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(54) Title: METHODS OF DETECTING TRICHOMONAS VAGINALIS

(57) Abstract: Compositions and methods for detecting Trichomonas vaginalis are provided.

METHODS OF DETECTING TRICHOMONAS VAGINALIS

1. FIELD OF THE INVENTION

[001] Compositions and methods for detecting *Trichomonas vaginalis* are provided.

2. BACKGROUND

[002] The protozoan *Trichomonas vaginalis* is responsible for trichomoniasis, which is a common sexually transmitted infection that can infect both men and women. There are 7.4 million cases of trichomoniasis annually in the United States. Trichomoniasis infections can be symptomatic or asymptomatic. *See, e.g.,* Ginocchio et al., *J. Clin. Microbiol.* 2012, 50: 2601-2608. In women, trichomoniasis is one of a range of conditions that comprise vaginal discharge. *See, e.g.*, Centers for Disease Control and Prevention (CDC). CDC fact sheet: trichomoniasis. 2010. www.cdc.gov/std/trichomonas/STDFact-Trichomoniasis.htm. Symptoms in females can include itching, burning, redness, or soreness of the genitals, unusual odor, discomfort with urination, or a thin clear, white, yellow, or green discharge. *See id.* In men, trichomoniasis may cause non-gonococcal urethritis (NGU). Symptoms in males can include itching or burning inside the penis, burning after ejaculation or urination, or penile discharge. *See, e.g*, Workowski et al., Centers for Disease Control and Prevention. Sexually transmitted disease treatment guidelines, 2010. MMWR 2010;59 (RR-12):1–110; Centers for Disease Control and Prevention. Biosafety in Microbiological and Biomedical laboratories. www.cdc.gov/biosafety/publications/.

[003] Improved methods for detection of *Trichomonas vaginalis* (TV) are needed. In particular, a highly specific, accurate, and sensitive urine- or swab-based diagnostic test is needed.

3. SUMMARY

[004] In some embodiments, methods of detecting the presence or absence of *Trichomonas vaginalis* (TV) in a sample from a subject are provided. In some embodiments, methods of determining whether a subject has a *Trichomonas vaginalis* (TV) infection are provided. In some embodiments, the methods comprise detecting the presence or absence of the TV 40S ribosomal protein (Tv40Srp) gene or RNA in a sample from the subject.

[005] In some embodiments, the subject has not previously been treated for TV infection. In some embodiments, the subject has previously been treated for TV infection.

In some embodiments, the previous treatment comprised one or more doses of metronidazole or tinidazole. In some embodiments, the subject does not have any symptoms of TV infection. In some embodiments, the subject has one or more symptoms of TV infection. In some embodiments, the subject has one or more symptoms selected from vaginitis, urethritis, and cervicitis. In some embodiments, the subject is female and has one or more symptoms selected from itching, burning, redness, and/or soreness of the genitals; unusual odor of the genitals; discomfort with urination; and a thin clear, white, yellow, or green discharge. In some embodiments, the subject is pregnant. In some embodiments, the subject is male and has one or more symptoms selected from itching and/or burning inside the penis; burning after ejaculation and/or urination; and penile discharge.

[006] In some embodiments, the method comprises detecting an endogenous control. In some embodiments, the endogenous control is a sample adequacy control. In some embodiments, the endogenous control is a single-copy human gene. In some embodiments, the endogenous control is selected from HMBS, GAPDH, beta actin, and beta globin.

[007] In some embodiments, the method comprises detecting an exogenous control. In some embodiments, the exogenous control is a sample processing control. In some embodiments, the exogenous control comprises a DNA sequence that is not expected to be present in the sample. In some embodiments, the exogenous control is a bacterial gene.

[008] In some embodiments, the method comprises PCR. In some embodiments, the method comprises quantitative PCR. In some embodiments, the PCR reaction takes less than 2 hours, less than 1 hour, or less than 30 minutes from an initial denaturation step through a final extension step.

[009] In some embodiments, the the TV 40S ribosomal protein (Tv40Srp) gene comprises the sequence of SEQ ID NO: 4. In some embodiments, the method comprises contacting nucleic acids from the sample with a first primer pair for detecting the TV 40S ribosomal protein (Tv40Srp) gene or RNA. In some embodiments, the method comprises contacting nucleic acids from the sample with a second primer pair for detecting an endogenous control. In some embodiments, the method comprises contacting nucleic acids from the sample with a third primer pair for detecting an exogenous control.

[0010] In some embodiments, the first primer pair comprises a first primer and a second primer, wherein the first primer comprises a sequence that is at least 90%, at least 95%, or 100% identical to at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, or at least 25 contiguous nucleotides of

SEQ ID NO: 4, and wherein the second primer comprises a sequence that is at least 90%, at least 95%, or 100% complementary to at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, or at least 25 contiguous nucleotides of SEQ ID NO: 4. In some embodiments, the first primer and the second primer each independently comprises 0, 1, or 2 mismatches compared to SEQ ID NO: 4 or its complement. In some embodiments, the first primer pair comprises a first primer consisting of 15 to 30 nucleotides and a second primer consisting of 15 to 30 nucleotides. In some embodiments, the first primer pair comprises a first primer of SEQ ID NO: 1 and a second primer of SEQ ID NO: 2. In some embodiments, the first primer pair produces an amplicon that is 50 to 500 nucleotides long, 50 to 400 nucleotides long, 50 to 300 nucleotides long, 50 to 200 nucleotides long, 50 to 150 nucleotides long, 100 to 300 nucleotides long, 100 to 200 nucleotides long, or 100 to 150 nucleotides long.

[0011] In some embodiments, the method comprises forming the Tv40Srp amplicon. In some embodiments, the method comprises contacting the Tv40Srp amplicon with a first probe capable of selectively hybridizing with the Tv40Srp amplicon. In some embodiments, the first probe comprises a detectable label. In some embodiments, the first probe comprises a fluorescent dye and a quencher molecule. In some embodiments, the first probe comprises a sequence that is at least 90%, at least 95%, or 100% identical or complementary to at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, or at least 25 contiguous nucleotides of SEQ ID NO: 4 or SEQ ID NO: 5. In some embodiments, the first probe comprises 0, 1, or 2 mismatches compared to SEQ ID NO: 4 or its complement or compared to SEQ ID NO: 5 or its complement. In some embodiments, the first probe consists of 15 to 30 nucleotides. In some embodiments, the first probe has the sequence of SEQ ID NO: 3.

[0012] In some embodiments, the method comprises forming an endogenous control amplicon and/or an exogenous control amplicon. In some embodiments, the method comprises contacting the endogenous control amplicon with a second probe capable of selectively hybridizing with the engoenous control amplicon and/or contacting the exogenous control amplicon with a third probe capable of selectively hybridizing with the exoenous control amplicon. In some embodiments, the second probe and the third probe each comprise a detectable label, wherein the detectable labels may be the same or different. In some embodiments, the detectable labels of the second and third probes are detectably different from the detectable label of the first probe. In some embodiments, the method

comprises detecting the Tv40Srp gene or RNA, an endogenous control, and an exogenous control in a single multiplex reaction.

[0013] In some embodiments, the sample is selected from a urine sample, an endocervical swab sample, a vaginal swab sample, and a urethral swab sample.

[0014] In some embodiments, compositions comprising a first primer pair for detecting a *Trichomonas vaginalis* 40S ribosomal protein (Tv40Srp) gene or RNA are provided. In some embodiments, the composition comprises a second primer pair for detecting an endogenous control. In some embodiments, the endogenous control is a sample adequacy control. In some embodiments, the endogenous control is selected from HMBS, GAPDH, beta actin, and beta globin. In some embodiments, the composition comprises a third primer pair for detecting an exogenous control. In some embodiments, the exogenous control is a sample processing control. In some embodiments, the exogenous control is a bacterial gene.

[0015] In some embodiments, the first primer pair comprises a first primer and a second primer, wherein the first primer comprises a sequence that is at least 90%, at least 95%, or 100% identical to at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, or at least 25 contiguous nucleotides of SEQ ID NO: 4, and wherein the second primer comprises a sequence that is at least 90%, at least 95%, or 100% complementary to at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, or at least 25 contiguous nucleotides of SEQ ID NO: 4. In some embodiments, the first primer and the second primer each independently comprises 0, 1, or 2 mismatches compared to SEQ ID NO: 4 or its complement. In some embodiments, the first primer pair comprises a first primer consisting of 15 to 30 nucleotides and a second primer consisting of 15 to 30 nucleotides. In some embodiments, the first primer pair comprises a first primer of SEQ ID NO: 1 and a second primer of SEQ ID NO: 2.

[0016] In some embodiments, the composition comprises a first probe capable of selectively hybridizing to a Tv40Srp amplicon produced by the first primer pair. In some embodiments, the first probe comprises a detectable label. In some embodiments, the first probe comprises a fluorescent dye and a quencher molecule. In some embodiments, the first probe comprises a sequence that is at least 90%, at least 95%, or 100% identical or complementary to at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, or at least 25 contiguous nucleotides of SEQ ID NO: 4 or SEQ ID NO: 5. In some embodiments, the first probe comprises 0, 1, or 2

mismatches compared to SEQ ID NO: 4 or its complement or compared to SEQ ID NO: 5 or its complement. In some embodiments, the first probe consists of 15 to 30 nucleotides. In some embodiments, the first probe has the sequence of SEQ ID NO: 3. In some embodiments, the Tv40Srp amplicon has the sequence of SEQ ID NO: 5.

[0017] In some embodiments, the composition comprises a second probe capable of selectively hybridizing to an endogenous control amplicon produced by the second primer pair. In some embodiments, the endogenous control is a sample adequacy control. In some embodiments, the endogenous control is selected from HMBS, GAPDH, beta actin, and beta globin. In some embodiments, the composition comprises a third probe capable of selectively hybridizing to an exogenous control amplicon produced by the third primer pair. In some embodiments, the exogenous control is a sample processing control. In some embodiments, the exogenous control comprises a DNA sequence that is not expected to be present in the sample. In some embodiments, the exogenous control is a bacterial DNA.

[0018] In some embodiments, the composition is a lyophilized composition. In some embodiments, the composition is in solution. In some embodiments, the composition comprises nucleic acids from a sample from a subject being tested for the presence of absence of *Trichomonas vaginalis*.

[0019] In some embodiments, kits are provided comprising a first primer pair for detecting a *Trichomonas vaginalis* 40S ribosomal protein (Tv40Srp) gene or RNA. In some embodiments, the kit comprises a second primer pair for detecting an endogenous control, wherein the primer pair for detecting Tv40Srp and the second primer pair are in the same or different compositions in the kit. In some embodiments, the endogenous control is a sample adequacy control. In some embodiments, the endogenous control is selected from HMBS, GAPDH, beta actin, and beta globin. In some embodiments, the kit comprises a third primer pair for detecting an exogenous control, wherein the third primer pair is in the same or different composition from the primer pair for detecting Tv40Srp and the second primer pair. In some embodiments, the exogenous control is a sample processing control. In some embodiments, the exogenous control comprises a DNA sequence that is not expected to be present in the sample. In some embodiments, the exogenous control is a bacterial gene.

[0020] In some embodiments, the first primer pair comprises a first primer and a second primer, wherein the first primer comprises a sequence that is at least 90%, at least 95%, or 100% identical to at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, or at least 25 contiguous nucleotides of SEQ ID NO: 4, and wherein the second primer comprises a sequence that is at least 90%, at

least 95%, or 100% complementary to at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, or at least 25 contiguous nucleotides of SEQ ID NO: 4. In some embodiments, the first primer and the second primer each independently comprises 0, 1, or 2 mismatches compared to SEQ ID NO: 4 or its complement. In some embodiments, the first primer pair comprises a first primer consisting of 15 to 30 nucleotides and a second primer consisting of 15 to 30 nucleotides. In some embodiments, the first primer pair comprises a first primer of SEQ ID NO: 1 and a second primer of SEQ ID NO: 2.

[0021] In some embodiments, the kit comprises a first probe capable of selectively hybridizing to a Tv40Srp amplicon produced by the first primer pair, wherein the first probe is in the same or different composition from one or more of the primer pairs. In some embodiments, the first probe comprises a detectable label. In some embodiments, the first probe comprises a fluorescent dye and a quencher molecule. In some embodiments, the first probe comprises a sequence that is at least 90%, at least 95%, or 100% identical or complementary to at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, or at least 25 contiguous nucleotides of SEQ ID NO: 4 or SEQ ID NO: 5. In some embodiments, the first probe comprises 0, 1, or 2 mismatches compared to SEQ ID NO: 4 or its complement or compared to SEQ ID NO: 5 or its complement. In some embodiments, the first probe consists of 15 to 30 nucleotides. In some embodiments, the first probe has the sequence of SEQ ID NO: 3. In some embodiments, the Tv40Srp amplicon has the sequence of SEQ ID NO: 5.

[0022] In some embodiments, the kit comprises a second probe capable of selectively hybridizing to an endogenous control amplicon produced by the second primer pair, wherein the second probe is in the same or different composition from one or more of the primer pairs. In some embodiments, the kit comprises a third probe capable of selectively hybridizing to an exogenous control amplicon produced by the third primer pair, wherein the third probe is in the same or different composition from one or more of the primer pairs.

[0023] In some embodiments, the kit comprises dNTPs and/or a thermostable polymerase. In some embodiments, the kit comprises one or more lyophilized compositions.

[0024] In some embodiments, a primer is provided, wherein the primer consists of the sequence of SEQ ID NO: 1, wherein the primer comprises at least one modified nucleotide. In some embodiments, a primer is provided, wherein the primer consists of the

sequence of SEQ ID NO: 2, wherein the primer comprises at least one modified nucleotide. In some embodiments, a probe is provided, wherein the probe consists of the sequence of SEQ ID NO: 3, wherein the probe comprises at least one modified nucleotide and/or a detectable label. In some embodiments, the probe comprises a fluorescent dye and a quencher molecule. In some embodiments, the probe is a fluorescence resonance energy transfer (FRET) probe. In some embodiments, the probe comprises at least one modified nucleotide.

[0025] In some embodiments, a composition is provided, wherein the composition comprises a first primer consisting of the sequence of SEQ ID NO: 2 and a second primer consisting of the sequence of SEQ ID NO: 3, wherein the first primer and the second primer each comprises at least one modified nucleotide. In some embodiments, the composition comprises a probe consisting of the sequence of SEQ ID NO: 3, wherein the probe comprises at least one modified nucleotide and/or a detectable label. In some embodiments, the probe comprises a fluorescent dye and a quencher molecule. In some embodiments, the probe is a fluorescence resonance energy transfer (FRET) probe. In some embodiments, the probe comprises at least one modified nucleotide. In some embodiments, the composition is a lyophilized composition. In some embodiments, the composition is in solution. In some embodiments, the composition comprises nucleic acids of a sample from a subject.

[0026] Further embodiments and details of the inventions are described below.

4. DETAILED DESCRIPTION

4.1. Definitions

[0027] To facilitate an understanding of the present invention, a number of terms and phrases are defined below:

[0028] As used herein, the terms “detect”, “detecting” or “detection” may describe either the general act of discovering or discerning or the specific observation of a detectably labeled composition.

[0029] As used herein, the term “detectably different” refers to a set of labels (such as dyes) that can be detected and distinguished simultaneously.

[0030] As used herein, the terms “patient” and “subject” are used interchangeably to refer to a human. In some embodiments, the methods described herein may be used on samples from non-human animals.

[0031] “*Trichomonas vaginalis*” refers to the protozoan responsible for trichomoniasis, a common sexually transmitted infection that can infect both men and

women. Trichomoniasis may be symptomatic or asymptomatic. Symptoms of trichomoniasis include, but are not limited to, vaginitis, urethritis, and cervicitis. Symptoms in females include, but are not limited to, itching, burning, redness, or soreness of the genitals, unusual odor, discomfort with urination, or a thin clear, white, yellow, or green discharge. Symptoms in males include, but are not limited to, itching or burning inside the penis, burning after ejaculation or urination, or penile discharge.

[0032] As used herein, the terms "oligonucleotide," "polynucleotide," "nucleic acid molecule," and the like, refer to nucleic acid-containing molecules, including but not limited to, DNA or RNA. The term encompasses sequences that include any of the known base analogs of DNA and RNA including, but not limited to, 4-acetylcytosine, 8-hydroxy-N6-methyladenosine, aziridinylcytosine, pseudoisocytosine, 5-(carboxyhydroxylmethyl) uracil, 5-fluorouracil, 5-bromouracil, 5-carboxymethylaminomethyl-2-thiouracil, 5-carboxymethyl-aminomethyluracil, dihydrouracil, inosine, N6-isopentenyladenine, 1-methyladenine, 1-methylpseudouracil, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-methyladenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarbonylmethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid, oxybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, N-uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid, pseudouracil, queosine, 2-thiocytosine, and 2,6-diaminopurine.

[0033] As used herein, the term "oligonucleotide," refers to a single-stranded polynucleotide having fewer than 500 nucleotides. In some embodiments, an oligonucleotide is 8 to 200, 8 to 100, 12 to 200, 12 to 100, 12 to 75, or 12 to 50 nucleotides long. Oligonucleotides may be referred to by their length, for example, a 24 residue oligonucleotide may be referred to as a "24-mer."

[0034] As used herein, the term "complementary" to a target gene (or target region thereof), and the percentage of "complementarity" of the probe sequence to the target gene sequence is the percentage "identity" to the sequence of target gene or to the reverse complement of the sequence of the target gene. In determining the degree of "complementarity" between probes used in the compositions described herein (or regions thereof) and a target gene, such as those disclosed herein, the degree of "complementarity" is expressed as the percentage identity between the sequence of the probe (or region thereof)

and sequence of the target gene or the reverse complement of the sequence of the target gene that best aligns therewith. The percentage is calculated by counting the number of aligned bases that are identical as between the 2 sequences, dividing by the total number of contiguous nucleotides in the probe, and multiplying by 100. When the term "complementary" is used, the subject oligonucleotide is at least 90% complementary to the target molecule, unless indicated otherwise. In some embodiments, the subject oligonucleotide is at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% complementary to the target molecule.

[0035] A "primer" or "probe" as used herein, refers to an oligonucleotide that comprises a region that is complementary to a sequence of at least 8 contiguous nucleotides of a target nucleic acid molecule, such as DNA (e.g., a target gene) or an mRNA (or a DNA reverse-transcribed from an mRNA). In some embodiments, a primer or probe comprises a region that is complementary to a sequence of at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, or at least 30 contiguous nucleotides of a target molecule. When a primer or probe comprises a region that is "complementary to at least x contiguous nucleotides of a target molecule," the primer or probe is at least 95% complementary to at least x contiguous nucleotides of the target molecule. In some embodiments, the primer or probe is at least 96%, at least 97%, at least 98%, at least 99%, or 100% complementary to the target molecule.

[0036] The term "nucleic acid amplification," encompasses any means by which at least a part of at least one target nucleic acid is reproduced, typically in a template-dependent manner, including without limitation, a broad range of techniques for amplifying nucleic acid sequences, either linearly or exponentially. Exemplary means for performing an amplifying step include polymerase chain reaction (PCR), ligase chain reaction (LCR), ligase detection reaction (LDR), multiplex ligation-dependent probe amplification (MLPA), ligation followed by Q-replicase amplification, primer extension, strand displacement amplification (SDA), hyperbranched strand displacement amplification, multiple displacement amplification (MDA), nucleic acid strand-based amplification (NASBA), two-step multiplexed amplifications, rolling circle amplification (RCA), and the like, including multiplex versions and combinations thereof, for example but not limited to, OLA/PCR, PCR/OLA, LDR/PCR, PCR/PCR/LDR, PCR/LDR, LCR/PCR, PCR/LCR (also known as

combined chain reaction--CCR), digital amplification, and the like. Descriptions of such techniques can be found in, among other sources, Ausbel et al.; PCR Primer: A Laboratory Manual, Diffenbach, Ed., Cold Spring Harbor Press (1995); The Electronic Protocol Book, Chang Bioscience (2002); Msuih et al., J. Clin. Micro. 34:501-07 (1996); The Nucleic Acid Protocols Handbook, R. Rapley, ed., Humana Press, Totowa, N.J. (2002); Abramson et al., Curr Opin Biotechnol. 1993 Feb.;4(1):41-7, U.S. Pat. No. 6,027,998; U.S. Pat. No. 6,605,451, Barany et al., PCT Publication No. WO 97/31256; Wenz et al., PCT Publication No. WO 01/92579; Day et al., Genomics, 29(1): 152-162 (1995), Ehrlich et al., Science 252:1643-50 (1991); Innis et al., PCR Protocols: A Guide to Methods and Applications, Academic Press (1990); Favis et al., Nature Biotechnology 18:561-64 (2000); and Rabenau et al., Infection 28:97-102 (2000); Belgrader, Barany, and Lubin, Development of a Multiplex Ligation Detection Reaction DNA Typing Assay, Sixth International Symposium on Human Identification, 1995 (available on the world wide web at: promega.com/geneticidproc/ussymp6proc/blegrad.html); LCR Kit Instruction Manual, Cat. #200520, Rev. #050002, Stratagene, 2002; Barany, Proc. Natl. Acad. Sci. USA 88:188-93 (1991); Bi and Sambrook, Nucl. Acids Res. 25:2924-2951 (1997); Zirvi et al., Nucl. Acid Res. 27:e40i-viii (1999); Dean et al., Proc Natl Acad Sci USA 99:5261-66 (2002); Barany and Gelfand, Gene 109:1-11 (1991); Walker et al., Nucl. Acid Res. 20:1691-96 (1992); Polstra et al., BMC Inf. Dis. 2:18- (2002); Lage et al., Genome Res. 2003 Feb.;13(2):294-307, and Landegren et al., Science 241:1077-80 (1988), Demidov, V., Expert Rev Mol Diagn. 2002 Nov.;2(6):542-8., Cook et al., J Microbiol Methods. 2003 May;53(2):165-74, Schweitzer et al., Curr Opin Biotechnol. 2001 Feb.;12(1):21-7, U.S. Pat. No. 5,830,711, U.S. Pat. No. 6,027,889, U.S. Pat. No. 5,686,243, PCT Publication No. WO0056927A3, and PCT Publication No. WO9803673A1.

[0037] In some embodiments, amplification comprises at least one cycle of the sequential procedures of: annealing at least one primer with complementary or substantially complementary sequences in at least one target nucleic acid; synthesizing at least one strand of nucleotides in a template-dependent manner using a polymerase; and denaturing the newly-formed nucleic acid duplex to separate the strands. The cycle may or may not be repeated. Amplification can comprise thermocycling or can be performed isothermally.

[0038] Unless otherwise indicated, the term "hybridize" is used herein refer to "specific hybridization" which is the binding, duplexing, or hybridizing of a nucleic acid molecule preferentially to a particular nucleotide sequence, in some embodiments, under stringent conditions. The term "stringent conditions" refers to conditions under which a

probe will hybridize preferentially to its target sequence, and to a lesser extent to, or not at all to, other sequences. A "stringent hybridization" and "stringent hybridization wash conditions" in the context of nucleic acid hybridization (*e.g.*, as in array, Southern, or Northern hybridization) are sequence-dependent and are different under different environmental parameters. An extensive guide to the hybridization of nucleic acids is found in, e.g., Tijssen (1993) Laboratory Techniques in Biochemistry and Molecular Biology--Hybridization with Nucleic Acid Probes part I, Ch. 2, "Overview of principles of hybridization and the strategy of nucleic acid probe assays," Elsevier, NY ("Tijssen"). Generally, highly stringent hybridization and wash conditions for filter hybridizations are selected to be about 5° C. lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH. The T_m is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. Very stringent conditions are selected to be equal to the T_m for a particular probe. Dependency of hybridization stringency on buffer composition, temperature, and probe length are well known to those of skill in the art (*see, e.g.*, Sambrook and Russell (2001) Molecular Cloning: A Laboratory Manual (3rd ed.) Vol. 1-3, Cold Spring Harbor Laboratory, Cold Spring Harbor Press, NY).

[0039] A "sample," as used herein, includes urine samples (including samples derived from urine samples), endocervical swabs, and patient-collected vaginal swabs, and other types of human samples. In some embodiments, a urine sample is a "first catch" urine sample, which is a sample taken as a subject first begins to urinate. As used herein, urine samples include, but are not limited to, whole urine, a sample comprising cells from a urine sample, a sample comprising the cell pellet isolated by centrifugation of a urine sample, a sample comprising cells isolated by filtration of a urine sample, and the like. In some embodiments, a urine sample comprises a buffer, such as a preservative. In some embodiments, a sample is a human sample other than a urine sample, such as an endocervical swab or a vaginal swab, including a patient-collected vaginal swab, and a urethral swab. In some embodiments, a swab sample comprises a buffer, such as a preservative.

[0040] An "endogenous control," as used herein refers to a moiety that is naturally present in the sample to be used for detection. In some embodiments, an endogenous control is a "sample adequacy control" (SAC), which may be used to determine whether there was sufficient sample used in the assay, or whether the sample comprised sufficient

biological material, such as cells. In some embodiments, an SAC is a single copy human gene. In some embodiments, an endogenous control, such as an SAC, is selected that can be detected in the same manner as the target gene is detected and, in some embodiments, simultaneously with the target gene.

[0041] An "exogenous control," as used herein, refers to a moiety that is added to a sample or to an assay, such as a "sample processing control" (SPC). In some embodiments, an exogenous control is included with the assay reagents. An exogenous control is typically selected that is not expected to be present in the sample to be used for detection, or is present at very low levels in the sample such that the amount of the moiety naturally present in the sample is either undetectable or is detectable at a much lower level than the amount added to the sample as an exogenous control. In some embodiments, an exogenous control comprises a nucleotide sequence that is not expected to be present in the sample type used for detection of the target gene. In some embodiments, an exogenous control comprises a nucleotide sequence that is not known to be present in the species from whom the sample is taken. In some embodiments, an exogenous control comprises a nucleotide sequence from a different species than the subject from whom the sample was taken. In some embodiments, an exogenous control comprises a nucleotide sequence that is not known to be present in any species. In some embodiments, an exogenous control is selected that can be detected in the same manner as the target gene is detected and, in some embodiments, simultaneously with the target gene. In some embodiments, an exogenous control is a bacterial DNA. In some embodiments, the bacterium is a species not expected to be found in the sample type being tested.

[0042] In the sequences herein, "U" and "T" are used interchangeably, such that both letters indicate a uracil or thymine at that position. One skilled in the art will understand from the context and/or intended use whether a uracil or thymine is intended and/or should be used at that position in the sequence. For example, one skilled in the art would understand that native RNA molecules typically include uracil, while native DNA molecules typically include thymine. Thus, where an RNA sequence includes "T", one skilled in the art would understand that that position in the native RNA is likely a uracil.

[0043] In the present disclosure, "a sequence selected from" encompasses both "one sequence selected from" and "one or more sequences selected from." Thus, when "a sequence selected from" is used, it is to be understood that one, or more than one, of the listed sequences may be chosen.

4.2. Detecting *Trichomonas vaginalis*

[0044] The present inventors have developed an assay for detecting *Trichomonas vaginalis* (TV). In some embodiments, the assay comprises detecting the TV 40S ribosomal protein (Tv40Srp) gene. In some embodiments, the assay comprises detecting RNA transcribed from the TV 40S ribosomal protein (Tv40Srp) gene. The present assay relies on the polymerase chain reaction (PCR), and can be carried out in a substantially automated manner using a commercially available nucleic acid amplification system. Exemplary nonlimiting nucleic acid amplification systems that can be used to carry out the methods of the invention include the GeneXpert® system, a GeneXpert® Infinity system, and a Smartcycler System (Cepheid, Sunnyvale, CA). The present assay can be completed in under 3 hours, and in some embodiments, under 2 hours, using an automated system, for example, the GeneXpert® system.

4.2.1. General methods

[0045] Compositions and methods for detecting *Trichomonas vaginalis* (TV) are provided. In some embodiments, the method comprises detecting the TV 40S ribosomal protein (Tv40Srp) gene.

[0046] In some embodiments, a method of detecting *Trichomonas vaginalis* (TV) in a subject comprises detecting the presence of the TV 40S ribosomal protein (Tv40Srp) gene in a sample from the subject. In some embodiments, the sample is selected from a urine sample, an endocervical swab, and a vaginal swab. In some embodiments, the urine sample is a first catch urine sample.

[0047] In some embodiments, a method of detecting TV further comprises detecting at least one endogenous control, such as a sample adequacy control (SAC). In some embodiments, a method of detecting TV further comprises detecting at least one exogenous control, such as a sample processing control (SPC). In some embodiments, a method of detecting TV further comprises detecting at least one endogenous control and at least one exogenous control.

[0048] In some embodiments, a method of detecting TV comprises detecting the TV 40S ribosomal protein (Tv40Srp) gene in a sample. In some embodiments, a method of detecting TV further comprises detecting a sample adequacy control (SAC), such as a single copy human gene. In some embodiments, a method of detecting TV further comprises detecting a sample processing control (SPC), such as an exogenously added bacterial DNA.

