide.
4. The composition of claim 3, wherein the labeled polynucleotide comprises a sequence selected from the group consisting of nucleotides 5-34 of SEQ ID NO: 97, nucleotides 5-31 of SEQ ID NO: 98, nucleotides 3-31 of SEQ ID NO: 99, nucleotides 5-30 of SEQ ID NO: 100, nucleotides 6-29 of SEQ ID NO: 101, nucleotides 5-26 of SEQ ID NO: 103, nucleotides 4-27 of SEQ ID NO: 104, nucleotides 7-30 of SEQ ID NO: 105, nucleotides 5-27 of SEQ ID NO: 106, nucleotides 6-29 of SEQ ID NO: 107, nucleotides 6-29 of SEQ ID NO: 108, nucleotides 6-29 of SEQ ID NO: 109, nucleotides 6-30 of SEQ ID NO: 110, nucleotides 8-31 of SEQ ID NO: 111, nucleotides 6-29 of SEQ ID NO: 112, nucleotides 8-31 of SEQ ID NO: 113, nucleotides 5-29 of SEQ ID NO: 114, nucleotides 5-29 of SEQ ID NO: 115, nucleotides 4-28 of SEQ ID NO: 116, nucleotides 5-30 of SEQ ID NO: 117, nucleotides 8-28 of SEQ ID NO: 118, nucleotides 8-30 of SEQ ID NO: 119,

nucleotides 8-29 of SEQ ID NO: 120, nucleotides 4-33 of SEQ ID NO: 121, nucleotides 7-34 of SEQ ID NO: 122, nucleotides 9-34 of SEQ ID NO: 123, nucleotides 8-34 of SEQ ID NO: 124, nucleotides 6-28 of SEQ ID NO: 125, nucleotides 7-28 of SEQ ID NO: 126, nucleotides 3-27 of SEQ ID NO: 127, nucleotides 7-32 of SEQ ID NO: 128, nucleotides 4-27 of SEQ ID NO: 129, and nucleotides 6-27 of SEQ ID NO: 130.

5. The composition of claim 2, wherein the probe is a molecular beacon comprising a fluorophore, a quencher, and a polynucleotide.
6. The composition of claim 5, wherein the molecular beacon comprises a sequence selected from the group consisting of nucleotides 5-34 of SEQ ID NO: 97, nucleotides 5-31 of SEQ ID NO: 98, nucleotides 3-31 of SEQ ID NO: 99, nucleotides 5-30 of SEQ ID NO: 100, nucleotides 6-29 of SEQ ID NO: 101, nucleotides 5-26 of SEQ ID NO: 103, nucleotides 4-27 of SEQ ID NO: 104, nucleotides 7-30 of SEQ ID NO: 105, nucleotides 5-27 of SEQ ID NO: 106, nucleotides 6-29 of SEQ ID NO: 107, nucleotides 6-29 of SEQ ID NO: 108, nucleotides 6-29 of SEQ ID NO: 109, nucleotides 6-30 of SEQ ID NO: 110, nucleotides 8-31 of SEQ ID NO: 111, nucleotides 6-29 of SEQ ID NO: 112, nucleotides 8-31 of SEQ ID NO: 113, nucleotides 5-29 of SEQ ID NO: 114, nucleotides 5-29 of SEQ ID NO: 115, nucleotides 4-28 of SEQ ID NO: 116, nucleotides 5-30 of SEQ ID NO: 117, nucleotides 8-28 of SEQ ID NO: 118, nucleotides 8-30 of SEQ ID NO: 119, nucleotides 8-29 of SEQ ID NO: 120, nucleotides 4-33 of SEQ ID NO: 121, nucleotides 7-34 of SEQ ID NO: 122, nucleotides 9-34 of SEQ ID NO: 123, nucleotides 8-34 of SEQ ID NO: 124, nucleotides 6-28 of SEQ ID NO: 125, nucleotides 7-28 of SEQ ID NO: 126, nucleotides 3-27 of SEQ ID NO: 127, nucleotides 7-32 of SEQ ID NO: 128, nucleotides 4-27 of SEQ ID NO: 129, and nucleotides 6-27 of SEQ ID NO: 130.
7. The composition of claim 6, wherein the molecular beacon comprises a sequence selected from the group consisting of SEQ ID NOs: 97-101 and 103-130.
8. The composition of claim 7, wherein the polynucleotide sequence consists of SEQ ID NO: 115.
9. A kit comprising a composition according to claim 1.

10. The kit of claim 9, further comprising a strand displacement polymerase.
11. The kit of claim 9, further comprising a molecular beacon comprising a fluorophore, a quencher, and a polynucleotide, wherein the polynucleotide consists of a sequence selected from the group consisting of SEQ ID NOs: 97-101 and 103-130.
12. The kit of claim 11, wherein the polynucleotide consists of SEQ ID NO: 115.
13. The composition of claim 1, wherein the set of primers is selected from the group consisting of:
 - i. a primer set consisting of a forward outer primer consisting essentially of SEQ ID NO: 87, a backward outer primer consisting essentially of SEQ ID NO: 82, a forward inner primer consisting essentially of SEQ ID NO: 94, a backward inner primer consisting essentially of SEQ ID NO: 84, a forward loop primer consisting essentially of SEQ ID NO: 76, and a backward loop primer consisting essentially of SEQ ID NO: 86;
 - ii. a primer set consisting of a forward outer primer consisting essentially of SEQ ID NO: 87, a backward outer primer consisting essentially of SEQ ID NO: 82, a forward inner primer consisting essentially of SEQ ID NO: 94, and a backward inner primer consisting essentially of SEQ ID NO: 84; and
 - iii. a primer set consisting of a forward inner primer consisting essentially of SEQ ID NO: 94 and a backward inner primer consisting essentially of SEQ ID NO: 84.
14. A method of detecting *Chlamydia trachomatis* 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:
 - (i) a primer set comprising a forward outer primer comprising SEQ ID NO: 87, a backward outer primer comprising SEQ ID NO: 82,

a forward inner primer comprising SEQ ID NO: 94, a backward inner primer comprising SEQ ID NO: 84, a forward loop primer comprising SEQ ID NO: 76, and a backward loop primer comprising SEQ ID NO: 86,

- (ii) a primer set comprising a forward outer primer comprising SEQ ID NO: 87, a backward outer primer comprising SEQ ID NO: 82, a forward inner primer comprising SEQ ID NO: 94, and a backward inner primer comprising SEQ ID NO: 84, and
- (iii) a primer set comprising a forward inner primer comprising SEQ ID NO: 94 and a backward inner primer comprising SEQ ID NO: 84; 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 *Chlamydia trachomatis* in the test sample.

15. The method of claim 14, wherein the amplification in step (b) of the target sequence is performed at between about 60°C and about 67°C for less than fifteen minutes.

16. The method of claim 15, 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 5-34 of SEQ ID NO: 97, nucleotides 5-31 of SEQ ID NO: 98, nucleotides 3-31 of SEQ ID NO: 99, nucleotides 5-30 of SEQ ID NO: 100, nucleotides 6-29 of SEQ ID NO: 101, nucleotides 5-26 of SEQ ID NO: 103, nucleotides 4-27 of SEQ ID NO: 104, nucleotides 7-30 of SEQ ID NO: 105, nucleotides 5-27 of SEQ ID NO: 106, nucleotides 6-29 of SEQ ID NO: 107, nucleotides 6-29 of SEQ

ID NO: 108, nucleotides 6-29 of SEQ ID NO: 109, nucleotides 6-30 of SEQ ID NO: 110, nucleotides 8-31 of SEQ ID NO: 111, nucleotides 6-29 of SEQ ID NO: 112, nucleotides 8-31 of SEQ ID NO: 113, nucleotides 5-29 of SEQ ID NO: 114, nucleotides 5-29 of SEQ ID NO: 115, nucleotides 4-28 of SEQ ID NO: 116, nucleotides 5-30 of SEQ ID NO: 117, nucleotides 8-28 of SEQ ID NO: 118, nucleotides 8-30 of SEQ ID NO: 119, nucleotides 8-29 of SEQ ID NO: 120, nucleotides 4-33 of SEQ ID NO: 121, nucleotides 7-34 of SEQ ID NO: 122, nucleotides 9-34 of SEQ ID NO: 123, nucleotides 8-34 of SEQ ID NO: 124, nucleotides 6-28 of SEQ ID NO: 125, nucleotides 7-28 of SEQ ID NO: 126, nucleotides 3-27 of SEQ ID NO: 127, nucleotides 7-32 of SEQ ID NO: 128, nucleotides 4-27 of SEQ ID NO: 129, and nucleotides 6-27 of SEQ ID NO: 130.