In some embodiments, a method of detecting TV further comprises detecting an SAC and an SPC.

[0049] In the present disclosure, the term "target gene" is used for convenience to refer to the TV 40S ribosomal protein (Tv40Srp) gene, and also to exogenous and/or endogenous controls. Thus, it is to be understood that when a discussion is presented in terms of a target gene, that discussion is specifically intended to encompass the TV 40S ribosomal protein (Tv40Srp) gene, the endogenous control(s) (e.g., SAC), and the exogenous control(s) (e.g., SPC).

[0050] In some embodiments, the presence of the TV 40S ribosomal protein (Tv40Srp) gene is detected in a urine sample. In some embodiments, the target gene is detected in a urine sample to which a buffer (such as a preservative) has been added. In some embodiments, the buffer is added to a urine sample at a ratio of 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:9, or 1:10 buffer:urine. In some embodiments, the presence of the TV 40S ribosomal protein (Tv40Srp) gene is detected in an endocervical swab sample or a vaginal swab sample. In some embodiments, the vaginal swab is a patient-collected vaginal swab. In some embodiments, the target gene is detected in an endocervical swab sample or a vaginal swab sample that has been placed in a buffer (such as a preservative). In some embodiments, a swab is placed in 1 mL, 2 mL, 2.5 mL of buffer.

[0051] In some embodiments, detection of the TV 40S ribosomal protein (Tv40Srp) gene in a sample from a subject indicates the presence of *Trichomonas vaginalis* in the subject. In some embodiments, the detecting is done quantitatively. In other embodiments, the detecting is done qualitatively. In some embodiments, detecting a target gene comprises forming a complex comprising a polynucleotide and a nucleic acid selected from a target gene, a DNA amplicon of a target gene, and a complement of a target gene. In some embodiments, detecting a target gene comprises PCR. In some embodiments, detecting a target gene comprises quantitative PCR or real-time PCR. In some embodiments, a sample adequacy control (SAC) and/or a sample processing control (SPC) is detected in the same assay as the target gene. In some embodiments, if the TV 40S ribosomal protein (Tv40Srp) gene is detected, TV is considered to be detected even if the SPC and/or SAC are not detected in the assay. In some embodiments, if the TV 40S ribosomal protein (Tv40Srp) gene is not detected, TV is considered to be not detected only if the SPC and SAC are also detected in the assay.

[0052] In some embodiments, the presence of the TV 40S ribosomal protein (Tv40Srp) gene can be measured in samples collected at one or more times from a subject to

monitor treatment for TV infection in the subject. Treatments include, but are not limited to, a single dose or multiple doses of metronidazole or tinidazole. In some embodiments, a subject with a history of TV infection is monitored for recurrence of TV by detecting the presence or absence of the TV 40S ribosomal protein (Tv40Srp) gene at regular or semi-regular intervals. In some such embodiments, the patient is monitored by detecting the presence or absence of the TV 40S ribosomal protein (Tv40Srp) gene at least once per month, at least once every two months, at least once every three months, at least once every four months, at least once every five months, at least once every six months, at least once every nine months, at least once per year, or at least once every two years.

[0053] In some embodiments, the present assay may be used as part of routine and/or preventative healthcare for a subject. That is, in some embodiments, the present assay may be used to test an individual for TV infection whether or not the individual has exhibited symptoms of TV infection or has a history of TV infection. In some embodiments, the present assay is used to detect TV infection in subjects who are pregnant and/or who are attempting to become pregnant. In some instances, pregnant women with TV are more likely to experience pre-term delivery and/or have babies with low birth weight (less than 5.5 pounds).

[0054] In some embodiments, a sample to be tested is a urine sample (such as a first catch urine sample), or is derived from a urine sample. In some embodiments, a buffer (such as a preservative) is added to the urine sample. In some embodiments, the buffer is added to the urine sample within 1 hour, within 2 hours, within 3 hours, within 4 hours, within 5 hours, within 6 hours, within 7 hours, or within 8 hours of sample collection.

[0055] In some embodiments, a sample to be tested is an endocervical swab sample or a vaginal swab sample. In some embodiments, the swab is placed in a buffer. In some embodiments, the swab is immediately placed in the buffer. In some embodiments, the swab is placed in the buffer within 1 hour, within 2 hours, within 3 hours, within 4 hours, within 5 hours, within 6 hours, within 7 hours, or within 8 hours of sample collection.

[0056] In some embodiments, less than 5 ml, less than 4 ml, less than 3 ml, less than 2 ml, less than 1 ml, or less than 0.75 ml of urine are used in the present methods. In some embodiments, 0.1 ml to 1 ml of urine is used in the present methods.

[0057] In some embodiments, the sample to be tested is another bodily fluid, such as blood, sputum, mucus, saliva, vaginal or penile discharge, semen, etc.

[0058] The clinical sample to be tested is, in some embodiments, fresh (i.e., never frozen). In other embodiments, the sample is a frozen specimen. In some embodiments, the

sample is a tissue sample, such as a formalin-fixed paraffin embedded sample. In some embodiments, the sample is a liquid cytology sample.

[0059] In some embodiments, the sample to be tested is obtained from an individual who has one or more symptoms of TV infection. Nonlimiting exemplary symptoms of TV infection include vaginitis, urethritis, and cervicitis; in females: itching, burning, redness, or soreness of the genitals, unusual odor, discomfort with urination, and a thin clear, white, yellow, or green discharge; and in males: itching or burning inside the penis, burning after ejaculation or urination, and penile discharge. In some embodiments, the sample to be tested is obtained from an individual who has previously been diagnosed with TV infection. In some such embodiments, the individual is monitored for recurrence of TV infection.

[0060] In some embodiments, methods described herein can be used for routine screening of healthy individuals with no risk factors. In some embodiments, methods described herein are used to screen asymptomatic individuals, for example, during routine or preventative care. In some embodiments, methods described herein are used to screen women who are pregnant or who are attempting to become pregnant.

[0061] In some embodiments, the methods described herein can be used to assess the effectiveness of a treatment for TV infection in a patient.

[0062] In some embodiments, use of the the TV 40S ribosomal protein (Tv40Srp) gene for detecting TV infection is provided. In some embodiments, use of the the TV 40S ribosomal protein (Tv40Srp) gene for monitoring recurrence of TV infection is provided.

[0063] In any of the embodiments described herein, the TV 40S ribosomal protein (Tv40Srp) gene may be detected in the same assay reaction as a sample processing control (SPC) and/or sample adequacy control (SAC).

[0064] In some embodiments, a method of facilitating detection of TV infection in a subject is provided. Such methods comprise detecting the presence or absence of the TV 40S ribosomal protein (Tv40Srp) gene in a sample from the subject. In some embodiments, information concerning the presence or absence of the TV 40S ribosomal protein (Tv40Srp) gene in the sample from the subject is communicated to a medical practitioner. A "medical practitioner," as used herein, refers to an individual or entity that diagnoses and/or treats patients, such as a hospital, a clinic, a physician's office, a physician, a nurse, or an agent of any of the aforementioned entities and individuals. In some embodiments, detecting the presence or absence of TV 40S ribosomal protein (Tv40Srp) gene is carried out at a laboratory that has received the subject's sample from the medical practitioner or agent of the medical practitioner. The laboratory carries out the detection by any method, including

those described herein, and then communicates the results to the medical practitioner. A result is "communicated," as used herein, when it is provided by any means to the medical practitioner. In some embodiments, such communication may be oral or written, may be by telephone, in person, by e-mail, by mail or other courier, or may be made by directly depositing the information into, e.g., a database accessible by the medical practitioner, including databases not controlled by the medical practitioner. In some embodiments, the information is maintained in electronic form. In some embodiments, the information can be stored in a memory or other computer readable medium, such as RAM, ROM, EEPROM, flash memory, computer chips, digital video discs (DVD), compact discs (CDs), hard disk drives (HDD), magnetic tape, etc.

[0065] In some embodiments, methods of detecting TV are provided. In some embodiments, methods of diagnosing TV infection are provided. In some embodiments, the method comprises obtaining a sample from a subject and providing the sample to a laboratory for detection of the TV 40S ribosomal protein (Tv40Srp) gene in the sample. In some embodiments, the method further comprises receiving a communication from the laboratory that indicates the presence or absence of the TV 40S ribosomal protein (Tv40Srp) gene in the sample. A "laboratory," as used herein, is any facility that detects the target gene in a sample by any method, including the methods described herein, and communicates the result to a medical practitioner. In some embodiments, a laboratory is under the control of a medical practitioner. In some embodiments, a laboratory is not under the control of the medical practitioner.

[0066] When a laboratory communicates the result of detecting the presence or absence of the TV 40S ribosomal protein (Tv40Srp) gene to a medical practitioner, in some embodiments, the laboratory indicates whether or not the TV 40S ribosomal protein (Tv40Srp) gene was detected in the sample. In some embodiments, the laboratory indicates whether the sample comprises *Trichomonas vaginalis* (TV), by indicating, for example, "TV positive" or "TV negative" or "TV present" or "TV absent," and the like.

[0067] As used herein, when a method relates to detecting TV, determining the presence of TV, monitoring for TV, and/or diagnosing TV infection, the method includes activities in which the steps of the method are carried out, but the result is negative for the presence of TV. That is, detecting, determining, monitoring, and diagnosing TV or TV infection include instances of carrying out the methods that result in either positive or negative results.

[0068] In some embodiments, at least one endogenous control (e.g., an SAC) and/or at least one exogenous control (e.g., an SPC) are detected simultaneously with the TV 40S ribosomal protein (Tv40Srp) gene in a single reaction.

4.2.2. Exemplary controls

[0069] In some embodiments, an assay described herein comprises detecting the TV 40S ribosomal protein (Tv40Srp) gene and at least one endogenous control. In some embodiments, the endogenous control is a sample adequacy control (SAC). In some such embodiments, if the TV 40S ribosomal protein (Tv40Srp) gene is not detected in a sample, and the SAC is also not detected in the sample, the assay result is considered "invalid" because the sample may have been insufficient. While not intending to be bound by any particular theory, an insufficient sample may be too dilute, contain too little cellular material, contain an assay inhibitor, etc. In some embodiments, the failure to detect an SAC may indicate that the assay reaction failed. In some embodiments, an endogenous control (such as an SAC) is a single-copy human gene. Nonlimiting exemplary SACs include human hydroxymethyl-bilane synthase (HMBS), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), beta actin, beta2-microglobin, cyclooxygenase 1, hypoxanthine phosphoribosyl-transferase, porphobilinogen deaminase, and the transferrin receptor.

[0070] In some embodiments, an assay described herein comprises detecting the TV 40S ribosomal protein (Tv40Srp) gene and at least one exogenous control. In some embodiments, the exogenous control is a sample processing control (SPC). In some such embodiments, if the TV 40S ribosomal protein (Tv40Srp) gene is not detected in a sample, and the SPC is also not detected in the sample, the assay result is considered "invalid" because there may have been an error in sample processing, including but not limited to, failure of the assay. Nonlimiting exemplary errors in sample processing include, inadequate sample processing, the presence of an assay inhibitor, compromised reagents, etc. In some embodiments, an exogenous control (such as an SPC) is added to a sample. In some embodiments, an exogenous control (such as an SPC) is added during performance of an assay, such as with one or more buffers or reagents. In some embodiments, when a GeneXpert® system is to be used, the SPC is included in the GeneXpert® cartridge. In some embodiments, an exogenous control (such as an SPC) is a DNA sequence that is not expected to be present in the sample being assayed. Nonlimiting exemplary SPCs include bacterial genes not expected to be present in the sample being assayed.

[0071] In some embodiments, an endogenous control and/or an exogenous control is detected contemporaneously, such as in the same assay, as detection of the TV 40S ribosomal protein (Tv40Srp) gene in a sample. In some embodiments, an assay comprises reagents for detecting the TV 40S ribosomal protein (Tv40Srp) gene, an exogenous control, and an endogenous control simultaneously in the same assay reaction. In some such embodiments, for example, an assay reaction comprises a primer set for amplifying the TV 40S ribosomal protein (Tv40Srp) gene, a primer set for amplifying an endogenous control, and a primer set for amplifying an exogenous control, and labeled probes for detecting the amplification products (such as, for example, TaqMan® probes).

4.2.3. Exemplary sample preparation

4.2.3.1. Exemplary buffers

[0072] In some embodiments, a buffer is added to a urine sample. In some embodiments, the buffer is added within one hour, two hours, three hours, or six hours of the time the urine sample was collected (e.g., voided). In some embodiments, a buffer is added to the urine sample within one hour, two hours, three hours, or six hours before the sample is analyzed by the methods described herein.

[0073] In some embodiments, a swab sample is placed in a buffer. In some embodiments, the swab sample is placed in the buffer within one hour, two hours, three hours, or six hours of the time the swab sample was collected. In some embodiments, the swab sample is placed in a buffer within one hour, two hours, three hours, or six hours before the sample is analyzed by the methods described herein.

[0074] Non-limiting exemplary commercial buffers include PreservCyt (Hologic, Bedford, MA), SurePath (BD, Franklin Lakes, NJ), and CyMol (Copan Diagnostics, Murrietta, CA).

4.2.3.2. Exemplary DNA preparation

[0075] Sample DNA can be prepared by any appropriate method. In some embodiments, target DNA is prepared by contacting a sample with a lysis buffer and binding DNA to a DNA binding substrate, such as a glass or silica substrate. The binding substrate may have any suitable form, such as a particulate, porous solid, or membrane form. For example, the support may comprise hydroxycellulose, glass fiber, cellulose, nitrocellulose, zirconium hydroxide, titanium (IV) oxide, silicon dioxide, zirconium silicate, or silica particles (e.g., see U.S. Patent No. 5,234,809). Many such DNA binding substrates are known in the art.

[0076] In some embodiments, DNA is detected in a lysate without first isolating or separating the DNA. In some some embodiments, the sample is subject to a lysis step to release the DNA. Non-limiting exemplary lysis methods include sonication (for example, for 2-15 seconds, 8-18 μm at 36 kHz); chemical lysis, for example, using a detergent; and various commercially available lysis reagents. In some embodiments, DNA is detected are measured in a sample in which DNA has been isolated or separated from at least some other cellular components.

[0077] When the methods discussed herein indicate that a target gene is detected, such detection may be carried out on a complement of a target gene instead of, or in addition to, the target gene sequence shown herein. In some embodiments, when the complement of a target gene is detected, a polynucleotide for detection is used that is complementary to the complement of the target gene. In some some embodiments, a polynucleotide for detection comprises at least a portion that is at least 90%, at least 95%, or 100% identical in sequence to the target gene, although it may comprise modified nucleotides.

4.2.4. Exemplary analytical methods

[0078] As described above, methods are presented for detecting *Trichomonas vaginalis*. The methods comprise detecting the presence of the TV 40S ribosomal protein (Tv40Srp) gene in a sample from a subject. In some embodiments, the method further comprises detecting at least one endogenous control (such as an SAC) and/or at least one exogenous control (such as an SPC). In some embodiments, detection of the TV 40S ribosomal protein (Tv40Srp) gene indicates the presence of TV, even if the endogenous control and/or exogenous control is not detected in the assay. In some embodiments, if the TV 40S ribosomal protein (Tv40Srp) gene is not detected, the result is considered to be negative for TV only if the controls are detected. In some embodiments, if the TV 40S ribosomal protein (Tv40Srp) gene is not detected, the result is considered to be negative for TV only if the endogenous control and exogenous control are detected.

[0079] Any analytical procedure capable of permitting specific detection of a target gene may be used in the methods herein presented. Exemplary nonlimiting analytical procedures include, but are not limited to, nucleic acid amplification methods, PCR methods, isothermal amplification methods, and other analytical detection methods known to those skilled in the art.

[0080] In some embodiments, the method of detecting a target gene, such as the TV 40S ribosomal protein (Tv40Srp) gene, comprises amplifying the gene and/or a complement thereof. Such amplification can be accomplished by any method. Exemplary methods include, but are not limited to, isothermal amplification, real time PCR, endpoint PCR, and amplification using T7 polymerase from a T7 promoter annealed to a DNA, such as provided by the SenseAmp Plus™ Kit available at Implen, Germany.

[0081] When a target gene is amplified, in some embodiments, an amplicon of the target gene is formed. An amplicon may be single stranded or double-stranded. In some embodiments, when an amplicon is single-stranded, the sequence of the amplicon is related to the target gene in either the sense or antisense orientation. In some embodiments, an amplicon of a target gene is detected rather than the target gene itself. Thus, when the methods discussed herein indicate that a target gene is detected, such detection may be carried out on an amplicon of the target gene instead of, or in addition to, the target gene itself. In some embodiments, when the amplicon of the target gene is detected rather than the target gene, a polynucleotide for detection is used that is complementary to the complement of the target gene. In some embodiments, when the amplicon of the target gene is detected rather than the target gene, a polynucleotide for detection is used that is complementary to the target gene. Further, in some embodiments, multiple polynucleotides for detection may be used, and some polynucleotides may be complementary to the target gene and some polynucleotides may be complementary to the complement of the target gene.

[0082] In some embodiments, the method of detecting the TV 40S ribosomal protein (Tv40Srp) gene comprises PCR, as described below. In some embodiments, detecting one or more target genes comprises real-time monitoring of a PCR reaction, which can be accomplished by any method. Such methods include, but are not limited to, the use of TaqMan®, molecular beacons, or Scorpion probes (i.e., energy transfer (ET) probes, such as FRET probes) and the use of intercalating dyes, such as SYBR green, EvaGreen, thiazole orange, YO-PRO, TO-PRO, etc.

[0083] Nonlimiting exemplary conditions for amplifying a target gene are as follows. An exemplary cycle comprises an initial denaturation at 90°C to 100°C for 30 seconds to 5 minutes, followed by cycling that comprises denaturation at 90°C to 100°C for 1 to 10 seconds, followed by annealing and amplification at 60°C to 75°C for 10 to 30 seconds. A further exemplary cycle comprises 1 minute at 95°C, followed by up to 40 cycles of 5 seconds at 92.5°C, 20 seconds at 68°C. In some embodiments, for the first

cycle following the initial denaturation step, the cycle denaturation step is omitted. In some embodiments, Taq polymerase is used for amplification. In some embodiments, the cycle is carried out at least 10 times, at least 15 times, at least 20 times, at least 25 times, at least 30 times, at least 35 times, at least 40 times, or at least 45 times. In some embodiments, Taq is used with a hot start function. In some embodiments, the amplification reaction occurs in a GeneXpert® cartridge, and amplification of the TV 40S ribosomal protein (Tv40Srp) gene, an endogenous control, and an exogenous control occurs in the same reaction. In some embodiments, detection of the TV 40S ribosomal protein (Tv40Srp) gene occurs in less than 3 hours, less than 2.5 hours, less than 2 hours, less than 1 hour, or less than 30 minutes from initial denaturation through the last extension.

[0084] In some embodiments, detection of a target gene comprises forming a complex comprising a polynucleotide that is complementary to a target gene or to a complement thereof, and a nucleic acid selected from the target gene, a DNA amplicon of the target gene, and a complement of the target gene. Thus, in some embodiments, the polynucleotide forms a complex with a target gene. In some embodiments, the polynucleotide forms a complex with a complement of the target gene. In some embodiments, the polynucleotide forms a complex with a DNA amplicon of the target gene. When a double-stranded DNA amplicon is part of a complex, as used herein, the complex may comprise one or both strands of the DNA amplicon. Thus, in some embodiments, a complex comprises only one strand of the DNA amplicon. In some embodiments, a complex is a triplex and comprises the polynucleotide and both strands of the DNA amplicon. In some embodiments, the complex is formed by hybridization between the polynucleotide and the target gene, complement of the target gene, or DNA amplicon of the target gene. The polynucleotide, in some embodiments, is a primer or probe.

[0085] In some embodiments, a method comprises detecting the complex. In some embodiments, the complex does not have to be associated at the time of detection. That is, in some embodiments, a complex is formed, the complex is then dissociated or destroyed in some manner, and components from the complex are detected. An example of such a system is a TaqMan® assay. In some embodiments, when the polynucleotide is a primer, detection of the complex may comprise amplification of the target gene, a complement of the target gene, or a DNA amplicon of the target gene.

[0086] In some embodiments the analytical method used for detecting at least one target gene in the methods set forth herein includes real-time quantitative PCR. In some embodiments, the analytical method used for detecting at least one target gene includes the

use of a TaqMan® probe. The assay uses energy transfer ("ET"), such as fluorescence resonance energy transfer ("FRET"), to detect and quantitate the synthesized PCR product. Typically, the TaqMan® probe comprises a fluorescent dye molecule coupled to the 5'-end and a quencher molecule coupled to the 3'-end, such that the dye and the quencher are in close proximity, allowing the quencher to suppress the fluorescence signal of the dye via FRET. When the polymerase replicates the chimeric amplicon template to which the TaqMan® probe is bound, the 5'-nuclease of the polymerase cleaves the probe, decoupling the dye and the quencher so that the dye signal (such as fluorescence) is detected. Signal (such as fluorescence) increases with each PCR cycle proportionally to the amount of probe that is cleaved.

[0087] In some embodiments, a target gene is considered to be detected if any signal is generated from the TaqMan probe during the PCR cycling. For example, in some embodiments, if the PCR includes 40 cycles, if a signal is generated at any cycle during the amplification, the target gene is considered to be present and detected. In some some embodiments, if no signal is generated by the end of the PCR cycling, the target gene is considered to be absent and not detected.

[0088] In some embodiments, quantitation of the results of real-time PCR assays is done by constructing a standard curve from a nucleic acid of known concentration and then extrapolating quantitative information for target genes of unknown concentration. In some embodiments, the nucleic acid used for generating a standard curve is a DNA (for example, an endogenous control, or an exogenous control). In some embodiments, the nucleic acid used for generating a standard curve is a purified double-stranded plasmid DNA or a single-stranded DNA generated in vitro.

[0089] In some embodiments, in order for an assay to indicate that TV is not present in a sample, the Ct values for an endogenous control (such as an SAC) and/or an exogenous control (such as an SPC) must be within a previously-determined valid range. That is, in some embodiments, the absence of TV cannot be confirmed unless the controls are detected, indicating that the assay was successful. Ct values are inversely proportional to the amount of nucleic acid target in a sample.

[0090] In some embodiments, a threshold Ct (or a "cutoff Ct") value for a target gene (including an endogenous control and/or exogenous control), below which the gene is considered to be detected, has previously been determined. In some embodiments, a threshold Ct is determined using substantially the same assay conditions and system (such as a GeneXpert®) on which the samples will be tested.

[0091] In addition to the TaqMan® assays, other real-time PCR chemistries useful for detecting and quantitating PCR products in the methods presented herein include, but are not limited to, Molecular Beacons, Scorpion probes and intercalating dyes, such as SYBR Green, EvaGreen, thiazole orange, YO-PRO, TO-PRO, etc., which are discussed below.

[0092] In various embodiments, real-time PCR detection is utilized to detect, in a single multiplex reaction, the TV 40S ribosomal protein (Tv40Srp) gene, an endogenous control, and an exogenous control. In some multiplex embodiments, a plurality of probes, such as TaqMan® probes, each specific for a different target, is used. In some embodiments, each target gene-specific probe is spectrally distinguishable from the other probes used in the same multiplex reaction.

[0093] Real-time PCR is performed using any PCR instrumentation available in the art. Typically, instrumentation used in real-time PCR data collection and analysis comprises a thermal cycler, optics for fluorescence excitation and emission collection, and optionally a computer and data acquisition and analysis software.

[0094] In some embodiments, detection and/or quantitation of real-time PCR products is accomplished using a dye that binds to double-stranded DNA products, such as SYBR Green, EvaGreen, thiazole orange, YO-PRO, TO-PRO, etc. In some embodiments, the analytical method used in the methods described herein is a DASL® (DNA-mediated Annealing, Selection, Extension, and Ligation) Assay. In some embodiments, the analytical method used for detecting and quantifying the target genes in the methods described herein is a bead-based flow cytometric assay. *See* Lu J. et al. (2005) Nature 435:834-838, which is incorporated herein by reference in its entirety. An example of a bead-based flow cytometric assay is the xMAP® technology of Luminex, Inc. *See* www.luminexcorp.com/technology/index.html. In some embodiments, the analytical method used for detecting and quantifying the levels of the at least one target gene in the methods described herein is by gel electrophoresis and detection with labeled probes (e.g., probes labeled with a radioactive or chemiluminescent label), such as by northern blotting. In some embodiments, exemplary probes contain one or more affinity-enhancing nucleotide analogs as discussed below, such as locked nucleic acid ("LNA") analogs, which contain a bicyclic sugar moiety instead of deoxyribose or ribose sugars. *See, e.g.*, Várallyay, E. et al. (2008) Nature Protocols 3(2):190-196, which is incorporated herein by reference in its entirety. In some embodiments, detection and quantification of one or more target genes is accomplished using microfluidic devices and single-molecule detection.

[0095] Optionally, the sample DNA is modified before hybridization. The target DNA/probe duplex is then passed through channels in a microfluidic device and that comprise detectors that record the unique signal of the 3 labels. In this way, individual molecules are detected by their unique signal and counted. See U.S. Patent Nos. 7,402,422 and 7,351,538 to Fuchs et al., U.S. Genomics, Inc., each of which is incorporated herein by reference in its entirety.

4.2.5. Exemplary Automation and Systems

[0096] In some embodiments, gene expression is detected using an automated sample handling and/or analysis platform. In some embodiments, commercially available automated analysis platforms are utilized. For example, in some embodiments, the GeneXpert® system (Cepheid, Sunnyvale, CA) is utilized.

[0097] The present invention is illustrated for use with the GeneXpert system. Exemplary sample preparation and analysis methods are described below. However, the present invention is not limited to a particular detection method or analysis platform. One of skill in the art recognizes that any number of platforms and methods may be utilized.

[0098] The GeneXpert® utilizes a self-contained, single use cartridge. Sample extraction, amplification, and detection may all carried out within this self-contained "laboratory in a cartridge." (See e.g., US Patents 5,958,349, 6,403,037, 6,440,725, 6,783,736, 6,818,185; each of which is herein incorporated by reference in its entirety.)

[0099] Components of the cartridge include, but are not limited to, processing chambers containing reagents, filters, and capture technologies useful to extract, purify, and amplify target nucleic acids. A valve enables fluid transfer from chamber to chamber and contain nucleic acids lysis and filtration components. An optical window enables real-time optical detection. A reaction tube enables very rapid thermal cycling.

[00100] In some embodiments, the GenXpert® system includes a plurality of modules for scalability. Each module includes a plurality of cartridges, along with sample handling and analysis components.

[00101] After the sample is added to the cartridge, the sample is contacted with lysis buffer and released DNA is bound to a DNA-binding substrate such as a silica or glass substrate. The sample supernatant is then removed and the DNA eluted in an elution buffer such as a Tris/EDTA buffer. The eluate may then be processed in the cartridge to detect target genes as described herein. In some embodiments, the eluate is used to reconstitute at least some of the PCR reagents, which are present in the cartridge as lyophilized particles.

[00102] In some embodiments, PCR is used to amplify and analyze the presence of the target genes. In some embodiments, the PCR uses Taq polymerase with hot start function, such as AptaTaq (Roche). In some embodiments, the initial denaturation is at 90°C to 100°C for 30 seconds to 5 minutes; the cycling denaturation temperature is 90°C to 100°C for 1 to 10 seconds; the cycling anneal and amplification temperature is 60°C to 75°C for 10 to 30 seconds; and up to 50 cycles are performed.

[00103] In some embodiments, a double-denature method is used to amplify low copy number targets. A double-denature method comprises, in some embodiments, a first denaturation step followed by addition of primers and/or probes for detecting target genes. All or a substantial portion of the DNA-containing sample (such as a DNA eluate) is then denatured a second time before, in some instances, a portion of the sample is aliquotted for cycling and detection of the target genes. While not intending to be bound by any particular theory, the double-denature protocol may increase the chances that a low copy number target gene (or its complement) will be present in the aliquot selected for cycling and detection because the second denaturation effectively doubles the number of targets (i.e., it separates the target and its complement into two separate templates) before an aliquot is selected for cycling. In some embodiments, the first denaturation step comprises heating to a temperature of 90°C to 100°C for a total time of 30 seconds to 5 minutes. In some embodiments, the second denaturation step comprises heating to a temperature of 90°C to 100°C for a total time of 5 seconds to 3 minutes. In some embodiments, the first denaturation step and/or the second denaturation step is carried out by heating aliquots of the sample separately. In some embodiments, each aliquot may be heated for the times listed above. As a non-limiting example, a first denaturation step for a DNA-containing sample (such as a DNA eluate) may comprise heating at least one, at least two, at least three, or at least four aliquots of the sample separately (either sequentially or simultaneously) to a temperature of 90°C to 100°C for 60 seconds each. As a non-limiting example, a second denaturation step for a DNA-containing sample (such as a DNA eluate) containing enzyme, primers, and probes may comprise heating at least one, at least two, at least three, or at least four aliquots of the eluate separately (either sequentially or simultaneously) to a temperature of 90°C to 100°C for 5 seconds each. In some embodiments, an aliquot is the entire DNA-containing sample (such as a DNA eluate). In some embodiments, an aliquot is less than the entire DNA-containing sample (such as a DNA eluate).

[00104] In some embodiments, target genes in a DNA-containing sample, such as a DNA eluate, are detected using the following protocol: One or more aliquots of the DNA-containing sample are heated separately to 95°C for 60 seconds each. The enzyme and primers and probes are added to the DNA-containing sample and one or more aliquots are heated separately to 95°C for 5 seconds each. At least one aliquot of the DNA-containing sample containing enzyme, primers, and probes is then heated to 94°C for 60 seconds. The aliquot is then cycled 45 times with the following 2-step cycle: (1) 94°C for 5 seconds, (2) 66°C for 30 seconds.

[00105] The present invention is not limited to particular primer and/or probe sequences. Exemplary amplification primers and detection probes are described in the Examples.

[00106] In some embodiments, an off-line centrifugation is used, for example, with samples with low cellular content. The sample, with or without a buffer added, is centrifuged and the supernatant removed. The pellet is then resuspended in a smaller volume of either supernatant or the buffer. The resuspended pellet is then analyzed as described herein.

4.2.6. Exemplary Data Analysis

[00107] In some embodiments, the presence of TV is detected if the Ct value for the TV 40S ribosomal protein (Tv40Srp) gene is below a certain threshold. In some embodiments the valid range of Ct values is 9 to 39.9 Ct. In some such embodiments, if no amplification above background is observed from the TV-specific primers after 40 cycles, the sample is considered to be negative for TV.

[00108] In some embodiments, a computer-based analysis program is used to translate the raw data generated by the detection assay into data of predictive value for a clinician. The clinician can access the predictive data using any suitable means. Thus, in some embodiments, the present invention provides the further benefit that the clinician, who is not likely to be trained in genetics or molecular biology, need not understand the raw data. The data is presented directly to the clinician in its most useful form. The clinician is then able to immediately utilize the information in order to optimize the care of the subject.

[00109] The present invention contemplates any method capable of receiving, processing, and transmitting the information to and from laboratories conducting the assays, information provides, medical personal, and subjects. For example, in some embodiments of the present invention, a sample (*e.g.*, a biopsy or a serum or urine sample) is obtained

from a subject and submitted to a profiling service (*e.g.*, clinical lab at a medical facility, genomic profiling business, etc.), located in any part of the world (*e.g.*, in a country different than the country where the subject resides or where the information is ultimately used) to generate raw data. Where the sample comprises a tissue or other biological sample, the subject may visit a medical center to have the sample obtained and sent to the profiling center, or subjects may collect the sample themselves (*e.g.*, a urine sample) and directly send it to a profiling center. Where the sample comprises previously determined biological information, the information may be directly sent to the profiling service by the subject (*e.g.*, an information card containing the information may be scanned by a computer and the data transmitted to a computer of the profiling center using an electronic communication systems). Once received by the profiling service, the sample is processed and a profile is produced (*i.e.*, expression data), specific for the diagnostic or prognostic information desired for the subject.

[00110] The profile data is then prepared in a format suitable for interpretation by a treating clinician. For example, rather than providing raw expression data, the prepared format may represent a diagnosis or risk assessment (*e.g.*, presence of TV) for the subject, with or without recommendations for particular treatment options. The data may be displayed to the clinician by any suitable method. For example, in some embodiments, the profiling service generates a report that can be printed for the clinician (*e.g.*, at the point of care) or displayed to the clinician on a computer monitor.

[00111] In some embodiments, the information is first analyzed at the point of care or at a regional facility. The raw data is then sent to a central processing facility for further analysis and/or to convert the raw data to information useful for a clinician or patient. The central processing facility provides the advantage of privacy (all data is stored in a central facility with uniform security protocols), speed, and uniformity of data analysis. The central processing facility can then control the fate of the data following treatment of the subject. For example, using an electronic communication system, the central facility can provide data to the clinician, the subject, or researchers.

[00112] In some embodiments, the subject is able to directly access the data using the electronic communication system. The subject may chose further intervention or counseling based on the results. In some embodiments, the data is used for research use. For example, the data may be used to further optimize the inclusion or elimination of markers as useful indicators of a particular condition or stage of disease or as a companion diagnostic to determine a treatment course of action.

4.2.7. Exemplary polynucleotides

[00113] In some embodiments, polynucleotides are provided. In some embodiments, synthetic polynucleotides are provided. Synthetic polynucleotides, as used herein, refer to polynucleotides that have been synthesized in vitro either chemically or enzymatically. Chemical synthesis of polynucleotides includes, but is not limited to, synthesis using polynucleotide synthesizers, such as OligoPilot (GE Healthcare), ABI 3900 DNA Synthesizer (Applied Biosystems), and the like. Enzymatic synthesis includes, but is not limited, to producing polynucleotides by enzymatic amplification, e.g., PCR. A polynucleotide may comprise one or more nucleotide analogs (i.e., modified nucleotides) discussed herein.

[00114] In some embodiments, a polynucleotide is provided that comprises a region that is at least 90%, at least 95%, or 100% identical to, or at least 90%, at least 95%, or 100% complementary to, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at elast 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, or at least 30 contiguous nucleotides of the TV 40S ribosomal protein (Tv40Srp) gene. In some embodiments, a polynucleotide is provided that comprises a region that is at least 90%, at least 95%, or 100% identical to, or complementary to, a span of 6 to 100, 8 to 100, 8 to 75, 8 to 50, 8 to 40, or 8 to 30 contiguous nucleotides of the TV 40S ribosomal protein (Tv40Srp) gene. Nonlimiting exemplary polynucleotides are shown in Table 1.

[00115] In various embodiments, a polynucleotide comprises fewer than 500, fewer than 300, fewer than 200, fewer than 150, fewer than 100, fewer than 75, fewer than 50, fewer than 40, or fewer than 30 nucleotides. In various embodiments, a polynucleotide is between 6 and 200, between 8 and 200, between 8 and 150, between 8 and 100, between 8 and 75, between 8 and 50, between 8 and 40, between 8 and 30, between 15 and 100, between 15 and 75, between 15 and 50, between 15 and 40, or between 15 and 30 nucleotides long.

[00116] In some embodiments, the polynucleotide is a primer. In some embodiments, the primer is labeled with a detectable moiety. In some embodiments, a primer is not labeled. A primer, as used herein, is a polynucleotide that is capable of selectively hybridizing to a target gene or to an amplicon that has been amplified from a target gene (collectively referred to as "template"), and, in the presence of the template, a polymerase and suitable buffers and reagents, can be extended to form a primer extension product.

[00117] In some embodiments, the polynucleotide is a probe. In some embodiments, the probe is labeled with a detectable moiety. A detectable moiety, as used herein, includes both directly detectable moieties, such as fluorescent dyes, and indirectly detectable moieties, such as members of binding pairs. When the detectable moiety is a member of a binding pair, in some embodiments, the probe can be detectable by incubating the probe with a detectable label bound to the second member of the binding pair. In some embodiments, a probe is not labeled, such as when a probe is a capture probe, e.g., on a microarray or bead. In some embodiments, a probe is not extendable, e.g., by a polymerase. In other embodiments, a probe is extendable.

[00118] In some embodiments, the polynucleotide is a FRET probe that in some embodiments is labeled at the 5'-end with a fluorescent dye (donor) and at the 3'-end with a quencher (acceptor), a chemical group that absorbs (i.e., suppresses) fluorescence emission from the dye when the groups are in close proximity (i.e., attached to the same probe). Thus, in some embodiments, the emission spectrum of the dye should overlap considerably with the absorption spectrum of the quencher. In other embodiments, the dye and quencher are not at the ends of the FRET probe.

4.2.7.1. Exemplary polynucleotide modifications

[00119] In some embodiments, the methods of detecting at least one target gene described herein employ one or more polynucleotides that have been modified, such as polynucleotides comprising one or more affinity-enhancing nucleotide analogs. Modified polynucleotides useful in the methods described herein include primers for reverse transcription, PCR amplification primers, and probes. In some embodiments, the incorporation of affinity-enhancing nucleotides increases the binding affinity and specificity of a polynucleotide for its target nucleic acid as compared to polynucleotides that contain only deoxyribonucleotides, and allows for the use of shorter polynucleotides or for shorter regions of complementarity between the polynucleotide and the target nucleic acid.

[00120] In some embodiments, affinity-enhancing nucleotide analogs include nucleotides comprising one or more base modifications, sugar modifications and/or backbone modifications.

[00121] In some embodiments, modified bases for use in affinity-enhancing nucleotide analogs include 5-methylcytosine, isocytosine, pseudoisocytosine, 5-bromouracil, 5-propynyluracil, 6-aminopurine, 2-aminopurine, inosine, diaminopurine, 2-chloro-6-aminopurine, xanthine and hypoxanthine.

[00122] In some embodiments, affinity-enhancing nucleotide analogs include nucleotides having modified sugars such as 2'-substituted sugars, such as 2'-O-alkyl-ribose sugars, 2'-amino-deoxyribose sugars, 2'-fluoro- deoxyribose sugars, 2'-fluoro-arabinose sugars, and 2'-O-methoxyethyl-ribose (2'MOE) sugars. In some embodiments, modified sugars are arabinose sugars, or d-arabino-hexitol sugars.

[00123] In some embodiments, affinity-enhancing nucleotide analogs include backbone modifications such as the use of peptide nucleic acids (PNA; *e.g.,* an oligomer including nucleobases linked together by an amino acid backbone). Other backbone modifications include phosphorothioate linkages, phosphodiester modified nucleic acids, combinations of phosphodiester and phosphorothioate nucleic acid, methylphosphonate, alkylphosphonates, phosphate esters, alkylphosphonothioates, phosphoramidates, carbamates, carbonates, phosphate triesters, acetamidates, carboxymethyl esters, methylphosphorothioate, phosphorodithioate, p-ethoxy, and combinations thereof.

[00124] In some embodiments, a polynucleotide includes at least one affinity-enhancing nucleotide analog that has a modified base, at least nucleotide (which may be the same nucleotide) that has a modified sugar, and/or at least one internucleotide linkage that is non-naturally occurring.

[00125] In some embodiments, an affinity-enhancing nucleotide analog contains a locked nucleic acid ("LNA") sugar, which is a bicyclic sugar. In some embodiments, a polynucleotide for use in the methods described herein comprises one or more nucleotides having an LNA sugar. In some embodiments, a polynucleotide contains one or more regions consisting of nucleotides with LNA sugars. In other embodiments, a polynucleotide contains nucleotides with LNA sugars interspersed with deoxyribonucleotides. *See, e.g.,* Frieden, M. *et al.* (2008) Curr. Pharm. Des. 14(11):1138-1142.

4.2.7.2. Exemplary primers

[00126] In some embodiments, a primer is provided. In some embodiments, a primer is at least 90%, at least 95%, or 100% identical to, or at least 90%, at least 95%, or 100% complementary to, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at elast 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, or at least 30 contiguous nucleotides of the TV 40S ribosomal protein (Tv40Srp) gene. In some embodiments, a primer is provided that comprises a region that is at least 90%, at least

95%, or 100% identical to, or complementary to, a span of 6 to 100, 8 to 100, 8 to 75, 8 to 50, 8 to 40, or 8 to 30 contiguous nucleotides of the TV 40S ribosomal protein (Tv40Srp) gene. Nonlimiting exemplary primers are shown in Table 1. In some embodiments, a primer may also comprise portions or regions that are not identical or complementary to the target gene. In some embodiments, a region of a primer that is at least 90%, at least 95%, or 100% identical or complementary to a target gene is contiguous, such that any region of a primer that is not identical or complementary to the target gene does not disrupt the identical or complementary region.

[00127] In some embodiments, a primer comprises a portion that is at least 90%, at least 95%, or 100% identical to a region of a target gene. In some such embodiments, a primer that comprises a region that is at least 90%, at least 95%, or 100% identical to a region of the target gene is capable of selectively hybridizing to an amplicon that has been produced by amplification of the target gene. In some embodiments, the primer is complementary to a sufficient portion of the amplicon such that it selectively hybridizes to the amplicon under the conditions of the particular assay being used.

[00128] As used herein, "selectively hybridize" means that a polynucleotide, such as a primer or probe, will hybridize to a particular nucleic acid in a sample with at least 5-fold greater affinity than it will hybridize to another nucleic acid present in the same sample that has a different nucleotide sequence in the hybridizing region. Exemplary hybridization conditions are discussed herein, for example, in the context of a reverse transcription reaction or a PCR amplification reaction. In some embodiments, a polynucleotide will hybridize to a particular nucleic acid in a sample with at least 10-fold greater affinity than it will hybridize to another nucleic acid present in the same sample that has a different nucleotide sequence in the hybridizing region.

[00129] In some embodiments, a primer comprises a detectable moiety.

[00130] In some embodiments, primer pairs are provided. Such primer pairs are designed to amplify a portion of a target gene, such as the TV 40S ribosomal protein (Tv40Srp) gene, or an endogenous control such as a sample adequacy control (SAC), or an exogenous control such as a sample processing control (SPC). In some embodiments, a primer pair is designed to produce an amplicon that is 50 to 1500 nucleotides long, 50 to 1000 nucleotides long, 50 to 750 nucleotides long, 50 to 500 nucleotides long, 50 to 400 nucleotides long, 50 to 300 nucleotides long, 50 to 200 nucleotides long, 50 to 150 nucleotides long, 100 to 300 nucleotides long, 100 to 200 nucleotides long, or 100 to 150 nucleotides long. Nonlimiting exemplary primer pairs are shown in Table 1.

4.2.7.3. Exemplary probes

[00131] In various embodiments, methods of detecting the presence of *Trichomonas vaginalis* comprise hybridizing nucleic acids of a sample with a probe. In some embodiments, the probe comprises a portion that is complementary to a target gene, such as the TV 40S ribosomal protein (Tv40Srp) gene, or an endogenous control such as a sample adequacy control (SAC), or an exogenous control such as a sample processing control (SPC). In some embodiments, the probe comprises a portion that is at least 90%, at least 95%, or 100% identical to a region of the target gene. In some such embodiments, a probe that is at least 90%, at least 95%, or 100% complementary to a target gene is complementary to a sufficient portion of the target gene such that it selectively hybridizes to the target gene under the conditions of the particular assay being used. In some embodiments, a probe that is complementary to a target gene comprises a region that is at least 90%, at least 95%, or 100% complementary to at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, or at least 30 contiguous nucleotides of the target gene. Nonlimiting exemplary probes are shown in Table 1. A probe that is at least 90%, at least 95%, or 100% complementary to a target gene may also comprise portions or regions that are not complementary to the target gene. In some embodiments, a region of a probe that is at least 90%, at least 95%, or 100% complementary to a target gene is contiguous, such that any region of a probe that is not complementary to the target gene does not disrupt the complementary region.

[00132] In some embodiments, the probe comprises a portion that is at least 90%, at least 95%, or 100% identical to a region of the target gene, such as the TV 40S ribosomal protein (Tv40Srp) gene, or an endogenous control such as a sample adequacy control (SAC), or an exogenous control such as a sample processing control (SPC). In some such embodiments, a probe that comprises a region that is at least 90%, at least 95%, or 100% identical to a region of the target gene is capable of selectively hybridizing to an amplicon that has been produced by amplification of the target gene. In some embodiments, the probe is at least 90%, at least 95%, or 100% complementary to a sufficient portion of the amplicon such that it selectively hybridizes to the amplicon under the conditions of the particular assay being used. In some embodiments, a probe that is complementary to a amplicon comprises a region that is at least 90%, at least 95%, or 100% complementary to at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at

least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, or at least 30 contiguous nucleotides of the amplicon. A probe that is at least 90%, at least 95%, or 100% complementary to an amplicon may also comprise portions or regions that are not complementary to the amplicon. In some embodiments, a region of a probe that is at least 90%, at least 95%, or 100% complementary to an amplicon is contiguous, such that any region of a probe that is not complementary to the amplicon does not disrupt the complementary region.

[00133] In some embodiments, the method of detecting one or more target genes comprises: (a) amplifying a region of the target gene; and (b) detecting the amplified region using real time PCR and a detection probe (which may be simultaneous with the amplification step (a)).

[00134] As described above, in some embodiments, real time PCR detection may be performed using a FRET probe, which includes, but is not limited to, a TaqMan® probe, a Molecular beacon probe and a Scorpion probe. In some embodiments, the real time PCR detection is performed with a TaqMan® probe, *i.e.,* a linear probe that typically has a fluorescent dye covalently bound at one end of the DNA and a quencher molecule covalently bound elsewhere, such as at the other end of, the DNA. The FRET probe comprises a sequence that is complementary to a region of the amplicon such that, when the FRET probe is hybridized to the amplicon, the dye fluorescence is quenched, and when the probe is digested during amplification of the amplicon, the dye is released from the probe and produces a fluorescence signal. In some embodiments, the amount of target gene in the sample is proportional to the amount of fluorescence measured during amplification.

[00135] The TaqMan® probe typically comprises a region of contiguous nucleotides having a sequence that is at least 90%, at least 95%, or 100% identical or complementary to a region of a target gene such that the probe is selectively hybridizable to a PCR amplicon of a region of the target gene. In some embodiments, the probe comprises a region of at least 6 contiguous nucleotides having a sequence that is fully complementary to or identically present in a region of a target gene. In some embodiments, the probe comprises a region that is at least 90%, at least 95%, or 100% identical or complementary to at least 8 contiguous nucleotides, at least 10 contiguous nucleotides, at least 12 contiguous nucleotides, at least 14 contiguous nucleotides, or at least 16 contiguous nucleotides of a target gene to be detected.

[00136] In some embodiments, the region of the amplicon that has a sequence that is at least 90%, at least 95%, or 100% complementary to the TaqMan® probe sequence is at or near the center of the amplicon molecule. In some embodiments, there are independently at least 2 nucleotides, such as at least 3 nucleotides, such as at least 4 nucleotides, such as at least 5 nucleotides of the amplicon at the 5'-end and at the 3'-end of the region of complementarity.

[00137] In some embodiments, Molecular Beacons can be used to detect PCR products. Like TaqMan® probes, Molecular Beacons use FRET to detect a PCR product via a probe having a fluorescent dye and a quencher attached at the ends of the probe. Unlike TaqMan® probes, Molecular Beacons remain intact during the PCR cycles. Molecular Beacon probes form a stem-loop structure when free in solution, thereby allowing the dye and quencher to be in close enough proximity to cause fluorescence quenching. When the Molecular Beacon hybridizes to a target, the stem-loop structure is abolished so that the dye and the quencher become separated in space and the dye fluoresces. Molecular Beacons are available, e.g., from Gene Link™ (*see* www.genelink.com/newsite/products/mbintro.asp).

[00138] In some embodiments, Scorpion probes can be used as both sequence-specific primers and for PCR product detection. Like Molecular Beacons, Scorpion probes form a stem-loop structure when not hybridized to a target nucleic acid. However, unlike Molecular Beacons, a Scorpion probe achieves both sequence-specific priming and PCR product detection. A fluorescent dye molecule is attached to the 5'-end of the Scorpion probe, and a quencher is attached elsewhere, such as to the 3'-end. The 3' portion of the probe is complementary to the extension product of the PCR primer, and this complementary portion is linked to the 5'-end of the probe by a non-amplifiable moiety. After the Scorpion primer is extended, the target-specific sequence of the probe binds to its complement within the extended amplicon, thus opening up the stem-loop structure and allowing the dye on the 5'-end to fluoresce and generate a signal. Scorpion probes are available from, e.g, Premier Biosoft International (*see* www.premierbiosoft.com/tech_notes/Scorpion.html).

[00139] In some embodiments, labels that can be used on the FRET probes include colorimetric and fluorescent dyes such as Alexa Fluor dyes, BODIPY dyes, such as BODIPY FL; Cascade Blue; Cascade Yellow; coumarin and its derivatives, such as 7-amino-4-methylcoumarin, aminocoumarin and hydroxycoumarin; cyanine dyes, such as Cy3 and Cy5; eosins and erythrosins; fluorescein and its derivatives, such as fluorescein isothiocyanate; macrocyclic chelates of lanthanide ions, such as Quantum Dye™; Marina

Blue; Oregon Green; rhodamine dyes, such as rhodamine red, tetramethylrhodamine and rhodamine 6G; Texas Red; fluorescent energy transfer dyes, such as thiazole orange-ethidium heterodimer; and, TOTAB.

[00140] Specific examples of dyes include, but are not limited to, those identified above and the following: Alexa Fluor 350, Alexa Fluor 405, Alexa Fluor 430, Alexa Fluor 488, Alexa Fluor 500. Alexa Fluor 514, Alexa Fluor 532, Alexa Fluor 546, Alexa Fluor 555, Alexa Fluor 568, Alexa Fluor 594, Alexa Fluor 610, Alexa Fluor 633, Alexa Fluor 647, Alexa Fluor 660, Alexa Fluor 680, Alexa Fluor 700, and, Alexa Fluor 750; amine-reactive BODIPY dyes, such as BODIPY 493/503, BODIPY 530/550, BODIPY 558/568, BODIPY 564/570, BODIPY 576/589, BODIPY 581/591, BODIPY 630/650, BODIPY 650/655, BODIPY FL, BODIPY R6G, BODIPY TMR, and, BODIPY-TR; Cy3, Cy5, 6-FAM, Fluorescein Isothiocyanate, HEX, 6-JOE, Oregon Green 488, Oregon Green 500, Oregon Green 514, Pacific Blue, REG, Rhodamine Green, Rhodamine Red, Renographin, ROX, SYPRO, TAMRA, 2', 4',5',7'-Tetrabromosulfonefluorescein, and TET.

[00141] Examples of dye/quencher pairs (i.e., donor/acceptor pairs) include, but are not limited to, fluorescein/tetramethylrhodamine; IAEDANS/fluorescein; EDANS/dabcyl; fluorescein/fluorescein; BODIPY FL/BODIPY FL; fluorescein/QSY 7 or QSY 9 dyes. When the donor and acceptor are the same, FRET may be detected, in some embodiments, by fluorescence depolarization. Certain specific examples of dye/quencher pairs (i.e., donor/acceptor pairs) include, but are not limited to, Alexa Fluor 350/Alexa Fluor488; Alexa Fluor 488/Alexa Fluor 546; Alexa Fluor 488/Alexa Fluor 555; Alexa Fluor 488/Alexa Fluor 568; Alexa Fluor 488/Alexa Fluor 594; Alexa Fluor 488/Alexa Fluor 647; Alexa Fluor 546/Alexa Fluor 568; Alexa Fluor 546/Alexa Fluor 594; Alexa Fluor 546/Alexa Fluor 647; Alexa Fluor 555/Alexa Fluor 594; Alexa Fluor 555/Alexa Fluor 647; Alexa Fluor 568/Alexa Fluor 647; Alexa Fluor 594/Alexa Fluor 647; Alexa Fluor 350/QSY35; Alexa Fluor 350/dabcyl; Alexa Fluor 488/QSY 35; Alexa Fluor 488/dabcyl; Alexa Fluor 488/QSY 7 or QSY 9; Alexa Fluor 555/QSY 7 or QSY9; Alexa Fluor 568/QSY 7 or QSY 9; Alexa Fluor 568/QSY 21; Alexa Fluor 594/QSY 21; and Alexa Fluor 647/QSY 21. In some instances, the same quencher may be used for multiple dyes, for example, a broad spectrum quencher, such as an Iowa Black® quencher (Integrated DNA Technologies, Coralville, IA) or a Black Hole Quencher™ (BHQ™; Sigma-Aldrich, St. Louis, MO).

[00142] In some embodiments, for example, in a multiplex reaction in which two or more moieties (such as amplicons) are detected simultaneously, each probe

comprises a detectably different dye such that the dyes may be distinguished when detected simultaneously in the same reaction. One skilled in the art can select a set of detectably different dyes for use in a multiplex reaction.

[00143] Specific examples of fluorescently labeled ribonucleotides useful in the preparation of PCR probes for use in some embodiments of the methods described herein are available from Molecular Probes (Invitrogen), and these include, Alexa Fluor 488-5-UTP, Fluorescein-12-UTP, BODIPY FL-14-UTP, BODIPY TMR-14-UTP, Tetramethylrhodamine-6-UTP, Alexa Fluor 546-14-UTP, Texas Red-5-UTP, and BODIPY TR-14-UTP. Other fluorescent ribonucleotides are available from Amersham Biosciences (GE Healthcare), such as Cy3-UTP and Cy5-UTP.

[00144] Examples of fluorescently labeled deoxyribonucleotides useful in the preparation of PCR probes for use in the methods described herein include Dinitrophenyl (DNP)-1'-dUTP, Cascade Blue-7-dUTP, Alexa Fluor 488-5-dUTP, Fluorescein-12-dUTP, Oregon Green 488-5-dUTP, BODIPY FL-14-dUTP, Rhodamine Green-5-dUTP, Alexa Fluor 532-5-dUTP, BODIPY TMR-14-dUTP, Tetramethylrhodamine-6-dUTP, Alexa Fluor 546-14-dUTP, Alexa Fluor 568-5-dUTP, Texas Red-12-dUTP, Texas Red-5-dUTP, BODIPY TR-14-dUTP, Alexa Fluor 594-5-dUTP, BODIPY 630/650-14-dUTP, BODIPY 650/665-14-dUTP; Alexa Fluor 488-7-OBEA-dCTP, Alexa Fluor 546-16-OBEA-dCTP, Alexa Fluor 594-7-OBEA-dCTP, Alexa Fluor 647-12-OBEA-dCTP. Fluorescently labeled nucleotides are commercially available and can be purchased from, e.g., Invitrogen.

[00145] In some embodiments, dyes and other moieties, such as quenchers, are introduced into polynucleotide used in the methods described herein, such as FRET probes, via modified nucleotides. A "modified nucleotide" refers to a nucleotide that has been chemically modified, but still functions as a nucleotide. In some embodiments, the modified nucleotide has a chemical moiety, such as a dye or quencher, covalently attached, and can be introduced into a polynucleotide, for example, by way of solid phase synthesis of the polynucleotide. In other embodiments, the modified nucleotide includes one or more reactive groups that can react with a dye or quencher before, during, or after incorporation of the modified nucleotide into the nucleic acid. In specific embodiments, the modified nucleotide is an amine-modified nucleotide, *i.e.,* a nucleotide that has been modified to have a reactive amine group. In some embodiments, the modified nucleotide comprises a modified base moiety, such as uridine, adenosine, guanosine, and/or cytosine. In specific embodiments, the amine-modified nucleotide is selected from 5-(3-aminoallyl)-UTP; 8-[(4-amino)butyl]-amino-ATP and 8-[(6-amino)butyl]-amino-ATP; N6-(4-amino)butyl-ATP,

N6-(6-amino)butyl-ATP, N4-[2,2-oxy-bis-(ethylamine)]-CTP; N6-(6-Amino)hexyl-ATP; 8-[(6-Amino)hexyl]-amino-ATP; 5-propargylamino-CTP, 5-propargylamino-UTP. In some embodiments, nucleotides with different nucleobase moieties are similarly modified, for example, 5-(3-aminoallyl)-GTP instead of 5-(3-aminoallyl)-UTP. Many amine modified nucleotides are commercially available from, e.g., Applied Biosystems, Sigma, Jena Bioscience and TriLink.

[00146] Exemplary detectable moieties also include, but are not limited to, members of binding pairs. In some such embodiments, a first member of a binding pair is linked to a polynucleotide. The second member of the binding pair is linked to a detectable label, such as a fluorescent label. When the polynucleotide linked to the first member of the binding pair is incubated with the second member of the binding pair linked to the detectable label, the first and second members of the binding pair associate and the polynucleotide can be detected. Exemplary binding pairs include, but are not limited to, biotin and streptavidin, antibodies and antigens, etc.

[00147] In some embodiments, multiple target genes are detected in a single multiplex reaction. In some such embodiments, each probe that is targeted to a unique amplicon is spectrally distinguishable when released from the probe, in which case each target gene is detected by a unique fluorescence signal. In some embodiments, two or more target genes are detected using the same fluorescent signal, in which case detection of that signal indicates the presence of either of the target genes or both.

[00148] One skilled in the art can select a suitable detection method for a selected assay, e.g., a real-time PCR assay. The selected detection method need not be a method described above, and may be any method.

4.3. Exemplary compositions and kits

[00149] In another aspect, compositions are provided. In some embodiments, compositions are provided for use in the methods described herein.