20. The method of claim 19, wherein the labeled polynucleotide comprises a sequence selected from the group consisting of SEQ ID NOs: 97-101 and 103-130.

21. The method of claim 20, wherein the sequence of the labeled polynucleotide is SEQ ID NO: 115.

22. The method of claim 14, wherein *Chlamydia trachomatis* is present in the test sample at a concentration of < 100 IFU/mL.

23. The method of claim 22, wherein *Chlamydia trachomatis* is present in the test sample at a concentration of < 5 IFU/ml and the amplification step is performed for less than 15 minutes.

24. The method of claim 22, wherein *Chlamydia trachomatis* is present in the test sample at a concentration of < 10 IFU/ml and the amplification step is performed for less than six minutes.

25. A method of detecting *Chlamydia trachomatis* 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 ten minutes with a reaction mixture comprising a strand displacement DNA polymerase and a sequence-specific LAMP primer set comprising a forward outer primer comprising SEQ ID NO: 87, a backward

outer primer comprising SEQ ID NO: 82, a forward inner primer comprising SEQ ID NO: 94, a backward inner primer comprising SEQ ID NO: 84, a forward loop primer comprising SEQ ID NO: 76, and a backward loop primer comprising SEQ ID NO: 86; 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 *Chlamydia trachomatis* in the test sample.

26. The method of claim 25, wherein detecting the presence or absence of the amplification product comprises hybridizing the amplified product with a molecular beacon comprising a polynucleotide sequence selected from the group consisting of nucleotides 5-34 of SEQ ID NO: 97, nucleotides 5-31 of SEQ ID NO: 98, nucleotides 3-31 of SEQ ID NO: 99, nucleotides 5-30 of SEQ ID NO: 100, nucleotides 6-29 of SEQ ID NO: 101, nucleotides 5-26 of SEQ ID NO: 103, nucleotides 4-27 of SEQ ID NO: 104, nucleotides 7-30 of SEQ ID NO: 105, nucleotides 5-27 of SEQ ID NO: 106, nucleotides 6-29 of SEQ ID NO: 107, nucleotides 6-29 of SEQ ID NO: 108, nucleotides 6-29 of SEQ ID NO: 109, nucleotides 6-30 of SEQ ID NO: 110, nucleotides 8-31 of SEQ ID NO: 111, nucleotides 6-29 of SEQ ID NO: 112, nucleotides 8-31 of SEQ ID NO: 113, nucleotides 5-29 of SEQ ID NO: 114, nucleotides 5-29 of SEQ ID NO: 115, nucleotides 4-28 of SEQ ID NO: 116, nucleotides 5-30 of SEQ ID NO: 117, nucleotides 8-28 of SEQ ID NO: 118, nucleotides 8-30 of SEQ ID NO: 119, nucleotides 8-29 of SEQ ID NO: 120, nucleotides 4-33 of SEQ ID NO: 121, nucleotides 7-34 of SEQ ID NO: 122, nucleotides 9-34 of SEQ ID NO: 123, nucleotides 8-34 of SEQ ID NO: 124, nucleotides 6-28 of SEQ ID NO: 125, nucleotides 7-28 of SEQ ID NO: 126, nucleotides 3-27 of SEQ ID NO: 127, nucleotides 7-32 of SEQ ID NO: 128, nucleotides 4-27 of SEQ ID NO: 129, and nucleotides 6-27 of SEQ ID NO: 130.

27. The method of claim 25, wherein detecting the presence or absence of the amplification product comprises hybridizing the amplified product with a molecular beacon comprising a polynucleotide sequence selected from the group consisting of SEQ ID NOs: 97-101 and 103-130.