[00150] In some embodiments, compositions are provided that comprise at least one target gene-specific primer. The term "target gene-specific primer" encompasses primers that have a region of contiguous nucleotides having a sequence that is (i) at least 90%, at least 95%, or 100% identical to a region of a target gene, or (ii) at least 90%, at least 95%, or 100% complementary to the sequence of a region of contiguous nucleotides found in a target gene. In some embodiments, a composition is provided that comprises at least one pair of target gene-specific primers. The term "pair of target gene-specific primers"

encompasses pairs of primers that are suitable for amplifying a defined region of a target gene. A pair of target gene-specific primers typically comprises a first primer that comprises a sequence that is at least 90%, at least 95%, or 100% identical to the sequence of a region of a target gene and a second primer that comprises a sequence that is at least 90%, at least 95%, or 100% complementary to a region of a target gene. A pair of primers is typically suitable for amplifying a region of a target gene that is 50 to 1500 nucleotides long, 50 to 1000 nucleotides long, 50 to 750 nucleotides long, 50 to 500 nucleotides long, 50 to 400 nucleotides long, 50 to 300 nucleotides long, 50 to 200 nucleotides long, 50 t0 150 nucleotides long, 100 to 300 nucleotides long, 100 to 200 nucleotides long, or 100 to 150 nucleotides long. Nonlimiting exemplary primers, and pairs of primers, are shown in Table 1.

[00151] In some embodiments, a composition comprises at least one pair of target gene-specific primers. In some embodiments, a composition additionally comprises a pair of target gene-specific primers for amplifying an endogenous control (such as an SAC) and/or one pair of target gene-specific primers for amplifying an exogenous control (such as an SPC).

[00152] In some embodiments, a composition comprises at least one target gene-specific probe. The term "target gene-specific probe" encompasses probes that have a region of contiguous nucleotides having a sequence that is (i) at least 90%, at least 95%, or 100% identical to a region of a target gene, or (ii) at least 90%, at least 95%, or 100% complementary to the sequence of a region of contiguous nucleotides found in a target gene. Nonlimiting exemplary target-specific probes are shown in Table 1.

[00153] In some embodiments, a composition (including a composition described above that comprises one or more pairs of target gene-specific primers) comprises one or more probes for detecting the target genes. In some embodiments, a composition comprises a probe for detecting an endogenous control (such as an SAC) and/or a probe for detecting an exogenous control (such as an SPC).

[00154] In some embodiments, a composition is an aqueous composition. In some embodiments, the aqueous composition comprises a buffering component, such as phosphate, tris, HEPES, etc., and/or additional components, as discussed below. In some embodiments, a composition is dry, for example, lyophilized, and suitable for reconstitution by addition of fluid. A dry composition may include one or more buffering components and/or additional components.

[00155] In some embodiments, a composition further comprises one or more additional components. Additional components include, but are not limited to, salts, such as NaCl , KCl,and $MgCl_2$; polymerases, including thermostable polymerases such as Taq; dNTPs; bovine serum albumin (BSA) and the like; reducing agents, such as β-mercaptoethanol; EDTA and the like; etc. One skilled in the art can select suitable composition components depending on the intended use of the composition.

[00156] In some embodiments, compositions are provided that comprise at least one polynucleotide for detecting at least one target gene. In some embodiments, the polynucleotide is used as a primer for a reverse transcriptase reaction. In some embodiments, the polynucleotide is used as a primer for amplification. In some embodiments, the polynucleotide is used as a primer for PCR. In some embodiments, the polynucleotide is used as a probe for detecting at least one target gene. In some embodiments, the polynucleotide is detectably labeled. In some embodiments, the polynucleotide is a FRET probe. In some embodiments, the polynucleotide is a TaqMan® probe, a Molecular Beacon, or a Scorpion probe.

[00157] In some embodiments, a composition comprises at least one FRET probe having a sequence that is at least 90%, at least 95%, or 100% identical, or at least 90%, at least 95%, or 100% complementary, to a region of, the TV 40S ribosomal protein (Tv40Srp) gene. In some embodiments, a FRET probe is labeled with a donor/acceptor pair such that when the probe is digested during the PCR reaction, it produces a unique fluorescence emission that is associated with a specific target gene. In some embodiments, when a composition comprises multiple FRET probes, each probe is labeled with a different donor/acceptor pair such that when the probe is digested during the PCR reaction, each one produces a unique fluorescence emission that is associated with a specific probe sequence and/or target gene. In some embodiments, the sequence of the FRET probe is complementary to a target region of a target gene. In other embodiments, the FRET probe has a sequence that comprises one or more base mismatches when compared to the sequence of the best-aligned target region of a target gene.

[00158] In some embodiments, a composition comprises a FRET probe consisting of at least 8, at least 9, at least 10, at least 11, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, or at least 25 nucleotides, wherein at least a portion of the sequence is at least 90%, at least 95%, or 100% identical, or at least 90%, at least 95%, or 100% complementary, to a region of, the TV 40S ribosomal protein (Tv40Srp) gene. In some embodiments, at least 8,

at least 9, at least 10, at least 11, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, or at least 25 nucleotides of the FRET probe are identically present in, or complementary to a region of, the TV 40S ribosomal protein (Tv40Srp) gene. In some embodiments, the FRET probe has a sequence with one, two or three base mismatches when compared to the sequence or complement of the TV 40S ribosomal protein (Tv40Srp) gene.

[00159] In some embodiments, a kit comprises a polynucleotide discussed above. In some embodiments, a kit comprises at least one primer and/or probe discussed above. In some embodiments, a kit comprises at least one polymerase, such as a thermostable polymerase. In some embodiments, a kit comprises dNTPs. In some embodiments, kits for use in the real time PCR methods described herein comprise one or more target gene-specific FRET probes and/or one or more primers for amplification of target genes.

[00160] In some embodiments, one or more of the primers and/or probes is "linear". A "linear" primer refers to a polynucleotide that is a single stranded molecule, and typically does not comprise a short region of, for example, at least 3, 4 or 5 contiguous nucleotides, which are complementary to another region within the same polynucleotide such that the primer forms an internal duplex. In some embodiments, the primers for use in reverse transcription comprise a region of at least 4, such as at least 5, such as at least 6, such as at least 7 or more contiguous nucleotides at the 3'-end that has a sequence that is complementary to region of at least 4, such as at least 5, such as at least 6, such as at least 7 or more contiguous nucleotides at the 5'-end of a target gene.

[00161] In some embodiments, a kit comprises one or more pairs of linear primers (a "forward primer" and a "reverse primer") for amplification of a target gene. Accordingly, in some embodiments, a first primer comprises a region of at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, or at least 25 contiguous nucleotides having a sequence that is at least 90%, at least 95%, or 100% identical to the sequence of a region of at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, or at least 25 contiguous nucleotides at a first location in the target gene. Furthermore, in some embodiments, a second primer comprises a region of at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at

least 22, at least 23, at least 24, or at least 25 contiguous nucleotides having a sequence that is at least 90%, at least 95%, or 100% complementary to the sequence of a region of at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, or at least 25 contiguous nucleotides at a second location in the target gene, such that a PCR reaction using the two primers results in an amplicon extending from the first location of the target gene to the second location of the target gene.

[00162] In some embodiments, the kit comprises at least two, at least three, or at least four sets of primers, each of which is for amplification of a different target gene, such as an endogenous control and/or an exogenous control.

[00163] In some embodiments, probes and/or primers for use in the compositions described herein comprise deoxyribonucleotides. In some embodiments, probes and/or primers for use in the compositions described herein comprise deoxyribonucleotides and one or more nucleotide analogs, such as LNA analogs or other duplex-stabilizing nucleotide analogs described above. In some embodiments, probes and/or primers for use in the compositions described herein comprise all nucleotide analogs. In some embodiments, the probes and/or primers comprise one or more duplex-stabilizing nucleotide analogs, such as LNA analogs, in the region of complementarity.

[00164] In some embodiments, the kits for use in real time PCR methods described herein further comprise reagents for use in the reverse transcription and amplification reactions. In some embodiments, the kits comprise enzymes such as heat stable DNA polymerases, such as Taq polymerase. In some embodiments, the kits further comprise deoxyribonucleotide triphosphates (dNTP) for use in amplification. In further embodiments, the kits comprise buffers optimized for specific hybridization of the probes and primers.

[00165] A kit generally includes a package with one or more containers holding the reagents, as one or more separate compositions or, optionally, as an admixture where the compatibility of the reagents will allow. The kit can also include other material(s) that may be desirable from a user standpoint, such as a buffer(s), a diluent(s), a standard(s), and/or any other material useful in sample processing, washing, or conducting any other step of the assay.

[00166] Kits preferably include instructions for carrying out one or more of the methods described herein. Instructions included in kits can be affixed to packaging material or can be included as a package insert. While the instructions are typically written or

printed materials they are not limited to such. Any medium capable of storing such instructions and communicating them to an end user is contemplated by this invention. Such media include, but are not limited to, electronic storage media (e.g., magnetic discs, tapes, cartridges, chips), optical media (e.g., CD ROM), and the like. As used herein, the term "instructions" can include the address of an internet site that provides the instructions.

[00167] In some embodiments, the kit can comprise the reagents described above provided in one or more GeneXpert®Sample cartridge(s). These cartridges permit extraction, amplification, and detection to be be carried out within this self-contained "laboratory in a cartridge." (See e.g., US Patents 5,958,349, 6,403,037, 6,440,725, 6,783,736, 6,818,185; each of which is herein incorporated by reference in its entirety.) Reagents for measuring genomic copy number level and detecting a pathogen could be provided in separate cartridges within a kit or these reagents (adapted for multiplex detection) could be provide in a single cartridge.

[00168] Any of the kits described here can include, in some embodiments, a receptacle for a urine sample and/or a swab for collecting a urethral swab sample, a vaginal swab sample, or an endocervical swab sample.

[00169] The following examples are for illustration purposes only, and are not meant to be limiting in any way.

5. EXAMPLES

5.1. Example 1: Detection of *Trichomonas vaginalis*

[00170] An assay was designed to detect the gene for the 40S ribosomal protein (Tv40Srp) of *Trichomonas vaginalis* (TV) by PCR, using the primers and probe shown in Table 1. In addition to the TV-specific primers and probe, primers and probe were included to detect a single-copy human gene used as a sample adequacy control (SAC) target. Primers and probe were also included to detect a bacterial gene, which was included in the multiplex reaction as a sample processing control (SPC) target.

Table 1: Primer and probe sequences

oligo name	target	sequence	SEQ ID NO	Amplicon SEQ ID NO
TV forward	Tv40Srp gene	GTAACAACCTTGGAGTTCTTCTTAAG	1	5
TV reverse	Tv40Srp gene	ACATCAATCTACAAGACACCACTTGA	2	
TV probe	Tv40Srp gene	F1-AGTTTGGCTGCTTAGCTTCGAC-Q1	3	

[00171] The final primer and probe compositions of the multiplex assay are shown in Table 2.

Table 2: Primer and probe concentrations

Target	Label	Purpose	Final conc. Forw. Primer	Final conc. Rev primer	Final conc. Probe
Tv40Srp	F1	TV detection	0.3μM	0.3μM	0.5 μM
single-copy human gene	F2	SAC	0.35 μM	0.35 μM	0.35 μM
bacterial gene	F2	SPC	0.4 μM	0.4 μM	0.2 μM

F1 and F2 are detectably different dyes that can be detected and distinguished simultaneously in a multiplex reaction. Each probe also comprises a quencher (e.g., Q1, above).

[00172] Each reaction contained 42-58 mM KCl, 3.5-5.0 mM $MgCl_2$, 250-350 μM dNTPs, 50 mM Tris, pH 8.6, and 0.01% sodium azide. AptaTaq (0.27-0.37 units/μl; Roche) was used for amplification.

[00173] For each sample to be tested, approximately 7 mL of first catch, voided urine was added to 1 mL of buffer, preferably within 2 hours of sample collection. Physician-collected endocervical swabs or self-collected (in a clinical setting) vaginal swabs were immediately placed into 2.5mL of buffer.

[00174] 500 μL of buffered urine or swab sample was loaded into a GeneXpert® cartridge for analysis. The sample was mixed with a lysis reagent to release nucleic acids. After lysis, the released nucleic acid from the sample was captured on a DNA-binding substrate. The nucleic acid was eluted from the substrate and used to reconstitute the reagents used for real-time PCR (described above). The reaction cycle used was: 1 minute at 95°C, followed by up to 40 cycles of 5 seconds at 92.5°C, 20 seconds at 68°C using a GeneXpert® cartridge in a GeneXpert® system.

[00175] The results of the assay were interpreted as shown in Table 3. The valid range of Ct values for the TV, SAC, and SPC targets were 9-39.9 Ct.

Table 3: Xpert TV assay results and interpretation

Result	Interpretation
TV DETECTED	*Trichomonas* target DNA is detected. • The *Trichomonas* target has a Ct within the valid range and a fluorescence endpoint above the threshold setting. • SPC – Not applicable. SPC is ignored because the *Trichomonas* target amplification may compete with this control. • SAC – Not applicable. SAC is ignored because the *Trichomonas* target amplification may compete with this control. • PCC – PASS. All probe check results pass.
TV NOT DETECTED	*Trichomonas* target DNA is not detected. SPC meets acceptance criteria. • *Trichomonas* target DNA is not detected. • SPC – PASS. SPC has a Ct within the valid range and fluorescence endpoint above the threshold setting. • SAC – PASS. SAC has a Ct within the valid range and a fluorescence endpoint above the threshold setting. • PCC – PASS. All probe check results pass.
INVALID	Presence or absence of *Trichomonas* target DNA cannot be determined. Repeat test according to the instructions in Section 11.2, Retest Procedure. • SPC – FAIL. SPC Ct is not within valid range and the fluorescence endpoint is below the threshold setting. • SAC – PASS. SAC has a Ct within the valid range and fluorescence endpoint in the above threshold setting. • PCC – PASS. all probe check results pass. *Or* • SPC – PASS. SPC has a Ct within the valid range and fluorescence endpoint above the threshold setting. • SAC – FAIL. SAC Ct is not within valid range and fluorescence endpoint is below the threshold setting. • PCC – PASS. all probe check results pass. *Or* • SPC – FAIL. SPC Ct is not within valid range and fluorescence endpoint is below the threshold setting. • SAC – FAIL. SAC Ct is not within valid range and fluorescence endpoint is below the threshold setting. • PCC – PASS. All probe check results pass.
ERROR	Presence or absence of *Trichomonas* target DNA cannot be determined. Repeat test according to the instructions in Section 11.2, Retest Procedure. • TRICHOMONAS – NO RESULT • SPC – NO RESULT • SAC – NO RESULT • PCC – FAIL.* All or one of the probe check results fail. * If the probe check passed, the error is caused by the maximum pressure limit exceeding the acceptable range or by a system component failure.
NO RESULT	Presence or absence of *Trichomonas* target DNA cannot be determined. Repeat test according to the instructions in Section 11.2, Retest Procedure. A NO RESULT indicates that insufficient data were collected. For example, the operator stopped a test that was in progress or a power failure occurred. • TRICHOMONAS – NO RESULT • SPC – NO RESULT • SAC – NO RESULT • PCC – Not applicable

5.2. Example 2: Clinical performance

[00176] Performance characteristics of the Xpert TV Assay were evaluated at 13 institutions in the U.S. Due to the low prevalence of *Trichomonas vaginalis* and the difficulty in obtaining fresh *Trichomonas vaginalis*-positive specimens from male subjects,

the specimen population for this study was supplemented with contrived male urine specimens.

[00177] Subjects included consenting asymptomatic and symptomatic, sexually active males and females seen in locations including, but not limited to: OB/GYN, sexually transmitted disease (STD), teen, public health, and family planning clinics.

[00178] The study specimens consisted of prospectively collected male urine, female urine, endocervical swabs, and patient-collected vaginal swabs (collected in a clinical setting). Contrived male urine specimens were included to supplement the male sample size.

[00179] The Xpert TV Assay performance was compared to an FDA-cleared *in vitro* qualitative nucleic acid amplification comparator assay that detects the ribosomal RNA of Trichomonas vaginalis using a transcription-mediated assay (APTIMA® Trichomonas vaginalis assay, GenProbe Hologic, San Diego, USA). Samples with discrepant results between the Xpert TV Assay and the comparator assay were analysed with bi-directional sequencing of a separate repetitive genomic DNA sequence. *See* Bandea, et al., *Jornal of Clinical Microbiology*. 2013, 51(4):1298-1300.

[00180] Of the Xpert TV Assays runs performed with eligible specimens, 97.3% (5327/5474) of these specimens were successful on the first attempt. The remaining 147 gave indeterminate results on the first attempt (91 ERROR, 44 INVALID and 12 NO RESULT). One hundred nineteen of the147 specimens yielded valid results after a single retest; 17 of the specimens were indeterminate on the second attempt and 11 specimens were not retested. The overall assay success rate was 99.5% (5446/5474).

[00181] Results from the Xpert TV Assay were compared to the comparator assay, with bi-directional sequencing of discrepants. Sensitivity and specificity by gender, specimen type and symptom status are presented in Table 4.

Table 4: Xpert TV assay vs. reference NAAT test plus sequencing

Specimen		Sx Status	n	TP	FP	TN	FN	Prev %	Sensitivity % (95 CI)	Specificity % (95 CI)	PPV % (95 CI)	NPV % (95 CI)
Female	PC-VS	Sym	717	63	0	649	5	9.4	92.6 (83.7-97.6)	100 (99.4-100)	100 (94.3-100)	99.2 (98.1-99.8)
		Asym	857	50	0	806	1	6.1	98.0 (89.6-100)	100 (99.5-100)	100 (92.9-100)	99.9 (99.3-100)
		All	1574	113	0	1455	6	7.6	95.0 (89.3-98.1)	100 (99.7-100)	100 (96.8-100)	99.6 (99.1-99.8)
	ES	Sym	714	59	0	651	4	8.8	93.7 (84.5-98.2)	100 (99.4-100)	100 (93.9-100)	99.4 (98.4-99.8)
		Asym	859	49	0	809	1	5.8	98.0 (89.4-99.9)	100 (99.5-100)	100 (92.7-100)	99.9 (99.3-100)
		All	1573	108	0	1460	5	7.2	95.6 (90.0-98.5)	100 (99.7-100)	100 (96.6-100)	99.7 (99.2-99.9)
	UR	Sym	713	60	0	651	2	8.7	96.8 (88.8-99.6)	100 (99.4-100)	100 (94.0-100)	99.7 (98.9-100)
		Asym	856	48	0	806	2	5.8	96.0 (86.3-99.5)	100 (99.5-100)	100 (92.6-100)	99.8 (99.1-100)
		All	1569	108	0	1457	4	7.1	96.4 (91.1-99.0)	100 (99.7-100)	100 (96.6-100)	99.7 (99.3-99.9)
Male	UR	Sym	125	1	0	124	0	0.8	100 (2.5-100)	100 (97.1-100)	100 (2.5-100)	100 (97.1-100)
		Asym	411	13	0	398	0	3.2	100 (75.3-100)	100 (99.1-100)	100 (75.3-100)	100 (99.1-100)
		CS	183	62	19	99	3	NA	95.4 (87.1-99.0)	83.9 (76.0-90.0)	NA	NA
		All	719	76	19	621	3	NA	96.2 (89.3-99.2)	97.0 (95.4-98.2)	NA	NA

a. TP = true positive, FP = false positive, TN = true negative, FN = false negative, PC-VS = patient-collected vaginal swab, ES = endocervical swab, CS = contrived specimens, UR = urine

5.3. Example 3: Limit of detection

[00182] The analytical sensitivity or limit of detection (LoD) of the Xpert TV Assay was assessed using two *Trichomonas vaginalis* strains, one metronidazole susceptible (*T.vaginalis* ATCC® 30001™), and one metronidazole resistant (*T.vaginalis* ATCC® 30238™). Both strains were tested in *T. vaginalis*-negative pooled male urine (MU) mixed with buffer and *T. vaginalis*-negative pooled vaginal swab (VS) in buffer

[00183] The limit of detection (LoD) was estimated by testing replicates of 20 at a minimum of five concentrations for each strain and sample type over three days. LoDs were estimated by logistic regression. The LoD is defined as the lowest number of cells/mL that can be reproducibly distinguished from negative samples with 95% confidence or the lowest concentration at which 19 of 20 replicates were positive. The study was performed with two different lots of Xpert TV reagents and the claimed LoD for each strain is the higher of the two determinations (Table 5). For swab samples in buffer, the limit of detection is 5 cells/mL. For urine samples in buffer, the limit of detection is 6 cells/mL. The claimed LoDs were verified by analyzing at least 20 replicates diluted to the estimated LoD concentrations.

Table 5: Limit of detection of Trichomonas vaginalis using Xpert TV

Trichomonas vaginalis strain and matrix	LoD Estimates (Logit) (lower and upper 95% confidence intervals) (cells/mL)		Verified LoD (cells/mL)	Verification (Positives/20)	LoD Claim (cells/mL)
	Lot 1	Lot 2			
ATCC 30001 in Vaginal Swab	3.9 (3.0–6.0)	4.2 (3.3–6.3)	4.2	20/20	5
ATCC 30238 in Vaginal Swab	4.4 (3.5–6.5)	3.7 (2.9–5.5)	4.4	19/20	5
ATCC 30001 in Male Urine	5.8 (4.7–7.9)	3.2 (2.6–4.8)	5.8	20/20	6
ATCC 30238 in Male Urine	4.9 (4.0–6.6)	4.3 (3.4–6.2)	4.9	19/20	5

5.4. Example 4: Assay reproducibility

[00184] A panel of eight specimens with varying concentrations of *Trichomonas vaginalis* was tested on 12 different days by two different operators, at each of three sites (8 specimens x 1 times/day x 12 days x 2 operators x 3 sites). Three lots of Xpert TV Assay were used at each of the 3 testing sites. Xpert TV Assays were performed according to the Xpert TV Assay procedure. Results are summarized in Table 6.

[00185] The reproducibility of the Xpert TV Assay was also evaluated in terms of the fluorescence signal expressed in Ct values for each target detected. The mean, standard deviation (SD), and coefficient of variation (CV) between-sites, between-lots, between-days, between-operator, and within-assay for each panel member are presented in Table 7.

Table 6: Summary of reproducibility results

Sample	Site 1			Site 2			Site 3			% Total Agreement by Sample
	Op 1	Op 2	Site	Op 1	Op 2	Site	Op 1	Op 2	Site	
FS-Neg	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	100% (24/24)	100% (48/48)	100% (144/144)
FS-Mod Pos	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	100% (24/24)	100% (48/48)	100% (144/144)
FS-Low Pos	62.5% (15/24)	75.0% (18/24)	68.8% (33/48)	70.8% (17/24)	83.3% (20/24)	77.1% (37/48)	90.7% (17/24)	87.5% (21/24)	79.2% (38/48)	75.0% (108/144)
FS-LoD	91.7% (22/24)	100% (24/24)	95.8% (46/48)	95.8% (23/24)	95.8% (23/24)	95.8% (46/48)	95.8% (23/24)	100% (24/24)	97.9% (47/48)	96.5% (139/144)
UR-Neg	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	100% (24/24)	100% (48/48)	100% (144/144)
UR-Mod Pos	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	100% (24/24)	100% (48/48)	100% (144/144)
UR-Low Pos	87.5% (31/24)	45.8% (11/24)	66.7% (32/48)	70.8% (17/24)	70.8% (17/24)	70.8% (34/48)	79.2% (19/24)	66.7% (16/24)	72.9% (35/48)	70.1% (101/144)
UR-LoD	91.7% (22/24)	100% (24/24)	95.8% (46/48)	95.8% (23/24)	91.7% (22/24)	93.8% (45/48)	100% (24/24)	91.7% (22/24)	95.8% (46/48)	95.1% (137/144)

a. FS = female swab matrix; UR = male urine matrix

Table 7: Summary of reproducibility data

Sample	Assay Channel (Analyte)	N[a]	Mean Ct	Between-Site		Between-Lot		Between-Day		Between-Operator		Within-Assay		Total	
				SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
FS-Neg	SAC	144	24.61	0	0	0.12	0.5	0.15	0.6	0	0	0.31	1.3	0.37	1.5
FS-Mod Pos	TV	144	35.40	0.09	0.3	0.32	0.9	0.16	0.4	0	0	0.68	1.9	0.77	2.2
FS-Low Pos	TV	108	38.18	0	0	0	0	0	0	0.52	1.4	0.86	2.3	1.01	2.6
FS-LoD	TV	139	37.14	0.16	0.4	0.36	1.0	0.21	0.6	0	0	0.92	2.5	1.02	2.7
UR-Neg	SAC	144	34.10	0.06	0.2	0.20	0.6	0	0	0.17	0.5	0.28	0.8	0.39	1.1
UR-Mod Pos	TV	144	35.40	0	0	0.37	1.0	0.13	0.4	0.18	0.5	0.65	1.8	0.78	2.2
UR-Low Pos	TV	101	37.95	0	0	0	0	0.36	1.0	0.52	1.4	0.94	2.5	1.14	3.0
UR-LoD	TV	137	37.03	0	0	0.33	0.9	0	0	0	0	0.96	2.6	1.01	2.7

a. Results with non-zero Ct values out of 144

5.5. Example 5: Analytical inclusivity

[00186] The analytical inclusivity of the Xpert TV Assay was evaluated by testing 17 *T. vaginalis* strains in triplicate at a concentration no greater than 3x analytical limit of detection (3x LoD). Strains were tested in *T. vaginalis*-negative pooled vaginal swab (VS) in buffer, and male urine (MU) mixed with buffer. *See* Table 8. Under the conditions of this study, all strains reported TV DETECTED results. The Xpert TV assay demonstrated 100% inclusivity in both sample types.

Table 8: Analytical inclusivity panel

Isolate ATCC #	Isolation Source	Results Vaginal Swab	Results Male Urine
30001	Vaginal exudate	TV DETECTED	TV DETECTED
30184	Vaginal swab	TV DETECTED	TV DETECTED
30187	Endocervical swab	TV DETECTED	TV DETECTED
30188	Vagina	TV DETECTED	TV DETECTED
30236	Endocervical swab	TV DETECTED	TV DETECTED
30240	Vaginal pool	TV DETECTED	TV DETECTED
30245	Vaginal and Endocervical material	TV DETECTED	TV DETECTED
30247	Vagina	TV DETECTED	TV DETECTED
50138	human	TV DETECTED	TV DETECTED
50139	human	TV DETECTED	TV DETECTED
50141	human	TV DETECTED	TV DETECTED
50143	human	TV DETECTED	TV DETECTED
50147	human	TV DETECTED	TV DETECTED
50167	Vagina	TV DETECTED	TV DETECTED
50183	Prostatic fluid	TV DETECTED	TV DETECTED
PRA-95	Vaginal exudate	TV DETECTED	TV DETECTED
PRA-98	human	TV DETECTED	TV DETECTED

5.6. Example 6: Analytical specificity

[00187] A panel of 47 organisms, including bacteria, fungi, and viruses commonly found in the urogenital tract, as well as other closely related protozoans to *Trichomonas* were tested with the Xpert TV Assay. Each bacterial or fungal strain was tested at 1 x 10^7 cfu/mL or greater. Strains which did not produce countable colonies were diluted to 0.5 McFarland units, approximately equivalent to1.5 x 108 cfu per mL for *E. coli*. Viral strains were purchased as heat inactivated stocks from ZeptoMetrix Corp. and tested at 1 x 10^6 U/mL or 10^6 genomes/mL. Protozoans were cultured in growth media, visually enumerated by light microscopy and tested at 1 x 10^6 cells/mL. Tests were performed in triplicate. The organisms tested and the Xpert TV assay results are listed in Table 9.

[00188] One organism, *Trichomonas tenax*, reported a TV DETECTED result with the Xpert TV assay. Under the conditions of this study, the analytical specificity of the Xpert TV Assay was 98%.

Table 9: Analytical specificity panel

Species	Strain ID	Test Result
Acinetobacter lwoffi	ATCC 17925	TV NOT DETECTED
Actinomyces israelii	ATCC 12102	TV NOT DETECTED
Atopobium vaginae	ATCC BAA-55	TV NOT DETECTED
Bacteroides fragilis	ATCC 25285	TV NOT DETECTED
Bacteroides ureolyticus	ATCC 33387	TV NOT DETECTED
Bifidobacterium adolescentis	ATCC 15703	TV NOT DETECTED
Campylobacter jejuni	ATCC 33560	TV NOT DETECTED
Candida albicans	ATCC 14053	TV NOT DETECTED
Candida glabrata	ATCC 90030	TV NOT DETECTED
Candida parapsilosis	ATCC 90018	TV NOT DETECTED
Candida tropicalis	ATCC 13803	TV NOT DETECTED
Chlamydia trachomatis	ATCC VR-885	TV NOT DETECTED
Clostridium difficile	ATCC 43594	TV NOT DETECTED
Clostridium perfringens	ATCC 13124	TV NOT DETECTED
Corynebacterium genitalium	ATCC 33031	TV NOT DETECTED
Cryptococcus neoformans	ATCC 32045	TV NOT DETECTED
Cytomegalovirus	ZeptoMetrix 0810003CF	TV NOT DETECTED
Enterobacter aerogenes	ATCC 51697	TV NOT DETECTED
Enterococcus feacalis	ATCC 19433	TV NOT DETECTED
Escherichia coli	ATCC 24922	TV NOT DETECTED
Fusobacterium nucleatum	ATCC 31647	TV NOT DETECTED
Gardnerella vaginalis	ATCC 49145	TV NOT DETECTED
Haemophilus ducreyi	ATCC 33940	TV NOT DETECTED
Herpes simplex virus I	ZeptoMetrix 0810005CF	TV NOT DETECTED
Herpes simplex virus II	ZeptoMetrix 0810006CF	TV NOT DETECTED
HIV-1	ZeptoMetrix 0801032CF	TV NOT DETECTED
HPV 16 (Caski)	ZeptoMetrix 0810232	TV NOT DETECTED
Klebsiella oxytoca	ATCC 43165	TV NOT DETECTED
Lactobacillus acidophilus	ATCC 314	TV NOT DETECTED
Lactobacillus jensenii	ATCC 25258	TV NOT DETECTED
Lactobacillus vaginalis	ATCC 49540	TV NOT DETECTED

Listeria monocytogenes	ATCC 15313	TV NOT DETECTED
Mobiluncus curtisii	ATCC 35241	TV NOT DETECTED
Mycoplasma hominis	ATCC 23114	TV NOT DETECTED
Neisseria gonorrhoeae	ATCC 35201	TV NOT DETECTED
Pentatrichomonas hominis	ATCC 30000	TV NOT DETECTED
Peptostreptococcus anaerobius	ATCC 49031	TV NOT DETECTED
Prevotella bivia	ATCC 29303	TV NOT DETECTED
Propionibacterium acnes	ATCC 6919	TV NOT DETECTED
Proteus mirabilis	ATCC 25933	TV NOT DETECTED
Pseudomonas aeruginosa	ATCC 35554	TV NOT DETECTED
Staphylococcus aureus	ATCC 700699	TV NOT DETECTED
Staphylococcus epidermidis	ATCC 14990	TV NOT DETECTED
Streptococcus agalactiae	ATCC 13813	TV NOT DETECTED
Streptococcus pyogenes	ATCC 19615	TV NOT DETECTED
Trichomonas tenax	ATCC 30207	TV DETECTED
Ureaplasma urealyticum	ATCC 27618	TV NOT DETECTED

5.7. Example 7: Interfering substances

[00189] In a non-clinical study, potentially interfering endogenous and exogenous substances that may be within the urogenital tract and present in endocervical and vaginal swab or first catch urine samples were evaluated with the Xpert TV Assay.

[00190] Substances were individually diluted into a pooled negative vaginal swab matrix and a pooled negative male urine matrix. The substances were also tested in the same matrices spiked with *T. vaginalis* cells at no greater than three times the limit of detection for the respective sample type. Eight replicates of each set of negative and positive samples were tested with the Xpert TV assay and compared to the results obtained in a control of the same sample without the potential interfering substance added. The substances and test concentrations are listed in Table 10 and Table 11.

[00191] Under the conditions of the study, in tests with the substances diluted into negative urine matrix no invalid results were reported; all tests reported TV NOT DETECTED as expected. Assay interference was observed in tests with blood at 0.75% v/v and azithromycin at 1.8 mg/mL diluted into positive urine matrix. False negative results were not reported for tests with blood at 0.5% v/v and azithromycin at 1 mg/mL.

[00192] Under the conditions of the study, in tests with the substances diluted into pooled negative swab matrix no invalid results were reported; all tests reported TV NOT DETECTED as expected.

[00193] In testing of substances diluted into pooled positive swab matrix, no false negative TV results were reported. Testing with all the substances reported TV DETECTED results as expected.

Table 10: Potentially interfering substances in urine samples

Class/Substance	Active Ingredient	Concentration Tested
Blood	Blood	0.3% v/v
Seminal Fluid	Seminal Fluid	5.0% v/v
Mucus	Mucin	0.8% w/v
Analgesics & Antibiotics	Acetylsalicylic Acid 500mg	8 mg/mL
	Acetaminophen	3.2 mg/mL
	Azithromycin	1.0 mg/mL
	Doxycycline	0.5 mg/mL
OTC Deodorant & Powders	PEG-20; PEG-32; PEG-20 Stearate	0.25% w/v
	Nanoxynol-9	0.25% w/v
Albumin	BSA	10 mg/ml
Glucose	Glucose	10 mg/ml
Bilirubin	Bilirubin	1 mg/ml
Acidic Urine (pH 4.0)	Urine + N-Acetyl-L-Cysteine	pH 4.0
Alkaline Urine (pH 9.0)	Urine + Ammonium Citrate	pH 9.0
Leukocytes	Leukocytes	10^6 cells/mL
Intravaginal Hormones	Progesterone; Estradiol	7 mg/mL Progesterone + 0.07 mg/mL Beta Estradiol

Table 11: Potentially interfering substances in swab samples

Class/Substance	Active Ingredient	Concentration Tested
Blood	Blood	1.0% v/v
Seminal Fluid	Seminal Fluid	5.0% v/v
Mucus	Mucin	0.8% w/v
Over the counter (OTC) Vaginal Products; Contraceptives; Vaginal treatments	Benzocaine 5%; Resorcinol 2%	0.25% w/v
	Clotrimazole 2%	0.25% w/v
	Miconazole Nitrate 2%	0.25% w/v
	Tioconazole	0.25% w/v
	5% w/w Aciclovir	0.25% w/v
	Glycerin, Propylene glycol	0.25% w/v
	Glycerin; Carbomer	0.12% w/v
	Glycerin, Hydroxyethyl cellulose	0.25% w/v
	Goldenseal 3X HPUS; Kreosotum 12X HPUS	0.25% w/v
	Povidone-iodine 10%	0.25% v/v
	Nonoxynol-9 12.5%	0.25% w/v
Hemerrhoidal Cream	Glycerin 14%; Pramoxine HCl 1%	0.25% w/v
Leukocytes	Leukocytes	10^6 cells/mL
Intravaginal Hormones	Progesterone; Estradiol	7 mg/mL Progesterone + 0.07 mg/mL Beta Estradiol

5.8. Example 8: Carry-over contamination

[00194] The study consisted of repeated tests of a TV-negative vaginal swab pool in buffer sample processed within the same GeneXpert module immediately following a high (10^6 cells/mL) TV positive vaginal swab pool in buffer sample. The study consisted of a TV-negative vaginal swab pool in buffer sample processed within the same GeneXpert module immediately followed by a high (10^6 cells/mL) TV positive vaginal swab pool in buffer sample. This testing scheme was repeated a further 20 times on two GeneXpert modules for a total of 82 runs resulting in 40 positive and 42 negative samples. All 40 positive samples were correctly reported as TV DETECTED and all 42 negative samples were correctly reported as TV NOT DETECTED.

5.9. Example 9: Alternate primers and probes tested to detect TV

[00195] To develop the TV assay described herein, four different forward primer, two different reverse primers, and two different probes for detecting the TV 40S

ribosomal protein (Tv40Srp) gene were tested for sensitivity and specificity (e.g., cross-reactivity with other species) in the assay. Table 12 shows the tested primers and probes.

Table 12: Alternate primer and probe sequences

oligo name	SEQ ID NO	sequence	Results
TV forward	1	GTAACAACCTTGGAGTTCTTCTTAAG	Final design
TV reverse	2	ACATCAATCTACAAGACACCACTTGA	Final design
TV probe	3	F1-AGTTTGGCTGCTTAGCTTCGAC-Q1	Final design
TV forward ALT1	6	GAGTTCTTCTTAAGCTGAACAC	FW design 2
TV forward ALT2	7	GAGTTCTTCTTGAGCTGAACAC	FW design 2 with different SNP at position 12
TV forward ALT3	8	AACAACCTTGGAGTTCTTCTTA	FW design 3
TV reverse ALT1	9	ATCTACAAGACACCACTTGA	RV design 2
TV probe ALT1	10	F1-AGTTTGGCTGCTTGGCTTCGAC-Q1	PR design 2 with different SNP at position 14

[00196] It was found that TV forward ALT1 cross-reacted with *Pentatric homonas hominis* (Pth), another closely related trichomonad found in human gut. When TV forward ALT1 was used in an assay with 1000 copies of TV and an assay with 500,000 copies of Pth, TV was detected with a Ct of 30.7 and Pth was detected with a Ct of 26.3. TV forward ALT2 was less sensitive than the final design, detecting TV with a higher Ct value of 31.5. Similarly, TV forward ALT3 was less sensitive than the final design, also detecting TV with a higher Ct value. TV reverse ALT1 also resulted in a less sensitive assay, detecting TV with a higher Ct value. Finally, TV probe ALT1 was less sensitive and less consistent than the final design.

[00197] All publications, patents, patent applications and other documents cited in this application are hereby incorporated by reference in their entireties for all purposes to the same extent as if each individual publication, patent, patent application or other document were individually indicated to be incorporated by reference for all purposes.

[00198] While various specific embodiments have been illustrated and described, it will be appreciated that changes can be made without departing from the spirit and scope of the invention(s).

TABLE OF CERTAIN SEQUENCES

SEQ ID NO	Description	Sequence
4	*Trichomonas vaginalis* 40S ribosomal protein (Tv40Srp) gene	ggccggcctt tctgatgggt aagtctaaag cttgcggtcg tctcgctgct cgtaaactcc gtcttgcaca caagtccaac ttgtgggctt ccaacgcata ccgccgttcc cttggtacat caatctacaa gacaccactt gagggtacat caatggcatc tggcatcgtc gtcggcaagg tcgctgtcga agccaagcag ccaaactctg ctattcgtaa agctgtccgt gttcagctta agaagaactc taaggttgtc acagctttcg ttccacgcga tggttccctc cgtcttattg atgataacga ccgtgttctt attgccggta tgggtcgttc tggccgttct gtcggtgacc ttccaggatg ccgtttcaaa gttatcaagg tcgctggttt ctccctcctt gctctttggc tcggcaagaa ggagaagccg cgcagctaaa taaatactct tgggtttacc ggtaaataaa aacatatatt acgaaataca aatattat
5	Tv40Srp amplicon	ACATCAATCT ACAAGACACC ACTTGAAGGC ACCTCAATGG CCTCCGGCAT TGTTGTCGGC AAAGTTGCTG TCGAAGCTAA GCAGCCAAAC TCCGCTATTC GTAAAGCAGT TCGTGTTCAG CTTAAGAAGA ACTCTAAAGT TGTTAC

WHAT IS CLAIMED IS:

1. A method of detecting the presence or absence of *Trichomonas vaginalis* (TV) in a sample from a subject comprising detecting the presence or absence of the TV 40S ribosomal protein (Tv40Srp) gene or RNA in the sample.

2. A method of determining whether a subject has a *Trichomonas vaginalis* (TV) infection comprising detecting the presence or absence of the TV 40S ribosomal protein (Tv40Srp) gene or RNA in a sample from the subject.

3. The method of claim 1 or claim 2, wherein the subject has not previously been treated for TV infection.

4. The method of claim 1 or claim 2, wherein the subject has previously been treated for TV infection.

5. The method of claim 4, wherein the previous treatment comprised one or more doses of metronidazole or tinidazole.

6. The method of any one of the preceding claims, wherein the subject does not have any symptoms of TV infection.

7. The method of any one of claims 1 to 5, wherein the subject has one or more symptoms of TV infection.

8. The method of claim 7, wherein the subject has one or more symptoms selected from vaginitis, urethritis, and cervicitis.

9. The method of claim 7 or claim 8, wherein the subject is female and has one or more symptoms selected from itching, burning, redness, and/or soreness of the genitals; unusual odor of the genitals; discomfort with urination; and a thin clear, white, yellow, or green discharge.

10. The method of any one of the preceding claims, wherein the subject is pregnant.

11. The method of claim 7 or claim 8, wherein the subject is male and has one or more symptoms selected from itching and/or burning inside the penis; burning after ejaculation and/or urination; and penile discharge.

12. The method of any one of the preceding claims, wherein the method comprises detecting an endogenous control.

13. The method of claim 12, wherein the endogenous control is a sample adequacy control.

14. The method of claim 12 or claim 13, wherein the endogenous control is a single-copy human gene.

15. The method of claim 14, wherein the endogenous control is selected from HMBS, GAPDH, beta actin, and beta globin.

16. The method of any one of the preceding claims, wherein the method comprises detecting an exogenous control.

17. The method of claim 16, wherein the exogenous control is a sample processing control.

18. The method of claim 16 or claim 17, wherein the exogenous control comprises a DNA sequence that is not expected to be present in the sample.

19. The method of any one of claims 16 to 18, wherein the exogenous control is a bacterial gene.

20. The method of any one of the preceding claims, wherein the method comprises PCR.

21. The method of claim 20, wherein the method comprises quantitative PCR.

22. The method of claim 20 or claim 21, wherein the PCR reaction takes less than 2 hours from an initial denaturation step through a final extension step.

23. The method of any one of the preceding claims, wherein the the TV 40S ribosomal protein (Tv40Srp) gene comprises the sequence of SEQ ID NO: 4.

24. The method of any one of the preceding claims, wherein the method comprises contacting nucleic acids from the sample with a first primer pair for detecting the TV 40S ribosomal protein (Tv40Srp) gene or RNA.

25. The method of claim 24, wherein the method comprises contacting nucleic acids from the sample with a second primer pair for detecting an endogenous control.

26. The method of claim 24 or claim 25, wherein the method comprises contacting nucleic acids from the sample with a third primer pair for detecting an exogenous control.

27. The method of any one of claims 24 to 26, wherein the first primer pair comprises a first primer and a second primer, wherein the first primer comprises a sequence that is at least 90%, at least 95%, or 100% identical to at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, or at least 25 contiguous nucleotides of SEQ ID NO: 4, and wherein the second primer comprises a sequence that is at least 90%, at least 95%, or 100% complementary to at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, or at least 25 contiguous nucleotides of SEQ ID NO: 4.

28. The method of claim 27, wherein the first primer and the second primer each independently comprises 0, 1, or 2 mismatches compared to SEQ ID NO: 4 or its complement.

29. The method of any one of claims 24 to 28, wherein the first primer pair comprises a first primer consisting of 15 to 30 nucleotides and a second primer consisting of 15 to 30 nucleotides.

30. The method of any one of claims 24 to 29, wherein the first primer pair comprises a first primer of SEQ ID NO: 1 and a second primer of SEQ ID NO: 2.

31. The method of any one of claims 24 to 30, wherein the first primer pair produces an amplicon that is 50 to 500 nucleotides long, 50 to 400 nucleotides long, 50 to 300 nucleotides long, 50 to 200 nucleotides long, 50 to 150 nucleotides long, 100 to 300 nucleotides long, 100 to 200 nucleotides long, or 100 to 150 nucleotides long.

32. The method of claim 31, wherein the method comprises forming the Tv40Srp amplicon.

33. The method of claim 32, wherein the method comprises contacting the Tv40Srp amplicon with a first probe capable of selectively hybridizing with the Tv40Srp amplicon.

34. The method of claim 33, wherein the first probe comprises a detectable label.

35. The method of claim 34, wherein the first probe comprises a fluorescent dye and a quencher molecule.

36. The method of any one of claims 33 to 35, wherein the first probe comprises a sequence that is at least 90%, at least 95%, or 100% identical or complementary to at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, or at least 25 contiguous nucleotides of SEQ ID NO: 4 or SEQ ID NO: 5.

37. The method of claim 36, wherein the first probe comprises 0, 1, or 2 mismatches compared to SEQ ID NO: 4 or its complement or compared to SEQ ID NO: 5 or its complement.

38. The method of any one of claims 33 to 37, wherein the first probe consists of 15 to 30 nucleotides.

39. The method of any one of claims 33 to 38, wherein the first probe has the sequence of SEQ ID NO: 3.

40. The method of any one of claims 32 to 39, wherein the method comprises forming an endogenous control amplicon and/or an exogenous control amplicon.

41. The method of claim 40, wherein the method comprises contacting the endogenous control amplicon with a second probe capable of selectively hybridizing with the engoenous control amplicon and/or contacting the exogenous control amplicon with a third probe capable of selectively hybridizing with the exoenous control amplicon.

42. The method of claim 41, wherein the second probe and the third probe each comprise a detectable label, wherein the detectable labels may be the same or different.

43. The method of claim 42, wherein the detectable labels of the second and third probes are detectably different from the detectable label of the first probe.

44. The method of any one of the preceding claims, wherein the method comprises detecting the Tv40Srp gene or RNA, an endogenous control, and an exogenous control in a single multiplex reaction.

45. The method of any one of the preceding claims, wherein the sample is selected from a urine sample, an endocervical swab sample, a vaginal swab sample, and a urethral swab sample.

46. A composition comprising a first primer pair for detecting a *Trichomonas vaginalis* 40S ribosomal protein (Tv40Srp) gene or RNA.

47. The composition of claim 39, wherein the composition comprises a second primer pair for detecting an endogenous control.

48. The composition of claim 40, wherein the endogenous control is a sample adequacy control.

49. The composition of claim 40, wherein the endogenous control is selected from HMBS, GAPDH, beta actin, and beta globin.

50. The composition of any one of claims 39 to 42, comprising a third primer pair for detecting an exogenous control.

51. The composition of claim 43, wherein the exogenous control is a sample processing control.

52. The composition of claim 43, wherein the exogenous control is a bacterial gene.

53. The composition of any one of claims 46 to 52, wherein the first primer pair comprises a first primer and a second primer, wherein the first primer comprises a sequence that is at least 90%, at least 95%, or 100% identical to at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, or at least 25 contiguous nucleotides of SEQ ID NO: 4, and wherein the second primer comprises a sequence that is at least 90%, at least 95%, or 100% complementary to at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, or at least 25 contiguous nucleotides of SEQ ID NO: 4.

54. The composition of claim 53, wherein the first primer and the second primer each independently comprises 0, 1, or 2 mismatches compared to SEQ ID NO: 4 or its complement.

55. The composition of any one of claims 46 to 54, wherein the first primer pair comprises a first primer consisting of 15 to 30 nucleotides and a second primer consisting of 15 to 30 nucleotides.

56. The composition of any one of claims 46 to 55, wherein the first primer pair comprises a first primer of SEQ ID NO: 1 and a second primer of SEQ ID NO: 2.

57. The composition of any one of claims 46 to 56, wherein the composition comprises a first probe capable of selectively hybridizing to a Tv40Srp amplicon produced by the first primer pair.

58. The composition of claim 57, wherein the first probe comprises a detectable label.

59. The composition of claim 58, wherein the first probe comprises a fluorescent dye and a quencher molecule.

60. The composition of any one of claims 57 to 59, wherein the first probe comprises a sequence that is at least 90%, at least 95%, or 100% identical or complementary to at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, or at least 25 contiguous nucleotides of SEQ ID NO: 4 or SEQ ID NO: 5.

61. The composition of claim 60, wherein the first probe comprises 0, 1, or 2 mismatches compared to SEQ ID NO: 4 or its complement or compared to SEQ ID NO: 5 or its complement.

62. The composition of any one of claims 57 to 61, wherein the first probe consists of 15 to 30 nucleotides.

63. The composition of any one of claims 57 to 62, wherein the first probe has the sequence of SEQ ID NO: 3.

64. The composition of any one of claims 57 to 63, wherein the Tv40Srp amplicon has the sequence of SEQ ID NO: 5.

65. The composition of any one of claims 46 to 64, wherein the composition comprises a second probe capable of selectively hybridizing to an endogenous control amplicon produced by the second primer pair.

66. The composition of claim 65, wherein the endogenous control is a sample adequacy control.

67. The composition of claim 65, wherein the endogenous control is selected from HMBS, GAPDH, beta actin, and beta globin.

68. The composition of any one of claims 46 to 67, wherein the composition comprises a third probe capable of selectively hybridizing to an exogenous control amplicon produced by the third primer pair.

69. The composition of claim 68, wherein the exogenous control is a sample processing control.

70. The composition of claim 68, wherein the exogenous control comprises a DNA sequence that is not expected to be present in the sample.

71. The composition of any one of claims 68 to 70, wherein the exogenous control is a bacterial DNA.

72. The composition of any one of claims 46 to 71, wherein the composition is a lyophilized composition.

73. The composition of any one of claims 46 to 71, wherein the composition is in solution.

74. The composition of claim 73, wherein the composition comprises nucleic acids from a sample from a subject being tested for the presence of absence of *Trichomonas vaginalis*.

75. A kit comprising a first primer pair for detecting a *Trichomonas vaginalis* 40S ribosomal protein (Tv40Srp) gene or RNA.

76. The kit of claim 75, wherein the kit comprises a second primer pair for detecting an endogenous control, wherein the primer pair for detecting Tv40Srp and the second primer pair are in the same or different compositions in the kit.

77. The kit of claim 76, wherein the endogenous control is a sample adequacy control.

78. The kit of claim 76, wherein the endogenous control is selected from HMBS, GAPDH, beta actin, and beta globin.

79. The kit of any one of claims 75 to 78, comprising a third primer pair for detecting an exogenous control, wherein the third primer pair is in the same or different composition from the primer pair for detecting Tv40Srp and the second primer pair.

80. The kit of claim 79, wherein the exogenous control is a sample processing control.

81. The kit of claim 79 or claim 80, wherein the exogenous control comprises a DNA sequence that is not expected to be present in the sample.

82. The kit of any one of claims 79 to 81, wherein the exogenous control is a bacterial gene.

83. The kit of any one of claims 75 to 82, wherein the first primer pair comprises a first primer and a second primer, wherein the first primer comprises a sequence that is at least 90%, at least 95%, or 100% identical to at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, or at least 25 contiguous nucleotides of SEQ ID NO: 4, and wherein the second primer comprises a sequence that is at least 90%, at least 95%, or 100% complementary to at least 15, at least 16, at least 17, at least

18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, or at least 25 contiguous nucleotides of SEQ ID NO: 4.

84. The kit of claim 83, wherein the first primer and the second primer each independently comprises 0, 1, or 2 mismatches compared to SEQ ID NO: 4 or its complement.

85. The kit of any one of claims 75 to 84, wherein the first primer pair comprises a first primer consisting of 15 to 30 nucleotides and a second primer consisting of 15 to 30 nucleotides.

86. The kit of any one of claims 75 to 85, wherein the primer pair for detecting the Tv40Srp gene or RNA comprises a first primer of SEQ ID NO: 1 and a second primer of SEQ ID NO: 2.

87. The kit of any one of claims 75 to 86, wherein the kit comprises a first probe capable of selectively hybridizing to a Tv40Srp amplicon produced by the first primer pair, wherein the first probe is in the same or different composition from one or more of the primer pairs.

88. The kit of claim 87, wherein the first probe comprises a detectable label.

89. The kit of claim 88, wherein the first probe comprises a fluorescent dye and a quencher molecule.

90. The kit of any one of claims 87 to 89, wherein the first probe comprises a sequence that is at least 90%, at least 95%, or 100% identical or complementary to at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, or at least 25 contiguous nucleotides of SEQ ID NO: 4 or SEQ ID NO: 5.

91. The kit of claim 90, wherein the first probe comprises 0, 1, or 2 mismatches compared to SEQ ID NO: 4 or its complement or compared to SEQ ID NO: 5 or its complement.

92. The kit of any one of claims 87 to 91, wherein the first probe consists of 15 to 30 nucleotides.

93. The kit of any one of claims 87 to 92, wherein the first probe has the sequence of SEQ ID NO: 3.

94. The kit of any one of claims 87 or claim 93, wherein the Tv40Srb amplicon has the sequence of SEQ ID NO: 5.

95. The kit of any one of claims 75 to 94, wherein the kit comprises a second probe capable of selectively hybridizing to an endogenous control amplicon produced by the second primer pair, wherein the second probe is in the same or different composition from one or more of the primer pairs.

96. The kit of any one of claims 75 to 95, wherein the kit comprises a third probe capable of selectively hybridizing to an exogenous control amplicon produced by the third primer pair, wherein the third probe is in the same or different composition from one or more of the primer pairs.

97. The kit of any one of claims 75 to 96, wherein the kit comprises dNTPs and/or a thermostable polymerase.

98. The kit of any one of claims 75 to 97, wherein the kit comprises one or more lyophilized compositions.

99. A primer consisting of the sequence of SEQ ID NO: 1, wherein the primer comprises at least one modified nucleotide.

100. A primer consisting of the sequence of SEQ ID NO: 2, wherein the primer comprises at least one modified nucleotide.

101. A probe consisting of the sequence of SEQ ID NO: 3, wherein the probe comprises at least one modified nucleotide and/or a detectable label.

102. The probe of claim 101, wherein the probe comprises a fluorescent dye and a quencher molecule.

103. The probe of claim 102, which is a fluorescence resonance energy transfer (FRET) probe.

104. The probe of any one of claims 101 to 103, wherein the probe comprises at least one modified nucleotide.

105. A composition comprising a first primer consisting of the sequence of SEQ ID NO: 2 and a second primer consisting of the sequence of SEQ ID NO: 3, wherein the first primer and the second primer each comprises at least one modified nucleotide.

106. The composition of claim 105, wherein the composition comprises a probe consisting of the sequence of SEQ ID NO: 3, wherein the probe comprises at least one modified nucleotide and/or a detectable label.

107. The composition of claim 106, wherein the probe comprises a fluorescent dye and a quencher molecule.

108. The composition of claim 106, wherein the probe is a fluorescence resonance energy transfer (FRET) probe.

109. The composition of any one of claims 106 to 108, wherein the probe comprises at least one modified nucleotide.

110. The composition of any one of claims 105 to 109, wherein the composition is a lyophilized composition.

111. The composition of any one of claims 105 to 109, wherein the composition is in solution.

112. The composition of claim 111, wherein the composition comprises nucleic acids of a sample from a subject.

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2014/046653

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12Q1/68
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2005/031005 A2 (GEN PROBE INC [US]; WEISBURG WILLIAM G [US]; BUNGO JENNIFER J [US]) 7 April 2005 (2005-04-07) the whole document	1-112
A	WO 2010/083274 A1 (BECTON DICKINSON CO [US]; MC MILLIAN RAY A [US]) 22 July 2010 (2010-07-22) the whole document	1-112
A	WO 2012/075321 A2 (PROGRAM FOR APPROPRIATE TECHNOLOGY IN HEALTH [US]; BARFIELD CORI ANNE) 7 June 2012 (2012-06-07) the whole document	1-112

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
5 September 2014	24/09/2014

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Bradbrook, Derek

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Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	A. J. SMITH ET AL: "Novel Core Promoter Elements and a Cognate Transcription Factor in the Divergent Unicellular Eukaryote Trichomonas vaginalis", MOLECULAR AND CELLULAR BIOLOGY, vol. 31, no. 7, 18 January 2011 (2011-01-18), pages 1444-1458, XP055138513, ISSN: 0270-7306, DOI: 10.1128/MCB.00745-10 figure 2 -----	1-112

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2014/046653

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2005031005 A2	07-04-2005	AT 542917 T	15-02-2012
		AU 2004276722 A1	07-04-2005
		CA 2525413 A1	07-04-2005
		EP 1633893 A2	15-03-2006
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		EP 2292793 A1	09-03-2011
		JP 2006525812 A	16-11-2006
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WO 2010083274 A1	22-07-2010	EP 2396422 A1	21-12-2011
		US 2010184066 A1	22-07-2010
		WO 2010083274 A1	22-07-2010
WO 2012075321 A2	07-06-2012	US 2012142003 A1	07-06-2012
		WO 2012075321 A2	07-06-2012

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权利要求书6页 说明书41页
序列表3页

(54)发明名称

检测阴道毛滴虫的方法

(57)摘要

本发明提供检测阴道毛滴虫(Trichomonas vaginalis)的组合物和方法。

CN 106661611 A

1.检测来自受试者的样品中存在或不存在阴道毛滴虫(Trichomonasvaginalis)(TV)的方法,所述方法包括检测所述样品中存在或不存在TV40S核糖体蛋白(Tv40Srp)基因或RNA。

2.一种确定受试者是否具有阴道毛滴虫(TV)感染的方法,所述方法包括检测来自受试者的样品中存在或不存在TV 40S核糖体蛋白(Tv40Srp)基因或RNA。

3.权利要求1或权利要求2所述的方法,其中所述受试者之前未对TV感染进行治疗。

4.权利要求1或权利要求2所述的方法,其中所述受试者之前对TV感染进行治疗。

5.权利要求4所述的方法,其中所述之前的治疗包含一个或多个剂量的甲硝哒唑或替硝唑。

6.前述权利要求中任一项所述的方法,其中所述受试者不具有任何TV感染症状。

7.权利要求1至5中任一项所述的方法,其中所述受试者具有一种以上TV感染症状。

8.权利要求7所述的方法,其中所述受试者具有选自阴道炎、尿道炎和子宫颈炎的一种以上症状。

9.权利要求7或权利要求8所述的方法,其中所述受试者是女性并且具有选自以下各项中的一种以上症状:生殖器的瘙痒、烧灼、发红、和/或疼痛;生殖器的异味;排尿不适;和稀的澄清、白色、黄色或绿色排泄物。

10.前述权利要求中任一项所述的方法,其中所述受试者是怀孕的。

11.权利要求7或权利要求8所述的方法,其中所述受试者是男性并且具有选自以下各项中的一种以上症状:阴茎内瘙痒和/或烧灼;射精和/或排尿后烧灼;和阴茎排泄物。

12.前述权利要求中任一项所述的方法,其中所述方法包括检测内源性对照。

13.权利要求12所述的方法,其中所述内源性对照是样品充分性对照。

14.权利要求12或权利要求13所述的方法,其中所述内源性对照是单拷贝人基因。

15.权利要求14所述的方法,其中所述内源性对照选自HMBS、GAPDH、β肌动蛋白和β珠蛋白。

16.前述权利要求中任一项所述的方法,其中所述方法包括检测外源性对照。

17.权利要求16所述的方法,其中所述外源性对照是样品处理对照。

18.权利要求16或权利要求17所述的方法,其中所述外源性对照包括预期不存在于样品中的DNA序列。

19.权利要求16至18中任一项所述的方法,其中所述外源性对照是细菌基因。

20.前述权利要求中任一项所述的方法,其中所述方法包括PCR。

21.权利要求20所述的方法,其中所述方法包括定量PCR。

22.权利要求20或权利要求21所述的方法,其中PCR反应从起始变性步骤通过最终延伸步骤花费小于2小时。

23.前述权利要求中任一项所述的方法,其中所述TV 40S核糖体蛋白(Tv40Srp)基因包含SEQ ID NO:4的序列。

24.前述权利要求中任一项所述的方法,其中所述方法包括将来自样品的核酸与第一引物对接触,所述第一引物对用于检测TV 40S核糖体蛋白(Tv40Srp)基因或RNA。

25.权利要求24所述的方法,其中所述方法包括将来自样品的核酸与第二引物对接触,所述第二引物对用于检测内源性对照。

26.权利要求24或权利要求25所述的方法，其中所述方法包括将来自样品的核酸与第三引物对接触，所述第三引物对用于检测外源性对照。

27.权利要求24至26中任一项所述的方法，其中所述第一引物对包括第一引物和第二引物，其中所述第一引物包含与SEQ ID NO:4的至少15，至少16，至少17，至少18，至少19，至少20，至少21，至少22，至少23，至少24，或至少25个连续核苷酸至少90％，至少95％，或100％同一的序列，并且其中所述第二引物包含与SEQ ID NO:4的至少15，至少16，至少17，至少18，至少19，至少20，至少21，至少22，至少23，至少24，或至少25个连续核苷酸至少90％，至少95％，或100％互补的序列。

28.权利要求27所述的方法，其中，与SEQ ID NO:4或其互补物相比，所述第一引物和所述第二引物各自独立地包含0、1、或2个错配。

29.权利要求24至28中任一项所述的方法，其中所述第一引物对包括由15至30个核苷酸组成的第一引物和由15至30个核苷酸组成的第二引物。

30.权利要求24至29中任一项所述的方法，其中所述第一引物对包括SEQ ID NO:1的第一引物和SEQ ID NO:2的第二引物。

31.权利要求24至30中任一项所述的方法，其中所述第一引物对产生50至500个核苷酸长度，50至400个核苷酸长度，50至300个核苷酸长度，50至200个核苷酸长度，50至150个核苷酸长度，100至300个核苷酸长度，100至200个核苷酸长度，或100至150个核苷酸长度的扩增子。

32.权利要求31所述的方法，其中所述方法包括形成Tv40Srp扩增子。

33.权利要求32所述的方法，其中所述方法将所述Tv40Srp扩增子与能够选择性与Tv40Srp扩增子杂交的第一探针接触。

34.权利要求33所述的方法，其中所述第一探针包含可检测标记。

35.权利要求34所述的方法，其中所述第一探针包含荧光染料和猝灭剂分子。

36.权利要求33至35中任一项所述的方法，其中所述第一探针包含SEQ ID NO:4或SEQ ID NO:5的至少15，至少16，至少17，至少18，至少19，至少20，至少21，至少22，至少23，至少24，或至少25个连续核苷酸至少90％，至少95％，或100％同一或互补的序列。

37.权利要求36所述的方法，其中与SEQ ID NO:4或其互补物相比，或与SEQ ID NO:5或其互补物相比，所述第一探针包含0、1、或2个错配。

38.权利要求33至37中任一项所述的方法，其中所述第一探针由15至30个核苷酸组成。

39.权利要求33至38中任一项所述的方法，其中所述第一探针具有SEQ ID NO:3的序列。

40.权利要求32至39中任一项所述的方法，其中所述方法包括形成内源性对照扩增子和/或外源性对照扩增子。

41.权利要求40所述的方法，其中所述方法包括将内源性对照扩增子与能够选择性与内源性对照扩增子杂交的第二探针接触和/或将外源性对照扩增子与能够选择性与外源性对照扩增子杂交的第三探针接触。

42.权利要求41所述的方法，其中所述第二探针和第三探针各自包含可检测标记，其中所述可检测标记可以相同或不同。

43.权利要求42所述的方法，其中所述第二和第三探针的可检测标记是可区别于第一

探针的可检测标记而被检测的。

44.前述权利要求中任一项所述的方法，其中所述方法包括以单个多重反应检测Tv40Srp基因或RNA、内源性对照和外源性对照。

45.前述权利要求中任一项所述的方法，其中所述样品选自尿样品、子宫颈内拭子样品、阴道拭子样品和尿道拭子样品。

46.组合物，其包含用于检测阴道毛滴虫40S核糖体蛋白(Tv40Srp)基因或RNA的第一引物对。

47.权利要求39所述的组合物，其中所述组合物包含用于检测内源性对照的第二引物对。

48.权利要求40所述的组合物，其中所述内源性对照是样品充分性对照。

49.权利要求40所述的组合物，其中所述内源性对照选自HMBS、GAPDH、β肌动蛋白和β珠蛋白。

50.权利要求39至42中任一项所述的组合物，其包含用于检测外源性对照的第三引物对。

51.权利要求43所述的组合物，其中所述外源性对照是样品处理对照。

52.权利要求43所述的组合物，其中所述外源性对照是细菌基因。

53.权利要求46至52中任一项所述的组合物，其中所述第一引物对包含第一引物和第二引物，其中所述第一引物包含与SEQ ID NO:4的至少15，至少16，至少17，至少18，至少19，至少20，至少21，至少22，至少23，至少24，或至少25个连续核苷酸至少90％，至少95％，或100％同一的序列，并且其中所述第二引物包含与SEQ ID NO:4的至少15，至少16，至少17，至少18，至少19，至少20，至少21，至少22，至少23，至少24，或至少25个连续核苷酸至少90％，至少95％，或100％互补的序列。

54.权利要求53所述的组合物，其中与SEQ ID NO:4或其互补物相比，所述第一引物和第二引物各自独立地包含0、1、或2个错配。

55.权利要求46至54中任一项所述的组合物，其中所述第一引物对包含由15至30个核苷酸组成的第一引物和由15至30个核苷酸组成的第二引物。

56.权利要求46至55中任一项所述的组合物，其中所述第一引物对包含SEQ ID NO:1的第一引物和SEQ ID NO:2的第二引物。

57.权利要求46至56中任一项所述的组合物，其中所述组合物包含能够选择性与第一引物对产生的Tv40Srp扩增子杂交的第一探针。

58.权利要求57所述的组合物，其中所述第一探针包含可检测标记。

59.权利要求58所述的组合物，其中所述第一探针包含荧光染料和猝灭剂分子。

60.权利要求57至59中任一项所述的组合物，其中所述第一探针包含与SEQ ID NO:4或SEQ ID NO:5的至少15，至少16，至少17，至少18，至少19，至少20，至少21，至少22，至少23，至少24，或至少25个连续核苷酸至少90％，至少95％，或100％同一或互补的序列。

61.权利要求60所述的组合物，其中与SEQ ID NO:4或其互补物相比或与SEQ ID NO:5或其互补物相比，所述第一探针包含0、1、或2个错配。

62.权利要求57至61中任一项所述的组合物，其中所述第一探针由15至30个核苷酸组成。

63.权利要求57至62中任一项所述的组合物，其中所述第一探针具有SEQ ID NO:3的序列。

64.权利要求57至63中任一项所述的组合物，其中所述Tv40Srp扩增子具有SEQ ID NO:5的序列。

65.权利要求46至64中任一项所述的组合物，其中所述组合物包含能够选择性与由第二引物对产生的内源性对照扩增子杂交的第二探针。

66.权利要求65所述的组合物，其中所述内源性对照是样品充分性对照。

67.权利要求65所述的组合物，其中所述内源性对照选自HMBS、GAPDH、β肌动蛋白和β珠蛋白。

68.权利要求46至67中任一项所述的组合物，其中所述组合物包含能够选择性与由第三引物对产生的外源性对照扩增子杂交的第三探针。

69.权利要求68所述的组合物，其中所述外源性对照是样品处理对照。

70.权利要求68所述的组合物，其中所述外源性对照包含预期不存在于样品中的DNA序列。

71.权利要求68至70中任一项所述的组合物，其中所述外源性对照是细菌DNA。

72.权利要求46至71中任一项所述的组合物，其中所述组合物是冻干的组合物。

73.权利要求46至71中任一项所述的组合物，其中所述组合物在溶液中。

74.权利要求73所述的组合物，其中所述组合物包含来自被测试存在或不存在阴道毛滴虫的受试者的样品的核酸。

75.一种试剂盒，其包含用于检测阴道毛滴虫40S核糖体蛋白(Tv40Srp)基因或RNA的第一引物对。

76.权利要求75所述的试剂盒，其中所述试剂盒包含用于检测内源性对照的第二引物对，其中所述用于检测Tv40Srp的引物对和所述第二引物对在试剂盒中的相同或不同组合物中。

77.权利要求76所述的试剂盒，其中所述内源性对照是样品充分性对照。

78.权利要求76所述的试剂盒，其中所述内源性对照选自HMBS、GAPDH、β肌动蛋白和β珠蛋白。

79.权利要求75至78中任一项所述的试剂盒，所述试剂盒包含用于检测外源性对照的第三引物对，其中所述第三引物对与所述用于检测Tv40Srp的引物对和所述第二引物对在相同或不同组合物中。

80.权利要求79所述的试剂盒，其中所述外源性对照是样品处理对照。

81.权利要求79或权利要求80所述的试剂盒，其中所述外源性对照包含预期不存在于样品中的DNA序列。

82.权利要求79至81中任一项所述的试剂盒，其中所述外源性对照是细菌基因。

83.权利要求75至82中任一项所述的试剂盒，其中所述第一引物对包含第一引物和第二引物，其中所述第一引物包含与SEQ ID NO:4的至少15，至少16，至少17，至少18，至少19，至少20，至少21，至少22，至少23，至少24，或至少25个连续核苷酸至少90％，至少95％，或100％同一的序列，并且其中所述第二引物包含与SEQ ID NO:4的至少15，至少16，至少17，至少18，至少19，至少20，至少21，至少22，至少23，至少24，或至少25个连续核苷酸至少

90％，至少95％，或100％互补的序列。

84.权利要求83所述的试剂盒，其中与SEQ ID NO:4或其互补物相比，所述第一引物和第二引物各自独立地包含0、1、或2个错配。

85.权利要求75至84中任一项所述的试剂盒，其中所述第一引物对包含由15至30个核苷酸组成的第一引物和由15至30个核苷酸组成的第二引物。

86.权利要求75至85中任一项所述的试剂盒，其中所述用于检测Tv40Srp基因或RNA的引物对包含SEQ ID NO:1的第一引物和SEQ ID NO:2的第二引物。

87.权利要求75至86中任一项所述的试剂盒，其中所述试剂盒包含能够选择性与由第一引物对产生的Tv40Srp扩增子杂交的第一探针，其中所述第一探针与一种以上所述引物对在相同或不同组合物中。

88.权利要求87所述的试剂盒，其中所述第一探针包含可检测标记。

89.权利要求88所述的试剂盒，其中所述第一探针包含荧光染料和猝灭剂分子。

90.权利要求87至89中任一项所述的试剂盒，其中所述第一探针包含与SEQ ID NO:4或SEQ ID NO:5的至少15，至少16，至少17，至少18，至少19，至少20，至少21，至少22，至少23，至少24，或至少25个连续核苷酸至少90％，至少95％，或100％同一或互补的序列。

91.权利要求90所述的试剂盒，其中与SEQ ID NO:4或其互补物相比或与SEQ ID NO:5或其互补物相比，所述第一探针包含0、1、或2个错配。

92.权利要求87至91中任一项所述的试剂盒，其中所述第一探针由15至30个核苷酸组成。

93.权利要求87至92中任一项所述的试剂盒，其中所述第一探针具有SEQ ID NO:3的序列。

94.权利要求87或权利要求93中任一项所述的试剂盒，其中所述Tv40Srb扩增子具有SEQ ID NO:5的序列。

95.权利要求75至94中任一项所述的试剂盒，其中所述试剂盒包含能够选择性与由第二引物对产生的内源性对照扩增子杂交的第二探针，其中所述第二探针与一种以上所述引物对在相同或不同组合物中。

96.权利要求75至95中任一项所述的试剂盒，其中所述试剂盒包含能够选择性与由第三引物对产生的外源性对照扩增子杂交的第三探针，其中所述第三探针与一种以上所述引物对在相同或不同组合物中。

97.权利要求75至96中任一项所述的试剂盒，其中所述试剂盒包含dNTPs和/或热稳定的聚合酶。

98.权利要求75至97中任一项所述的试剂盒，其中所述试剂盒包含一种以上冻干的组合物。

99.由SEQ ID NO:1的序列组成的引物，其中所述引物包含至少一个修饰的核苷酸。

100.由SEQ ID NO:2的序列组成的引物，其中所述引物包含至少一个修饰的核苷酸。

101.SEQ ID NO:3的序列组成的探针，其中所述探针包含至少一个修饰的核苷酸和/或可检测标记。

102.权利要求101所述的探针，其中所述探针包含荧光染料和猝灭剂分子。

103.权利要求102所述的探针，其是荧光共振能量转移(FRET)探针。

104.权利要求101至103中任一项所述的探针,其中所述探针包含至少一个修饰的核苷酸。

105.一种组合物,其包含由SEQ ID NO:2的序列组成的第一引物和由SEQ ID NO:3的序列组成的第二引物,其中所述第一引物和所述第二引物各自包含至少一个修饰的核苷酸。

106.权利要求105所述的组合物,其中所述组合物包含由SEQ ID NO:3的序列组成的探针,其中所述探针包含至少一个修饰的核苷酸和/或可检测标记。

107.权利要求106所述的组合物,其中所述探针包含荧光染料和猝灭剂分子。

108.权利要求106所述的组合物,其中所述探针是荧光共振能量转移(FRET)探针。

109.权利要求106至108中任一项所述的组合物,其中所述探针包含至少一个修饰的核苷酸。

110.权利要求105至109中任一项所述的组合物,其中所述组合物是冻干的组合物。

111.权利要求105至109中任一项所述的组合物,其中所述组合物在溶液中。

112.权利要求111所述的组合物,其中所述组合物包含来自受试者的样品的核酸。

检测阴道毛滴虫的方法

1.发明领域

[0001] 提供用于检测阴道毛滴虫(Trichomonas vaginalis)的组合物和方法。

[0002] 2.背景

[0003] 原生动物阴道毛滴虫造成毛滴虫病(trichomoniasis),其是常见性传播感染,可以感染男性和女性。每年在美国有七百四十万例毛滴虫病。毛滴虫病感染可以是有症状的或无症状的。参见,例如,Ginocchio等人,J.Clin.Microbiol.2012,50:2601-2608。在女性中,毛滴虫病是一系列包含阴道排出物的病况中的一种。参见,例如,Centers for Disease Control andPrevention(CDC).CDC fact sheet:trichomoniasis.2010.www.cdc.gov/std/trichomonas/STDFact-Trichomoniasis.htm。女性中的症状可能包括生殖器瘙痒、烧灼、发红、或疼痛,异味,排尿不适,或稀的澄清、白色、黄色或绿色排出物。参见同上。在男性中,毛滴虫病可以引起非淋病双球菌的尿道炎(NGU)。男性中的症状可能包括阴茎内部瘙痒或烧灼,射精或排尿后烧灼,或阴茎排泄物。参见,例如,Workowski等人,Centers for Disease Control and Prevention.Sexually transmitted disease treatment guidelines,2010.MMWR 2010;59(RR-12):1-110;Centers for Disease Control and Prevention.Biosafety in Microbiological and Biomedical laboratories.www.cdc.gov/biosafety/publications/。

[0004] 需要改善的检测阴道毛滴虫(TV)的方法。尤其是,需要高度特异的、准确的和灵敏的基于尿或拭子的诊断测试。

[0005] 3.概述

[0006] 在一些实施方案中,提供检测来自受试者的样品中存在或不存在阴道毛滴虫(TV)的方法。在一些实施方案中,提供确定受试者是否具有阴道毛滴虫(TV)感染的方法。在一些实施方案中,所述方法包括检测受试者的样品中存在或不存在TV 40S核糖体蛋白(Tv40Srp)基因或RNA。

[0007] 在一些实施方案中,所述受试者之前未对TV感染进行治疗。在一些实施方案中,所述受试者之前对TV感染进行治疗。在一些实施方案中,之前的治疗包括一个以上剂量的甲硝哒唑或替硝唑。在一些实施方案中,所述受试者不具有任何TV感染症状。在一些实施方案中,所述受试者具有一种以上TV感染症状。在一些实施方案中,所述受试者具有选自阴道炎、尿道炎和子宫颈炎的一种以上症状。在一些实施方案中,所述受试者是女性并且具有选自以下各项的一种以上症状:生殖器瘙痒、烧灼、发红、和/或疼痛;生殖器异味;排尿不适;和稀的澄清、白色、黄色或绿色排出物。在一些实施方案中,所述受试者怀孕。在一些实施方案中,所述受试者是男性并且具有选自以下各项的一种以上症状:阴茎内瘙痒和/或烧灼;射精和/或排尿后烧灼;和阴茎排泄物。

[0008] 在一些实施方案中,所述方法包括检测内源性对照。在一些实施方案中,内源性对照是样品充分性对照。在一些实施方案中,内源性对照是单拷贝人基因。在一些实施方案中,内源性对照选自HMBS、GAPDH、β肌动蛋白和β珠蛋白。

[0009] 在一些实施方案中,所述方法包括检测外源性对照。在一些实施方案中,外源性对

照是样品处理对照。在一些实施方案中，外源性对照包含预期不存在于样品中的DNA序列。在一些实施方案中，外源性对照是细菌基因。

[0010] 在一些实施方案中，所述方法包括PCR。在一些实施方案中，所述方法包括定量PCR。在一些实施方案中，PCR反应从起始变性步骤通过最终延伸步骤耗费小于2小时，小于1小时，或小于30分钟。

[0011] 在一些实施方案中，TV 40S核糖体蛋白(Tv40Srp)基因包含SEQ IDNO:4的序列。在一些实施方案中，所述方法包括将来自样品的核酸与用于检测TV 40S核糖体蛋白(Tv40Srp)基因或RNA的第一引物对接触。在一些实施方案中，所述方法包括将来自样品的核酸与用于检测内源性对照的第二引物对接触。在一些实施方案中，所述方法包括将来自样品的核酸与用于检测外源性对照的第三引物对接触。

[0012] 在一些实施方案中，第一引物对包含第一引物和第二引物，其中所述第一引物包含与SEQ ID NO:4的至少15，至少16，至少17，至少18，至少19，至少20，至少21，至少22，至少23，至少24，或至少25个连续核苷酸至少90％，至少95％，或100％同一的序列，并且其中第二引物包含与SEQ ID NO:4的至少15，至少16，至少17，至少18，至少19，至少20，至少21，至少22，至少23，至少24，或至少25个连续核苷酸至少90％，至少95％，或100％互补的序列。在一些实施方案中，与SEQ ID NO:4或其互补物相比，第一引物和第二引物各自独立地包含0、1、或2个错配。在一些实施方案中，第一引物对包含由15至30个核苷酸组成的第一引物和由15至30个核苷酸组成的第二引物。在一些实施方案中，第一引物对包含SEQ ID NO:1的第一引物和SEQ ID NO:2的第二引物。在一些实施方案中，第一引物对产生50至500个核苷酸长度，50至400个核苷酸长度，50至300个核苷酸长度，50至200个核苷酸长度，50至150个核苷酸长度，100至300个核苷酸长度，100至200个核苷酸长度，或100至150个核苷酸长度的扩增子。

[0013] 在一些实施方案中，所述方法包括形成Tv40Srp扩增子。在一些实施方案中，所述方法包括将Tv40Srp扩增子与能够选择性与Tv40Srp扩增子杂交的第一探针接触。在一些实施方案中，第一探针包含可检测标记。在一些实施方案中，第一探针包含荧光染料和猝灭剂分子。在一些实施方案中，第一探针包含与SEQ ID NO:4或SEQ ID NO:5的至少15，至少16，至少17，至少18，至少19，至少20，至少21，至少22，至少23，至少24，或至少25个连续核苷酸至少90％，至少95％，或100％同一或互补的序列。在一些实施方案中，与SEQ ID NO:4或其互补物相比或与SEQ ID NO:5或其互补物相比，第一探针包含0、1、或2个错配。在一些实施方案中，第一探针由15至30个核苷酸组成。在一些实施方案中，第一探针具有SEQ ID NO:3的序列。

[0014] 在一些实施方案中，所述方法包括形成内源性对照扩增子和/或外源性对照扩增子。在一些实施方案中，所述方法包括将内源性对照扩增子与能够选择性与内源性对照扩增子杂交的第二探针接触和/或外源性对照扩增子与能够选择性与外源性对照扩增子杂交的第三探针接触。在一些实施方案中，第二探针和第三探针各自包含可检测标记，其中所述可检测标记可以相同或不同。在一些实施方案中，第二和第三探针的可检测标记在检测上不同于第一探针的可检测标记。在一些实施方案中，所述方法包括在单个多重反应中检测Tv40Srp基因或RNA、内源性对照和外源性对照。

[0015] 在一些实施方案中，所述样品选自尿样品、子宫颈内拭子样品、阴道拭子样品和尿

道拭子样品。

[0016] 在一些实施方案中，提供组合物，其包含用于检测阴道毛滴虫40S核糖体蛋白(Tv40Srp)基因或RNA的第一引物对。在一些实施方案中，组合物包含用于检测内源性对照的第二引物对。在一些实施方案中，内源性对照是样品充分性对照。在一些实施方案中，内源性对照选自HMBS、GAPDH、β肌动蛋白和β珠蛋白。在一些实施方案中，组合物包含用于检测外源性对照的第三引物对。在一些实施方案中，外源性对照是样品处理对照。在一些实施方案中，外源性对照是细菌基因。

[0017] 在一些实施方案中，第一引物对包含第一引物和第二引物，其中所述第一引物包含与SEQ ID NO:4的至少15，至少16，至少17，至少18，至少19，至少20，至少21，至少22，至少23，至少24，或至少25个连续核苷酸至少90％，至少95％，或100％同一的序列，并且其中第二引物包含与SEQ ID NO:4的至少15，至少16，至少17，至少18，至少19，至少20，至少21，至少22，至少23，至少24，或至少25个连续核苷酸至少90％，至少95％，或100％互补的序列。在一些实施方案中，与SEQ ID NO:4或其互补物相比，第一引物和第二引物各自独立地包含0、1、或2个错配。在一些实施方案中，第一引物对包含由15至30个核苷酸组成的第一引物和由15至30个核苷酸组成的第二引物。在一些实施方案中，第一引物对包含SEQ ID NO:1的第一引物和SEQ ID NO:2的第二引物。

[0018] 在一些实施方案中，组合物包含能够选择性与由第一引物对产生的Tv40Srp扩增子杂交的第一探针。在一些实施方案中，第一探针包含可检测标记。在一些实施方案中，第一探针包含荧光染料和猝灭剂分子。在一些实施方案中，第一探针包含与SEQ ID NO:4或SEQ ID NO:5的至少15，至少16，至少17，至少18，至少19，至少20，至少21，至少22，至少23，至少24，或至少25个连续核苷酸至少90％，至少95％，或100％同一或互补的序列。在一些实施方案中，与SEQ ID NO:4或其互补物相比或与SEQ ID NO:5或其互补物相比，第一探针包含0、1、或2个错配。在一些实施方案中，第一探针由15至30个核苷酸组成。在一些实施方案中，第一探针具有SEQ ID NO:3的序列。在一些实施方案中，Tv40Srp扩增子具有SEQ ID NO:5的序列。

[0019] 在一些实施方案中，组合物包含能够选择性与由第二引物对产生的内源性对照扩增子杂交的第二探针。在一些实施方案中，内源性对照是样品充分性对照。在一些实施方案中，内源性对照选自HMBS、GAPDH、β肌动蛋白和β珠蛋白。在一些实施方案中，组合物包含能够选择性与由第三引物对产生的外源性对照扩增子杂交的第三探针。在一些实施方案中，外源性对照是样品处理对照。在一些实施方案中，外源性对照包含预期不存在于样品中的DNA序列。在一些实施方案中，外源性对照是细菌DNA。

[0020] 在一些实施方案中，组合物是冻干的组合物。在一些实施方案中，组合物在溶液中。在一些实施方案中，组合物包含来自测试存在或不存在阴道毛滴虫的受试者的样品的核酸。

[0021] 在一些实施方案中，提供试剂盒，所述试剂盒包含用于检测阴道毛滴虫40S核糖体蛋白(Tv40Srp)基因或RNA的第一引物对。在一些实施方案中，所述试剂盒包含用于检测内源性对照的第二引物对，其中用于检测Tv40Srp的引物对和第二引物对在试剂盒中的相同或不同组合物中。在一些实施方案中，内源性对照是样品充分性对照。在一些实施方案中，内源性对照选自HMBS、GAPDH、β肌动蛋白和β珠蛋白。在一些实施方案中，所述试剂盒包含用

于检测外源性对照的第三引物对，其中第三引物对与用于检测Tv40Srp的引物对和第二引物对在相同或不同组合物中。在一些实施方案中，外源性对照是样品处理对照。在一些实施方案中，外源性对照包含预期不存在于样品中的DNA序列。在一些实施方案中，外源性对照是细菌基因。

[0022] 在一些实施方案中，第一引物对包含第一引物和第二引物，其中第一引物包含与SEQ ID NO:4的至少15，至少16，至少17，至少18，至少19，至少20，至少21，至少22，至少23，至少24，或至少25个连续核苷酸至少90％，至少95％，或100％同一的序列，并且其中第二引物包含与SEQ ID NO:4的至少15，至少16，至少17，至少18，至少19，至少20，至少21，至少22，至少23，至少24，或至少25个连续核苷酸至少90％，至少95％，或100％互补的序列。在一些实施方案中，与SEQ ID NO:4或其互补物相比，第一引物和第二引物各自独立地包含0、1、或2个错配。在一些实施方案中，第一引物对包含由15至30个核苷酸组成的第一引物和由15至30个核苷酸组成的第二引物。在一些实施方案中，第一引物对包含SEQ ID NO:1的第一引物和SEQ ID NO:2的第二引物。

[0023] 在一些实施方案中，所述试剂盒包含能够选择性与由第一引物对产生的Tv40Srp扩增子杂交的第一探针，其中所述第一探针与一种以上所述引物对在相同或不同组合物中。在一些实施方案中，第一探针包含可检测标记。在一些实施方案中，第一探针包含荧光染料和猝灭剂分子。在一些实施方案中，第一探针包含与SEQ ID NO:4或SEQ ID NO:5的至少15，至少16，至少17，至少18，至少19，至少20，至少21，至少22，至少23，至少24，或至少25个连续核苷酸至少90％，至少95％，或100％同一或互补的序列。在一些实施方案中，与SEQ ID NO:4或其互补物相比或与SEQ ID NO:5或其互补物相比，第一探针包含0、1、或2个错配。在一些实施方案中，第一探针由15至30个核苷酸组成。在一些实施方案中，第一探针具有SEQ ID NO:3的序列。在一些实施方案中，Tv40Srp扩增子具有SEQ ID NO:5的序列。

[0024] 在一些实施方案中，所述试剂盒包含能够选择性与由第二引物对产生的内源性对照扩增子杂交的第二探针，其中第二探针与一种以上所述引物对在相同或不同组合物中。在一些实施方案中，所述试剂盒包含能够选择性与由第三引物对产生的外源性对照扩增子杂交的第三探针，其中第三探针与一种以上所述引物对在相同或不同组合物中。

[0025] 在一些实施方案中，所述试剂盒包含dNTP和/或热稳定的聚合酶。在一些实施方案中，所述试剂盒包含一种以上冻干的组合物。

[0026] 在一些实施方案中，提供引物，其中所述引物由SEQ ID NO:1的序列组成，其中引物包含至少一个修饰的核苷酸。在一些实施方案中，提供引物，其中引物由SEQ ID NO:2的序列组成，其中引物包含至少一个修饰的核苷酸。在一些实施方案中，提供探针，其中探针由SEQ ID NO:3的序列组成，其中探针包含至少一个修饰的核苷酸和/或可检测标记。在一些实施方案中，探针包含荧光染料和猝灭剂分子。在一些实施方案中，探针是荧光共振能量转移(FRET)探针。在一些实施方案中，探针包含至少一个修饰的核苷酸。

[0027] 在一些实施方案中，提供组合物，其中组合物包含由SEQ ID NO:2的序列组成的第一引物和由SEQ ID NO:3的序列组成的第二引物，其中第一引物和第二引物各自包含至少一个修饰的核苷酸。在一些实施方案中，组合物包含由SEQ ID NO:3序列的组成的探针，其中探针包含至少一个修饰的核苷酸和/或可检测标记。在一些实施方案中，探针包含荧光染料和猝灭剂分子。在一些实施方案中，探针是荧光共振能量转移(FRET)探针。在一些实施方

案中，探针包含至少一个修饰的核苷酸。在一些实施方案中，组合物是冻干的组合物。在一些实施方案中，组合物在溶液中。在一些实施方案中，组合物包含来自受试者的样品的核酸。

[0028] 本发明的进一步实施方案和详述在下文描述。

[0029] 4.详述

[0030] 4.1定义

[0031] 为了帮助理解本发明，在下文定义多个术语和措辞：

[0032] 如本文中使用的，术语“检测(detect)”，“检测(detecting)”或“检测(detection)”可以描述发现或理解或具体观察检测标记的组合物的一般行为。

[0033] 如本文中使用的，术语“在检测上不同”是指可以同时检测和区分的一组标记(如染料)。

[0034] 如本文中使用的，术语“患者”和“受试者”可交替用于指人。在一些实施方案中，本文所述方法可以用在来自非人动物的样品上。

[0035] “阴道毛滴虫”是指造成毛滴虫病的原生动物，毛滴虫病是常见性传播的感染，可以感染男性和女性。毛滴虫病可以是有症状的或无症状的。毛滴虫病的症状包括，但不限于，阴道炎、尿道炎和子宫颈炎。女性中的症状包括，但不限于，生殖器瘙痒、烧灼、发红、或疼痛，异味，排尿不适，或烯的澄清、白色、黄色或绿色排出物。男性中症状包括，但不限于，阴茎内瘙痒或烧灼，射精或排尿后烧灼，或阴茎排泄物。

[0036] 如本文中使用的，术语“寡核苷酸”、“多核苷酸”、“核酸分子”等，是指含核酸分子，包括但不限于，DNA或RNA。该术语包含包括任意已知的DNA和RNA的碱基类似物的序列，但不限于，4-乙酰基胞嘧啶，8-羟基-N6-甲基腺苷，氮丙定基胞嘧啶，假异胞嘧啶，5-(羧基羟基甲基)尿嘧啶，5-氟尿嘧啶，5-溴尿嘧啶，5-羧基甲基氨基甲基-2-硫代尿嘧啶，5-羧基甲基氨基甲基尿嘧啶，二氢尿嘧啶，肌苷，N6-异戊烯基腺嘌呤，1-甲基腺嘌呤，1-甲基假尿嘧啶，1-甲基鸟嘌呤，1-甲基肌苷，2,2-二甲基鸟嘌呤，2-甲基腺嘌呤，2-甲基鸟嘌呤，3-甲基胞嘧啶，5-甲基胞嘧啶，N6-甲基腺嘌呤，7-甲基鸟嘌呤，5-甲基氨基甲基尿嘧啶，5-甲氧基氨基甲基-2-硫代尿嘧啶，β-D-甘露糖基queosine，5′-甲氧基羰基甲基尿嘧啶，5-甲氧基尿嘧啶，2-甲基硫代-N6-异戊烯基腺嘌呤，尿嘧啶-5-氧基乙酸甲酯，尿嘧啶-5-氧基乙酸，oxybutoxosine，假尿嘧啶，queosine，2-硫代胞嘧啶，5-甲基-2-硫代尿嘧啶，2-硫代尿嘧啶，4-硫代尿嘧啶，5-甲基尿嘧啶，N-尿嘧啶-5-氧基乙酸甲酯，尿嘧啶-5-氧基乙酸，假尿嘧啶，queosine，2-硫代胞嘧啶，和2,6-二氨基嘌呤。

[0037] 如本文中使用的，术语“寡核苷酸”是指具有少于500个核苷酸的单链多核苷酸。在一些实施方案中，寡核苷酸是8至200，8至100，12至200，12至100，12至75，或12至50个核苷酸长度。寡核苷酸可以以其长度指示，例如24残基寡核苷酸可以称为“24-mer(24聚体)”。

[0038] 如本文中使用的，术语与靶基因(或其靶区域)“互补”，并且探针序列与靶基因序列的“互补性”百分比是与靶基因序列或与靶基因序列的反向互补物的“同一性”百分比。在本文所述组合物中使用的探针(或其区域)和靶基因(如本文中公开的那些)之间的“互补性”程度的确定中，“互补性”的程度表达为探针序列(或其区域)和与其最佳比对的靶基因序列或靶基因序列的反向互补物之间的百分比同一性。百分比通过计数2条序列之间同一的比对碱基数，除以探针中总的连续核苷酸数，并乘以100来计算。当使用术语“互补”时，受

试寡核苷酸与靶分子至少90％互补，除非另有说明。在一些实施方案中，受试寡核苷酸与靶分子至少91％，至少92％，至少93％，至少94％，至少95％，至少96％，至少97％，至少98％，至少99％，或100％互补。

[0039] 如本文中使用的“引物”或“探针”，是指包含与靶核酸分子，如DNA(例如，靶基因)或mRNA(或从mRNA反转录的DNA)的至少8个连续核苷酸的序列互补的区域的寡核苷酸。在一些实施方案中，引物或探针包含与靶分子的至少9，至少10，至少11，至少12，至少13，至少14，至少15，至少16，至少17，至少18，至少19，至少20，至少21，至少22，至少23，至少24，至少25，至少26，至少27，至少28，至少29，或至少30个连续核苷酸的序列互补的区域。当引物或探针包含“与靶分子的至少x个连续核苷酸互补”的区域时，引物或探针与靶分子的至少x个连续核苷酸至少95％互补。在一些实施方案中，引物或探针与靶分子的至少96％，至少97％，至少98％，至少99％，或100％互补。

[0040] 术语“核酸扩增”包括至少一种靶核酸的至少一部分复制(通常以模板依赖性方式)所通过的任意方法，包括但不限于宽范围的扩增核酸序列的技术(线性或指数地)。进行扩增步骤的示例性方法包括聚合酶链式反应(PCR)，连接酶链式反应(LCR)，连接酶检测反应(LDR)，多重连接依赖性探针扩增(MLPA)，连接接着Q-复制酶扩增，引物延伸，链替代扩增(SDA)，超支化链替代扩增，多重替代扩增(MDA)，基于核酸链的扩增(NASBA)，两步骤多重扩增，滚动圆扩增(RCA)等，包括多种版本和其组合，例如但不限于，OLA/PCR，PCR/OLA，LDR/PCR，PCR/PCR/LDR，PCR/LDR，LCR/PCR，PCR/LCR(也称为组合链式反应--CCR)，数字扩增等。这样的技术的描述可以在其他来源中，Ausbel等人；PCR Primer:A Laboratory Manual，Diffenbach，编辑，Cold Spring Harbor Press(1995)；The Electronic Protocol Book，Chang Bioscience(2002)；Msuih等人，J.Clin.Micro.34:501-07(1996)；The Nucleic Acid Protocols Handbook，R.Rapley，编辑，Humana Press，Totowa，N.J.(2002)；Abramson等人，Curr Opin Biotechnol.1993Feb.；4(1):41-7，美国专利号6,027,998；美国专利号6,605,451，Barany等人，PCT公开号WO 97/31256；Wenz等人，PCT公开号WO 01/92579；Day等人，Genomics，29(1):152-162(1995)，Ehrlich等人，Science252:1643-50(1991)；Innis等人，PCR Protocols:A Guide to methods and Applications，Academic Press(1990)；Favis等人，Nature Biotechnology18:561-64(2000)；和Rabenau等人，Infection 28:97-102(2000)；Belgrader，Barany，和Lubin，Development of a Multiplex Ligation Detection ReactionDNA Typing Assay，Sixth International Symposium on Human Identification，1995(可在万维网址:promega.com/geneticidproc/ussymp6proc/blegrad.html上获得)；LCR Kit Instruction Manual，Cat.#200520，Rev.#050002，Stratagene，2002；Barany，Proc.Natl.Acad.Sci.USA 88:188-93(1991)；Bi和Sambrook，Nucl.Acids Res.25:2924-2951(1997)；Zirvi等人，Nucl.Acid Res.27:e40i-viii(1999)；Dean等人，Proc Natl Acad Sci USA 99:5261-66(2002)；Barany和Gelfand，Gene 109:1-11(1991)；Walker等人，Nucl.Acid Res.20:1691-96(1992)；Polstra等人，BMC Inf.Dis.2:18-(2002)；Lage等人，Genome Res.2003年2月；13(2):294-307，和Landegren等人，Science241:1077-80(1988)，Demidov，V.，Expert Rev Mol Diagn.2002Nov.；2(6):542-8.，Cook等人，J Microbiol Methods.2003年5月；53(2):165-74，Schweitzer等人，Curr Opin Biotechnol.2001年2月；12(1):21-7，美国专利号5,830,711，美国专利号6,027,889，

美国专利号5,686,243,PCT公开号WO0056927A3,和PCT公开号WO9803673A1中找到。

[0041] 在一些实施方案中,扩增包含以下各项中的顺序步骤中的至少一个循环:将至少一条引物与至少一个靶核酸中的互补或顺序互补序列退火;使用聚合酶以模板-依赖性方式合成核苷酸的至少一条链;并且将新形成的核酸双链体变性以分离链。循环可以或可以不重复。扩增可以包括热循环或可以等温条件进行。

[0042] 除非另有说明,本文中使用术语"杂交"表示"特异杂交",其是核酸分子优先对特定核苷酸序列结合、形成双链、或杂交,在一些实施方案中,在严格条件下。术语"严格条件"是指探针将优先杂交于其靶序列,而对其他序列杂交程度较低,或根本不杂交的条件。在核酸杂交(例如,在阵列、DNA印迹、或RNA印迹杂交)的情况下,"严格杂交"和"严格杂交洗涤条件"是序列-依赖性的并且在不同环境参数下不同。核酸杂交的广泛指引在,例如,Tijssen(1993)Laboratory Techniques in Biochemistry and Molecular Biology--hybridization with Nucleic Acid Probes第I部分,第2章,"Overview of principles of hyridization and the strategy of nucleic acid probes assays,"Elsevier,NY("Tijssen")中找到。通常,用于滤纸杂交的高度严格杂交和洗涤条件选择为约5℃,低于特定序列在限定的离子强度和pH的热解链点。T_m是50%的靶序列杂交于完全匹配的探针的温度(在限定的离子强度和pH)。非常严格条件选择为等于特定探针的T_m。杂交严格性对缓冲液组成、温度和探针长度的依赖性对于本领域技术人员已知(参见,例如,Sambrook和Russell(2001)Molecular Cloning:A Laboratory Manual(3rd ed.)第1-3卷,Cold Spring Harbor Laboratory,Cold Spring Harbor Press,NY)。

[0043] 如本文中使用的"样品",包括尿样品(包括源自尿样品的样品),子宫颈的拭子,和患者-收集的阴道拭子,以及其他类型的人样品。在一些实施方案中,尿样品是"首次(first catch)"尿样品,其是受试者首次开始排尿时采集的样品。如本文中使用的,尿样品包括,但不限于,全尿,包含来自尿样品的细胞的样品,包含通过将尿样品离心分离的细胞沉淀的样品,包含通过过滤尿样品分离的细胞的样品等。在一些实施方案中,尿样品包含缓冲液,如防腐剂。在一些实施方案中,样品是除了尿样品之外的人样品,如子宫颈内拭子或阴道拭子,包括患者-收集的阴道拭子,和尿道拭子。在一些实施方案中,拭子样品包含缓冲液,如防腐剂。

[0044] 如本文中使用的"内源性对照"是指天然存在于用于检测的样品中的部分。在一些实施方案中,内源性对照是"样品充分性对照"(sample adequacycontrol,SAC),其可以用于确定是否存在足够的用于测定的样品,或样品是否包含足够的生物材料,如细胞。在一些实施方案中,SAC是单拷贝人基因。在一些实施方案中,选择内源性对照,如SAC,其可以以与检测靶基因相同的方式进行检测,并且,在一些实施方案中,与靶基因同时检测。

[0045] 如本文中使用的"外源性对照",是指加入至样品或测定中的部分,如"样品处理对照"(sample processing control,SPC)。在一些实施方案中,外源性对照包括测定试剂。外源性对照通常选择为预期不存在于样品中的用于检测,或以非常低的水平存在于样品中从而天然存在于样品中的该部分的量不可检测或可在比作为外源性对照加入至样品中的量远低的水平检测到。在一些实施方案中,外源性对照包含预期不存在于用于检测靶基因的样品类型中的核苷酸序列。在一些实施方案中,外源性对照包含已知在样品采集自得物种中不存在的核苷酸序列。在一些实施方案中,外源性对照包含来自与样品采集自的受试者

不同的物种的核苷酸序列。在一些实施方案中，外源性对照包含已知不存在于任何物种中的核苷酸序列。在一些实施方案中，选择可以与检测靶基因相同的方式检测的外源性对照，并且，在一些实施方案中，与靶基因同时检测。在一些实施方案中，外源性对照是细菌DNA。在一些实施方案中，细菌是未预期在测试样品类型中发现的物种。

[0046] 在本文中的序列中，“U”和“T”可交替使用，从而两个字母表示在该位置的尿嘧啶或胸腺嘧啶。从上下文和/或预期用途，本领域技术人员将理解尿嘧啶或胸腺嘧啶是否意在和/或应该用在序列中的该位置。例如，本领域技术人员将理解，天然RNA分子通常包括尿嘧啶，而天然DNA分子通常包括胸腺嘧啶。因此，在RNA序列包括“T”的情况下，本领域技术人员会理解天然RNA中的位置可能是尿嘧啶。

[0047] 在本公开中，“选自...的序列”包括“选自...的一条序列”和“选自...的一条以上序列”。因此，当使用“选自...的序列”时，要理解，可以选择所列序列中的一条，或多于一条。

[0048] 4.2检测阴道毛滴虫

[0049] 本发明人开发了检测阴道毛滴虫(TV)的测定。在一些实施方案中，所述测定包括检测TV 40S核糖体蛋白(Tv40Srp)基因。在一些实施方案中，测定包括检测从TV 40S核糖体蛋白(Tv40Srp)基因转录的RNA。本测定依赖于聚合酶链式反应(PCR)，并且可以使用可商购核酸扩增系统，以顺序自动化方式进行。可以用于进行本发明的方法的示例性的非限制性核酸扩增系统包括GeneXpert®系统，GeneXpert® Infinity系统，和Smartcycler系统(Cepheid，Sunnyvale，CA)。本测定使用自动化系统，例如，GeneXpert®系统，可以在3小时内完成，并且在一些实施方案中，在2小时内完成。

[0050] 4.2.1一般方法

[0051] 提供用于检测阴道毛滴虫(TV)的组合物和方法。在一些实施方案中，所述方法包括检测TV 40S核糖体蛋白(Tv40Srp)基因。

[0052] 在一些实施方案中，检测受试者中阴道毛滴虫(TV)的方法包括检测来自受试者的样品中存在TV 40S核糖体蛋白(Tv40Srp)基因。在一些实施方案中，样品选自尿样品，子宫颈的拭子，和阴道拭子。在一些实施方案中，尿样品是首次尿样品。

[0053] 在一些实施方案中，检测TV的方法还包括检测至少一种内源性对照，如样品充分性对照(SAC)。在一些实施方案中，检测TV的方法还包括检测至少一种外源性对照，如样品处理对照(SPC)。在一些实施方案中，检测TV的方法还包括检测至少一种内源性对照和至少一种外源性对照。

[0054] 在一些实施方案中，检测TV的方法包括检测样品中的TV 40S核糖体蛋白(Tv40Srp)基因。在一些实施方案中，检测TV的方法还包括检测样品充分性对照(SAC)，如单拷贝人基因。在一些实施方案中，检测TV的方法还包括检测样品处理对照(SPC)，如外源添加的细菌DNA。在一些实施方案中，检测TV的方法还包括检测SAC和SPC。

[0055] 在本公开中，为了方便，使用术语“靶基因”表示TV 40S核糖体蛋白(Tv40Srp)基因，并且还表示外源和/或内源性对照。因此，要理解，当关于靶基因进行讨论时，该讨论具体意在包括TV 40S核糖体蛋白(Tv40Srp)基因，一个或多个内源性对照(例如，SAC)，和一个或多个外源性对照(例如，SPC)。

[0056] 在一些实施方案中，在尿样品中检测TV 40S核糖体蛋白(Tv40Srp)基因的存在。在一些实施方案中，在向其中添加缓冲液(如防腐剂)的尿样品中检测靶基因。在一些实施方案中，以1:1,1:2,1:3,1:4,1:5,1:6,1:7,1:8,1:9，或1:10缓冲液:尿的比例将缓冲液加入至尿样品。在一些实施方案中，在子宫颈内拭子样品或阴道拭子样品中检测TV 40S核糖体蛋白(Tv40Srp)基因的存在。在一些实施方案中，阴道拭子是患者-收集的阴道拭子。在一些实施方案中，在置于缓冲液(如防腐剂)中的子宫颈内拭子样品或阴道拭子样品中检测靶基因。在一些实施方案中，将拭子置于1mL,2mL,2.5mL的缓冲液中。

[0057] 在一些实施方案中，来自受试者的样品中的TV 40S核糖体蛋白(Tv40Srp)基因的检测表明受试者中存在阴道毛滴虫。在一些实施方案中，检测定量进行。在其他实施方案中，检测定性进行。在一些实施方案中，检测靶基因包括形成包含选自靶基因、靶基因的DNA扩增子和靶基因的互补物的多核苷酸和核酸的复合体。在一些实施方案中，检测靶基因包括PCR。在一些实施方案中，检测靶基因包括定量PCR或实时PCR。在一些实施方案中，样品充分性对照(SAC)和/或样品处理对照(SPC)以与靶基因相同的测定检测。在一些实施方案中，如果检测到TV 40S核糖体蛋白(Tv40Srp)基因，TV被认为检测到，即使在测定中未检测到SPC和/或SAC。在一些实施方案中，如果未检测到TV 40S核糖体蛋白(Tv40Srp)基因，仅在测定中还检测到SPC和SAC时TV被认为未检测到。

[0058] 在一些实施方案中，TV 40S核糖体蛋白(Tv40Srp)基因的存在在从受试者以一次以上收集的样品中测量以监测受试者中对TV感染的治疗。治疗包括，但不限于，单剂量或多剂量的甲硝哒唑或替硝唑。在一些实施方案中，通过以定期的或半定期间隔检测存在或不存在TV 40S核糖体蛋白(Tv40Srp)基因，监测具有TV感染史的受试者的TV复发。在一些这样的实施方案中，通过每月至少一次，至少每两月一次，至少每三月一次，至少每四月一次，至少每五月一次，至少每六月一次，至少每九月一次，至少一年一次，或至少每两年一次检测存在或不存在TV 40S核糖体蛋白(Tv40Srp)基因来监测患者。

[0059] 在一些实施方案中，本测定可以用作受试者的常规和/或预防性卫生保健的部分。即，在一些实施方案中，本测定可以用于对个体测试TV感染，不论所述个体是否显示TV感染症状或具有TV感染史。在一些实施方案中，本测定用于检测怀孕的和/或尝试怀孕的受试者中的TV感染。在一些情况中，患有TV的孕妇更可能经历早产和/或具有低出生体重婴儿(小于5.5磅)。

[0060] 在一些实施方案中，要测试的样品是尿样品(如首次尿样品)，或源自尿样品。在一些实施方案中，将缓冲液(如防腐剂)加入至尿样品中。在一些实施方案中，在收集样品1小时内、2小时内、3小时内、4小时内、5小时内、6小时内、7小时内或8小时内将缓冲液加入至尿样品中。

[0061] 在一些实施方案中，要测试的样品是子宫颈内拭子样品或阴道拭子样品。在一些实施方案中，拭子置于缓冲液中。在一些实施方案中，拭子立即置于缓冲液中。在一些实施方案中，在一些实施方案中，在收集样品1小时内、2小时内、3小时内、4小时内、5小时内、6小时内、7小时内或8小时内将拭子置于缓冲液中。

[0062] 在一些实施方案中，小于5ml，小于4ml，小于3ml，小于2ml，小于1ml，或小于0.75ml的尿用于本方法。在一些实施方案中，0.1ml至1ml的尿用于本方法。

[0063] 在一些实施方案中，要测试的样品是另一种体液，如血液、痰、粘液、唾液、阴道或

阴茎排泄物、精液等。

[0064] 在一些实施方案中，要测试的临床样品是新鲜的(即，未冷冻)。在其他实施方案中，样品是冷冻样本。在一些实施方案中，样品是组织样品，如福尔马林固定的蜡包埋样品。在一些实施方案中，样品是液体细胞学样品。

[0065] 在一些实施方案中，要测试的样品获自具有一种以上TV感染症状的个体。TV感染的非限制性示例性症状包括阴道炎、尿道炎、和子宫颈炎；在女性中：生殖器瘙痒、烧灼、发红、或疼痛，异味，排尿不适，和烯的澄清、白色、黄色或绿色排出物；并且在男性中：阴茎内瘙痒或烧灼，射精或排尿后烧灼，和阴茎排泄物。在一些实施方案中，要测试的样品获自之前诊断有TV感染的个体。在一些这样的实施方案中，对个体监测TV感染的复发。

[0066] 在一些实施方案中，本文中所述方法可以用于常规筛选不具有风险因素的健康个体。在一些实施方案中，本文中所述方法用于例如，在常规或预防保健中筛选无症状个体。在一些实施方案中，本文中所述方法用于筛选怀孕或试图怀孕的女性。

[0067] 在一些实施方案中，本文中所述方法可以用于评估患者中对TV感染的治疗的有效性。

[0068] 在一些实施方案中，提供使用TV 40S核糖体蛋白(Tv40Srp)基因用于检测TV感染。在一些实施方案中，提供使用TV 40S核糖体蛋白(Tv40Srp)基因用于监测TV感染复发。

[0069] 在本文所述的任何实施方案中，TV 40S核糖体蛋白(Tv40Srp)基因可以在与样品处理对照(SPC)和/或样品充分性对照(SAC)相同的测定反应中检测。

[0070] 在一些实施方案中，提供帮助检测受试者中TV感染的方法。所述方法包括检测来自受试者的样品中存在或不存在TV 40S核糖体蛋白(Tv40Srp)基因。在一些实施方案中，关于来自受试者的样品中存在或不存在TV 40S核糖体蛋白(Tv40Srp)基因的信息传达给医学从业者。如本文中使用的“医学从业者”是指诊断和/或治疗患者的个人或实体，如医院、诊所、医生办公室、医生、护士或任意前述实体或个人的机构。在一些实施方案中，检测存在或不存在TV 40S核糖体蛋白(Tv40Srp)基因在实验室进行，所述实验室从医学从业者或医学从业者的机构接收受试者的样品。实验室通过任意方法进行检测，包括本文中所述的那些，并且随后将结果传达给医学从业者。当通过任意方式提供给医学从业者时，如本文中使用的结果“被传达”。在一些实施方案中，所述传达可以是口头的或书面的，可以通过电话、个人、电子邮件或其他信差，或可以通过将信息直接储存于，例如，医学从业者可进入的数据库传达，包括不由医学从业者控制的数据库。在一些实施方案中，信息以电子形式维持。在一些实施方案中，信息可以储存在存储器或其他计算机可读介质中，如RAM、ROM、EEPROM、闪存、计算机芯片、数字视频光盘(DVD)、压缩磁盘(CDs)、硬盘驱动器(HDD)、磁带等中。

[0071] 在一些实施方案中，提供检测TV的方法。在一些实施方案中，提供诊断TV感染的方法。在一些实施方案中，所述方法包括从受试者获得样品并且将样品提供给实验室用于检测样品中的TV 40S核糖体蛋白(Tv40Srp)基因。在一些实施方案中，所述方法还包括从实验室接受说明样品中存在或不存在TV 40S核糖体蛋白(Tv40Srp)基因的信息传达。如本文中使用的“实验室”，是通过任何方法(包括本文中所述方法)检测样品中靶基因并且将结果传达给医学从业者的任何装置。在一些实施方案中，实验室在医学从业者的控制下.在一些实施方案中，实验室不在医学从业者的控制下。

[0072] 当实验室将检测存在或不存在TV 40S核糖体蛋白(Tv40Srp)基因的结果传达给医

学从业者时，在一些实施方案中，实验室表明是否在样品中检测到TV 40S核糖体蛋白(Tv40Srp)基因。在一些实施方案中，实验室通过表明例如，“TV阳性”或“TV阴性”或“TV存在”或“TV不存在”等，表明样品是否包含阴道毛滴虫(TV)。

[0073] 如本文中使用的，当方法涉及检测TV，确定TV存在，监测TV，和/或诊断TV感染时，该方法包括其中进行方法的步骤，但对于TV存在的结果是阴性的活动。即，检测、确定、监测和诊断TV或TV感染包括进行导致阳性或阴性结果的方法的例子。

[0074] 在一些实施方案中，至少一个内源性对照(例如，SAC)和/或至少一个外源性对照(例如，SPC)在单个反应中与TV 40S核糖体蛋白(Tv40Srp)基因同时检测。

[0075] 4.2.2示例性对照

[0076] 在一些实施方案中，本文中所述的测定包括检测TV 40S核糖体蛋白(Tv40Srp)基因和至少一种内源性对照。在一些实施方案中，内源性对照是样品充分性对照(SAC)。在一些这样的实施方案中，如果在样品中未检测到TV 40S核糖体蛋白(Tv40Srp)基因，并且在样品中也未检测到SAC，测定结果被认为是“无效的”，因为样品可能不足够。而不意在受任何特定理论限制，不足够的样品可能太稀，含有太少细胞材料，含有测定抑制剂等。在一些实施方案中，未能检测SAC可以表明测定反应失败。在一些实施方案中，内源性对照(如SAC)是单拷贝人基因。非限制性示例性SACs包括人羟基甲基-胆色烷合成酶(HMBS)，甘油醛-3-磷酸脱氢酶(GAPDH)，β肌动蛋白，β2-微珠蛋白，环氧合酶1，次黄嘌呤磷酸核糖转移酶，胆色素原脱氨酶，和转铁蛋白受体。

[0077] 在一些实施方案中，本文所述的测定包括检测TV 40S核糖体蛋白(Tv40Srp)基因和至少一种外源性对照。在一些实施方案中，外源性对照是样品处理对照(SPC)。在一些这样的实施方案中，如果在样品中未检测到TV 40S核糖体蛋白(Tv40Srp)基因，并且在样品中也未检测到SPC，测定结果被认为是“无效的”，因为在样品处理中可能存在错误，包括但不限于，测定失败。样品处理中的非限制性示例性错误包括，不充分的样品处理，测定抑制剂的存在，受损的试剂等。在一些实施方案中，将外源性对照(如SPC)加入至样品。在一些实施方案中，在测定进行期间，如利用一种以上缓冲液或试剂将外源性对照(如SPC)加入。在一些实施方案中，当要使用GeneXpert®系统时，SPC包括在GeneXpert®筒中。在一些实施方案中，外源性对照(如SPC)是预期不存在于测定的样品中的DNA序列。非限制性示例性的SPCs包括预期不存在于测定的样品中的细菌基因。

[0078] 在一些实施方案中，在检测样品中TV 40S核糖体蛋白(Tv40Srp)基因时，同时检测内源性对照和/或外源性对照，如在同一测定中。在一些实施方案中，测定包含在同一测定反应中同时检测TV 40S核糖体蛋白(Tv40Srp)基因，外源性对照，和内源性对照的试剂。在一些这样的实施方案中，例如，测定反应包含扩增TV 40S核糖体蛋白(Tv40Srp)基因的引物组，扩增内源件对照的引物组，和扩增外源件对照的引物组，以及检测扩增产物的标记的探针(如，例如，TaqMan®探针)。

[0079] 4.2.3示例性的样品制备

[0080] 4.2.3.1示例性的缓冲液

[0081] 在一些实施方案中，将缓冲液加入至尿样品。在一些实施方案中，缓冲液在收集尿样品的时间的一小时、两小时、三小时或六小时内添加(例如，中空的)。在一些实施方案中，缓冲液在通过本文所述的方法分析样品前一小时、两小时、三小时或六小时内加入至尿样

品。

[0082] 在一些实施方案中，将拭子样品置于缓冲液中在一些实施方案中，拭子样品在收集拭子样品的时间的一小时、两小时、三小时或六小时内置于缓冲液中。在一些实施方案中，拭子样品在通过本文所述的方法分析样品前一小时、两小时、三小时或六小时内置于缓冲液中。

[0083] 非限制性示例性的商业缓冲液包括PreservCyt(Hologic，Bedford，MA)，SurePath(BD，Franklin Lakes，NJ)，和CyMol(Copan Diagnostics，Murrietta，CA)。

[0084] 4.2.3.2示例性的DNA制备

[0085] 可以通过任意适当的方法制备样品DNA。在一些实施方案中，通过将样品与裂解缓冲液接触和将DNA结合于DNA结合基材，如玻璃或二氧化硅基材制备靶DNA。所述结合基材可以具有任何合适的形式，如颗粒、多孔固体或膜形式。例如，支持物可以包含羟基纤维素、玻璃纤维、纤维素、硝基纤维素、氢氧化锆、氧化钛(IV)、二氧化硅、硅酸锆、或二氧化硅颗粒(例如，参见美国专利号5,234,809)。很多这样的DNA结合基材在本领域中已知。

[0086] 在一些实施方案中，在不首先分离或分开DNA的情况下在溶解产物中检测DNA。在一些实施方案中，将样品进行裂解步骤以释放DNA。非限制性示例性裂解方法包括超声(例如，2-15秒，8-18μm，在36kHz)；化学裂解，例如，使用去污剂；和各种可商购裂解试剂。在一些实施方案中，在其中DNA从至少一些其他细胞组分分离或分开的样品中检测DNA。

[0087] 当本文中讨论的方法表明检测靶基因时，所述检测可以在靶基因的互补物是上进行(而不是本文中所示的靶基因序列，或除了本文中所示的靶基因序列)。在一些实施方案中，当检测靶基因的互补物时，使用与靶基因的互补物互补的多核苷酸用于检测。在一些实施方案中，用于检测的多核苷酸包含序列上与靶基因至少90％，至少95％，或100％同一的至少一部分，尽管其可以包含修饰的核苷酸。

[0088] 4.2.4示例性的分析方法

[0089] 如上文所述的，提供用于检测阴道毛滴虫的方法。所述方法包括检测来自受试者的样品中TV 40S核糖体蛋白(Tv40Srp)基因的存在。在一些实施方案中，所述方法还包括检测至少一种内源性对照(如SAC)和/或至少一种外源性对照(如SPC)。在一些实施方案中，检测TV 40S核糖体蛋白(Tv40Srp)基因表明TV的存在，即使内源性对照和/或外源性对照在测定中未检测到。在一些实施方案中，如果未检测到TV 40S核糖体蛋白(Tv40Srp)基因，仅当检测到对照时，认为结果对于TV是阴性的。在一些实施方案中，如果未检测到TV 40S核糖体蛋白(Tv40Srp)基因，仅当检测到内源性对照和外源性对照时，认为结果对于TV是阴性的。

[0090] 任何能够允许特异检测靶基因的分析步骤可以用于本文中提供的方法。示例性的非限制性分析步骤包括，但不限于，核酸扩增方法、PCR方法、等温扩增方法和本领域技术人员已知的其他分析检测方法。

[0091] 在一些实施方案中，检测靶基因，如TV 40S核糖体蛋白(Tv40Srp)基因的方法，包括扩增基因和/或其互补物。所述扩增可以通过任意方法完成。示例性的方法包括，但不限于，等温扩增、实时PCR、终点PCR、和使用T7聚合酶从与DNA退火的T7启动子扩增，如由可在德国Implen获得的SenseAmp Plus™ Kit提供的。

[0092] 当扩增靶基因时，在一些实施方案中，形成靶基因的扩增子。扩增子可以是单链或双链的。在一些实施方案中，当扩增子为单链时，扩增子的序列以正义或反义取向与靶基因

相关。在一些实施方案中，检测靶基因的扩增子而不是靶基因本身。因此，当本文讨论的方法表明检测到靶基因时，该检测可以在靶基因的扩增子上进行(而不是靶基因本身，或除了靶基因本身之外)。在一些实施方案中，当检测靶基因的扩增子而不是靶基因时，使用与靶基因的互补物互补的多核苷酸用于检测。在一些实施方案中，当检测靶基因的扩增子而不是靶基因时，使用与靶基因互补的多核苷酸用于检测。此外，在一些实施方案中，可以使用多个多核苷酸用于检测，并且一些多核苷酸可以与靶基因互补并且一些多核苷酸可以与靶基因的互补物互补。

[0093] 在一些实施方案中，检测TV 40S核糖体蛋白(Tv40Srp)基因的方法包括PCR，如下文所述的。在一些实施方案中，检测一种以上靶基因包括实时监测PCR反应，其可以通过任何方法完成。所述方法包括，但不限于，使用TaqMan®，分子信标，或Scorpion探针(即，能量转移(ET)探针，如FRET探针)和使用增补染料，如SYBR绿，EvaGreen，噻唑橙，YO-PRO，TO-PRO等。

[0094] 用于扩增靶基因的非限制性示例性条件如下。示例性的循环包括在90℃至100℃起始变性30秒至5分钟，接着包括在90℃至100℃变性1至10秒，接着在60℃至75℃退火和扩增10至30秒的循环。进一步示例性的循环包括在95℃1分钟，接着在92.5℃5秒，在68℃20秒的多至40循环。在一些实施方案中，对于接着起始变性步骤的第一循环，省略循环变性步骤。在一些实施方案中，Taq聚合酶用于扩增。在一些实施方案中，循环进行至少10次，至少15次，至少20次，至少25次，至少30次，至少35次，至少40次，或至少45次。在一些实施方案中，使用具有热启动功能的Taq。在一些实施方案中，扩增反应在GeneXpert®筒中发生，并且TV 40S核糖体蛋白(Tv40Srp)基因、内源性对照和外源性对照的扩增在同一反应中发生。在一些实施方案中，TV 40S核糖体蛋白(Tv40Srp)基因的检测从起始变性到最后延伸在小于3小时，小于2.5小时，小于2小时，小于1小时，或小于30分钟内发生。

[0095] 在一些实施方案中，靶基因的检测包括形成包含与靶基因或其互补物互补的多核苷酸以及选自靶基因、靶基因的DNA扩增子和靶基因的互补物的核酸的复合物。因此，在一些实施方案中，多核苷酸与靶基因形成复合物。在一些实施方案中，多核苷酸与靶基因的互补物形成复合物。在一些实施方案中，多核苷酸与靶基因的DNA扩增子形成复合物。当双链DNA扩增子是复合物的部分时，如本文中使用的，复合物可以包含DNA扩增子的一条或两条链。因此，在一些实施方案中，复合物仅包含DNA扩增子的一条链。在一些实施方案中，复合物是三链体，并且包含多核苷酸以及DNA扩增子的两条链。在一些实施方案中，复合物通过在多核苷酸和靶基因、靶基因的互补物、或靶基因的DNA扩增子之间杂交形成。在一些实施方案中，多核苷酸是引物或探针。

[0096] 在一些实施方案中，方法包括检测复合物。在一些实施方案中，在检测时不必须将复合物关联。即，在一些实施方案中，形成复合物，然后复合物以某些方式解离或破坏，并且检测来自复合物的成分。该系统的实例是TaqMan®测定。在一些实施方案中，当多核苷酸是引物时，复合物的检测可以包括靶基因的扩增、靶基因的互补物、或靶基因的DNA扩增子。

[0097] 在一些实施方案中，用于在本文中所述方法中检测至少一种靶基因的分析方法包括实时定量PCR。在一些实施方案中，用于检测至少一种靶基因的分析方法包括使用TaqMan®探针。该测定使用能量转移(“ET”)，如荧光共振能量转移(“FRET”)，来检测和定

量合成的PCR产物。通常，TaqMan®探针包含偶联于5'-末端的荧光染料分子和偶联于3'-末端的猝灭剂分子，从而所述染料和猝灭剂紧密接近，允许猝灭剂经由FRET抑制染料的荧光信号。当聚合酶复制TaqMan®探针结合的嵌合扩增子模板时，聚合酶的5'-核酸酶裂解探针，解偶联染料和猝灭剂，从而检测到染料信号(如荧光)。信号(如荧光)随切割的探针的量成比例的每个PCR循环增加。

[0098] 在一些实施方案中，如果在PCR循环期间从TaqMan探针产生任何信号，认为检测到靶基因。例如，在一些实施方案中，如果PCR包括40个循环，如果在扩增期间在任何循环产生信号，认为存在和检测到靶基因。在一些实施方案中，如果在PCR循环的最后没有产生信号，认为不存在和检测不到靶基因。

[0099] 在一些实施方案中，实时PCR测定结果的定量通过从已知浓度的核酸构建标准曲线并随后推测未知浓度的靶基因的定量信息完成。在一些实施方案中，用于产生标准曲线的核酸是DNA(例如，内源性对照，或外源性对照)。在一些实施方案中，用于产生标准曲线的核酸是纯化的双链质粒DNA或在体外产生的单链DNA。

[0100] 在一些实施方案中，为了测定以表明TV不存在于样品中，对于内源性对照(如SAC)和/或外源性对照(如SPC)的Ct值必须在预先确定的有效范围内。即，在一些实施方案中，不能确认不存在TV，除非检测到对照，表明测定是成功的。Ct值与样品中的核酸靶标的量成反比。

[0101] 在一些实施方案中，对于靶基因(包括内源性对照和/或外源性对照)的阈值Ct(或“截取值Ct”)值(低于该值认为检测到)，在先确定。在一些实施方案中，使用基本上相同的测定条件和系统(如GeneXpert®)确定阈值Ct，基于此将测试样品。

[0102] 除了TaqMan®测定外，用于在本文中提供的方法中检测和定量PCR产物的其他实时PCR化学包括，但不限于，分子信标，Scorpion探针和增补染料，如SYBR绿，EvaGreen，噻唑橙，YO-PRO，TO-PRO，等，其在下文讨论。

[0103] 在各种实施方案中，在单个多重反应中，利用实时PCR检测来检测，TV 40S核糖体蛋白(Tv40Srp)基因、内源性对照和外源性对照。在一些多重实施方案中，使用多个探针，如TaqMan®探针(各自对不同靶标特异)。在一些实施方案中，每个靶基因-特异的探针在光谱上可与用于相同多重反应的其他探针相区分。

[0104] 使用本领域中可获得的任意PCR仪器进行实时PCR。通常，用于实时PCR数据收集和分析的仪器包含热循环仪，用于荧光激发和发射收集的光学器件，和任选地计算机和数据获得和分析软件。

[0105] 在一些实施方案中，实时PCR产物的检测和/或定量使用结合双链DNA产物的染料完成，如SYBR绿，EvaGreen，噻唑橙，YO-PRO，TO-PRO等。在一些实施方案中，用于本文所述的方法的分析方法是DASL®(DNA-介导的退火、选择、延伸和连接)测定。在一些实施方案中，用于检测和定量本文所述的方法中的靶基因的分析方法是基于珠子的流式细胞术测定。参见Lu J.等人(2005)Nature 435:834-838，其通过引用以其整体结合于本文。基于珠子的流式细胞术测定的实例是Luminex，Inc.的xMAP®技术。参见www.luminexcorp.com/technology/index.html。在一些实施方案中，用于检测和定量本文所述的方法中的至少一

种靶基因的水平的分析方法是通过凝胶电泳和用标记的探针(例如,用放射活性或化学发光标记标记的探针)检测,如通过RNA印迹。在一些实施方案中,示例性的探针含有一种以上如下文讨论的亲和力增强的核苷酸类似物,如锁定核酸("LNA")类似物,其含有双环糖部分,而不是脱氧核糖或核糖糖。参见,例如,Várallyay,E.等人(2008)Nature Protocols 3(2):190-196,其通过引用以其整体结合于本文。在一些实施方案中,一种以上靶基因的检测和定量使用微流体装置和单分子检测完成。

[0106] 任选地,在杂交之前修饰样品DNA。然后将靶DNA/探针双链体通过微流体装置中的通道,其包含记录3种标记的独特信号的检测器。以此方式,个体分子通过其独特信号检测并计数。参见Fuchs等人,U.S.Genomics,Inc.的美国专利号7,402,422和7,351,538,其各自通过引用以其整体结合于本文。

[0107] 4.2.5示例性的自动化和系统

[0108] 在一些实施方案中,使用自动化的样品处理和/或分析平台检测基因表达。在一些实施方案中,利用可商购自动化分析平台。例如,在一些实施方案中,利用GeneXpert®系统(Cepheid,Sunnyvale,CA)。

[0109] 利用GeneXpert系统说明本发明。示例性的样品制备和分析方法在下文描述。然而,本发明不限于特定检测方法或分析平台。本领域技术人员了解可以利用任意数量的平台和方法。

[0110] GeneXpert®利用自有的、单用途筒。样品提取、扩增和检测可以全部在该自有的"筒中实验室"内进行(参见例如,美国专利5,958,349,6,403,037,6,440,725,6,783,736,6,818,185;其各自通过引用以其整体结合于本文)。

[0111] 筒的组件包括,但不限于,含有试剂的处理室、滤器和用于提取、纯化和扩增靶核酸的捕获技术。阀使得流体能够在室间转移并且含有核酸裂解和过滤组件。光学窗口使得能够实时光学检测。反应管使得能够非常快速的热循环。

[0112] 在一些实施方案中,GenXpert®系统包括用于可扩展性的多个模块。每个模块包括多个筒,连同样品处理和分析组件。

[0113] 在将样品加入至筒中后,样品与裂解缓冲液接触并且释放的DNA结合于DNA-结合基材如二氧化硅或玻璃基材。然后去除样品上清液并且将DNA在洗脱缓冲液如Tris/EDTA缓冲液中洗脱。然后可以将洗脱物在筒中处理以如本文所述检测靶基因。在一些实施方案中,洗脱物用于重构至少一些PCR试剂,其作为冻干的颗粒存在于筒中。

[0114] 在一些实施方案中,PCR用于扩增和分析靶基因的存在。在一些实施方案中,PCR使用具有热启动功能的Taq聚合酶,如AptaTaq(Roche)。在一些实施方案中,起始变性在90℃至100℃30秒至5分钟;循环变性温度是90℃至100℃1至10秒;循环退火和扩增温度是60℃至75℃10至30秒;并且进行多至50个循环。

[0115] 在一些实施方案中,双重-变性方法用于扩增低拷贝数靶标。在一些实施方案中,双重-变性方法包括,第一变性步骤接着加入用于检测靶基因的引物和/或探针。然后将所有或大部分含DNA的样品(如DNA洗脱物)第二次变性,之后,在一些情况中,将一部分样品等分,用于循环和检测靶基因。而不意在受任何特定理论限制,双重变性方案可以增加低拷贝数靶基因(或其互补物)将存在于选择用于循环和检测的等分部分中的概率,因为第二变性

有效使靶标数量翻倍(即,其将靶标和其互补物分为两个分开的模板),之后选择等分部分用于循环。在一些实施方案中,第一变性步骤包含加热至90℃至100℃的温度达30秒至5分钟的总时间。在一些实施方案中,第二变性步骤包含加热至90℃至100℃的温度达5秒至3分钟的总时间。在一些实施方案中,第一变性步骤和/或第二变性步骤通过分开加热样品的等分部分进行。在一些实施方案中,每个等分部分可以加热上述次数。作为非限制性实例,用于含DNA的样品(如DNA洗脱物)的第一变性步骤可以包括将样品的至少一个,至少两个,至少三个,或至少四个等分部分(顺序或同时)分开加热至90℃至100℃的温度各自达60秒。作为非限制性实例,用于含有酶、引物和探针的含DNA的样品(如DNA洗脱物)的第二变性步骤可以包括将洗脱物的至少一个,至少两个,至少三个,或至少四个等分部分(顺序或同时)分开加热至90℃至100℃的温度各自达5秒。在一些实施方案中,等分部分是整个含DNA的样品(如DNA洗脱物)。在一些实施方案中,等分部分少于整个含DNA的样品(如DNA洗脱物)。

[0116] 在一些实施方案中,使用以下方案检测含DNA的样品,如DNA洗脱物中的靶基因:将含DNA的样品的一种以上等分部分分开加热至95℃各自达60秒。将酶和引物以及探针加入至含DNA的样品中并且将一种以上等分部分分开加热至95℃各自达5秒。然后将至少一个等分部分的含有酶、引物和探针的含DNA的样品加热至94℃达60秒。然后将等分部分以以下2步骤循环循环45次:(1)94℃5秒,(2)66℃30秒。

[0117] 本发明不限于特定引物和/或探针序列。示例性的扩增引物和检测探针在实例中描述。

[0118] 在一些实施方案中,使用线下离心,例如,利用具有低细胞含量的样品。将样品(有或没有缓冲液加入)离心并去除上清。然后将沉淀重悬在更小体积的上清或缓冲液。然后将重悬的沉淀如本文所述进行分析。

[0119] 4.2.6示例性的数据分析

[0120] 在一些实施方案中,如果对于TV 40S核糖体蛋白(Tv40Srp)基因的Ct值低于某阈值,则检测到TV的存在。在一些实施方案中,Ct值的有效范围是9至39.9Ct。在一些这样的实施方案中,如果在40个循环后未从TV-特异性引物观察到高于背景的扩增,认为样品对于TV阴性。

[0121] 在一些实施方案中,基于计算机的分析程序用于将检测测定产生的原始数据翻译为临床医生的预测值数据。临床医生可以使用任何合适的方式获取预测数据。因此,在一些实施方案中,本发明提供进一步益处,即可能未受遗传学或分子生物学训练的临床医生不需要理解原始数据。数据以其最有用的形式直接提供给临床医生。然后临床医生能够立即利用信息以优化受试者的护理。

[0122] 本发明考虑能够接收、处理、和传递信息至进行测定、信息提供、医学个体和受试者的实验室和从进行测定、信息提供、医学个体和受试者的实验室接收、处理、和传递信息的任意方法。例如,在本发明的一些实施方案中,样品(例如,活检或血清或尿样品)获自受试者并且进行特征分析(profiling)服务(例如,在医疗设施处的临床实验室,基因组分析业务等),其位于世界的任何部分(例如,不同于受试者居住的国家或信息最终使用的国家)以产生原始数据。在样品包含组织或其他生物样品的情况下,受试者可以访问医学中心已使得获得样品并送至分析中心,或受试者可以自己收集样品(例如,尿样品)并且直接将其送至分析中心。在样品包含之前确定的生物信息的情况下,信息可以直接由受试者送至分

析服务(例如,可以用计算机扫描含有信息的信息卡并且将使用电子通讯系统将数据传递至分析中心的计算机)。一旦由分析服务接收,样品被处理并且产生特征分析结果(profile)(即表达数据),特异于受试者所需的诊断或预后信息。

[0123] 然后将分析数据以适于由治疗临床医生解释的格式准备。例如,不是提供原始表达数据,准备的格式可以表示对受试者的诊断或风险评估(例如,存在TV),建议或不建议特定治疗选择。数据可以通过任何合适的方法展示给临床医生。例如,在一些实施方案中,分析服务产生可以打印给临床医生(例如,在护理点)或在计算机显示器上展示给临床医生的报告。

[0124] 在一些实施方案中,首先在护理点或在区域设施处分析信息。然后将原始数据送至中央处理设施用于进一步分析和/或将原始数据转变为用于临床医生或患者的信息。中央处理设施提供数据分析的隐私性(所有数据以统一的安全协议储存在中央设施中)、速度和均匀性的优势。然后中央处理设施可以在治疗受试者后控制数据的命运。例如,使用电子通讯系统,中央设施可以将数据提供给临床医生、受试者、或研究者。

[0125] 在一些实施方案中,受试者能够使用电子通讯系统直接获得数据。受试者可以选择基于该结果进一步干预或咨询。在一些实施方案中,数据用于研究用途。例如,数据可以用于进一步优化作为疾病的特定状况或阶段的有用指示物或作为伴随诊断的标志物的包含或排除以确定治疗过程。

[0126] 4.2.7示例性的多核苷酸

[0127] 在一些实施方案中,提供多核苷酸。在一些实施方案中,提供合成的多核苷酸。如本文中使用的,合成的多核苷酸,是指化学或酶法体外合成的多核苷酸。多核苷酸的化学合成包括,但不限于,using使用多核苷酸合成仪,如OligoPilot(GE Healthcare),ABI 3900DNA合成仪(Applied Biosystems)等合成。酶法合成包括,但不限于,通过酶法扩增,例如,PCR产生多核苷酸。多核苷酸可以包含本文中讨论的一种以上核苷酸类似物(即,修饰的核苷酸)。

[0128] 在一些实施方案中,提供包含与TV 40S核糖体蛋白(Tv40Srp)基因的至少8,至少9,至少10,至少11,至少12,至少13,至少14,至少15,至少16,至少17,至少18,至少19,至少20,至少21,至少22,至少23,至少24,至少25,至少26,至少27,至少28,至少29,或至少30个连续核苷酸至少90%,至少95%,或100%同一,或至少90%,至少95%,或100%互补的区域的多核苷酸。在一些实施方案中,提供包含与TV 40S核糖体蛋白(Tv40Srp)基因的6至100,8至100,8至75,8至50,8至40,或8至30个连续核苷酸至少90%,至少95%,或100%同一,或互补的区域的多核苷酸。非限制性示例性多核苷酸显示在表1中。

[0129] 在各种实施方案中,多核苷酸包含少于500,少于300,少于200,少于150,少于100,少于75,少于50,少于40,或少于30个核苷酸。在各种实施方案中,多核苷酸为6至200个之间,8至200个之间,8至150个之间,8至100个之间,8至75个之间,8至50个之间,8至40个之间,8至30个之间,15至100个之间,15至75个之间,15至50个之间,15至40个之间,或15至30个之间的核苷酸长度。

[0130] 在一些实施方案中,多核苷酸是引物。在一些实施方案中,引物用可检测部分标记。在一些实施方案中,引物未被标记。如本文中使用的引物是能够选择性与靶基因或与从靶基因扩增的扩增子(统称为“模板”)杂交的多核苷酸,并且,在模板的存在下,聚合酶和合

适的缓冲液以及试剂,可以延伸以形成引物延伸产物。

[0131] 在一些实施方案中,多核苷酸是探针。在一些实施方案中,探针用可检测部分标记。如本文中使用的可检测部分,包括直接可检测部分,如荧光染料,和间接可检测部分,如结合对成员。在一些实施方案中,当可检测部分是结合对的成员时,通过将探针与结合于结合对的第二成员的可检测标记温育,可以检测探针。在一些实施方案中,探针未标记,如当探针是捕获探针,例如,在微阵列上或珠子上时。在一些实施方案中,探针是不可延伸的,例如,由聚合酶延伸。在其他实施方案中,探针是可延伸的。

[0132] 在一些实施方案中,多核苷酸在一些实施方案中是在5'-末端用荧光染料(供体)和在3'-末端用猝灭剂(受体)标记的FRET探针,所述猝灭剂是当基团紧密接近(即,连接于同一探针)时从染料吸收(即,抑制)荧光发射的化学基团。因此,在一些实施方案中,染料的发射光谱应该与猝灭剂的吸收光谱相当程度上重叠。在其他实施方案中,染料和猝灭剂不在FRET探针末端。

[0133] 4.2.7.1示例性的多核苷酸修饰

[0134] 在一些实施方案中,检测本文所述的至少一种靶基因的方法利用一种以上经修饰的多核苷酸,如包含一种以上亲和力增强的核苷酸类似物的多核苷酸。用于本文所述的方法的修饰的多核苷酸包括用于反转录的引物、PCR扩增引物和探针。在一些实施方案中,与仅含有脱氧核糖核苷酸的多核苷酸相比,掺入亲和力增强核苷酸增加多核苷酸对其靶核酸的结合亲和力和特异性,并且允许使用更短的多核苷酸或多核苷酸和靶核酸之间的更短互补性区域。

[0135] 在一些实施方案中,亲和力增强核苷酸类似物包括一种以上碱基修饰、糖修饰和/或骨架修饰的核苷酸。

[0136] 在一些实施方案中,用于亲和力增强核苷酸类似物的修饰的碱基包括5-甲基胞嘧啶,异胞嘧啶,假异胞嘧啶,5-溴尿嘧啶,5-丙炔基尿嘧啶,6-氨基嘌呤,2-氨基嘌呤,肌苷,二氨基嘌呤,2-氯-6-氨基嘌呤,黄嘌呤和次黄嘌呤。

[0137] 在一些实施方案中,亲和力增强核苷酸类似物包括具有修饰的糖的核苷酸如2'-取代的糖,如2'-O-烷基-核糖糖,2'-氨基-脱氧核糖糖,2'-氟-脱氧核糖糖,2'-氟-阿拉伯糖糖,和2'-O-甲氧基乙基-核糖(2'MOE)糖。在一些实施方案中,修饰的糖是阿拉伯糖糖,或d-阿拉伯糖基-己糖醇糖。

[0138] 在一些实施方案中,亲和力增强核苷酸类似物包括骨架修饰如使用肽核酸(PNA;例如,包括由氨基酸骨架连接在一起的核碱基的寡聚物)。其他骨架修饰包括磷硫酰连接,磷酸二酯修饰的核酸,磷酸二酯和磷硫酰核酸的组合,膦酸甲酯,膦酸烷基酯,磷酸酯,硫代膦酸烷基酯,氨基磷酸酯,氨基甲酸酯,碳酸脂,磷酸三酯,乙酰胺酯,羧基甲酯,甲基磷硫酰,二硫代磷酸酯,对乙氧基,和其组合。

[0139] 在一些实施方案中,多核苷酸包括至少一种具有修饰的碱基的亲和力增强核苷酸类似物,至少具有修饰的糖的核苷酸(其可以是相同的核苷酸),和/或至少一种非天然存在的核苷酸间连接。

[0140] 在一些实施方案中,亲和力增强核苷酸类似物含有锁定的核酸("LNA")糖,其是双环糖。在一些实施方案中,用于本文所述的方法的多核苷酸包含一种以上具有LNA糖的核苷酸。在一些实施方案中,多核苷酸含有一个以上由具有LNA糖的核苷酸组成的区域。在其他

实施方案中，多核苷酸含有具有散布有脱氧核糖核苷酸的LNA糖的核苷酸。参见，例如，Frieden,M.等人(2008)Curr.Pharm.Des.14(11):1138-1142。

[0141] 4.2.7.2示例性的引物

[0142] 在一些实施方案中，提供引物。在一些实施方案中，引物与TV 40S核糖体蛋白(Tv40Srp)基因的至少8，至少9，至少10，至少11，至少12，至少13，至少14，至少15，至少16，至少17，至少18，至少19，至少20，至少21，至少22，至少23，至少24，至少25，至少26，至少27，至少28，至少29，或至少30个连续核苷酸至少90％，至少95％，或100％同一，或至少90％，至少95％，或100％互补。在一些实施方案中，提供包含与TV 40S核糖体蛋白(Tv40Srp)基因的6至100，8至100，8至75，8至50，8至40，或8至30个连续核苷酸至少90％，至少95％，或100％同一，或互补的区域的引物。非限制性示例性的引物显示在表1中。在一些实施方案中，引物还可以包含不与靶基因同一或互补的部分或区域。在一些实施方案中，与靶基因至少90％，至少95％，或100％同一或互补的引物的区域是连续的，从而与靶基因不同一或互补的引物的任意区域不破坏同一或互补区域。

[0143] 在一些实施方案中，引物包含与靶基因的区域至少90％，至少95％，或100％同一的部分。在一些这样的实施方案中，包含与靶基因的区域至少90％，至少95％，或100％同一的区域的引物能够选择性与通过靶基因的扩增产生的扩增子杂交。在一些实施方案中，引物与扩增子的足够部分互补从而其在使用的特定测定条件下选择性与扩增子杂交。

[0144] 如本文中使用的，“选择性杂交”意为，多核苷酸，如引物或探针，将以比将在相同样品中存在的在杂交区域具有不同核苷酸序列的另一核酸杂交至少大5倍的亲和力与样品中的特定核酸杂交。示例性的杂条件在本文中讨论，例如，在反转录反应或PCR扩增反应的情况下。在一些实施方案中，多核苷酸将以比将在相同样品中存在的在杂交区域具有不同核苷酸序列的另一核酸杂交至少大10倍的亲和力与样品中的特定核酸杂交。

[0145] 在一些实施方案中，引物包含可检测部分。

[0146] 在一些实施方案中，提供引物对。设计这样的引物对以扩增靶基因，如TV 40S核糖体蛋白(Tv40Srp)基因，或内源性对照如样品充分性对照(SAC)，或外源性对照如样品处理对照(SPC)的一部分。在一些实施方案中，引物对设计为产生50至1500个核苷酸长度，50至1000个核苷酸长度，50至750个核苷酸长度，50至500个核苷酸长度，50至400个核苷酸长度，50至300个核苷酸长度，50至200个核苷酸长度，50至150个核苷酸长度，100至300个核苷酸长度，100至200个核苷酸长度，或100至150个核苷酸长度的扩增子。非限制性示例性的引物对显示在表1中。

[0147] 4.2.7.3示例性的探针

[0148] 在各种实施方案中，检测阴道毛滴虫的存在的方法包括将样品的核酸与探针杂交。在一些实施方案中，探针包含与靶基因，如TV 40S核糖体蛋白(Tv40Srp)基因，或内源性对照如样品充分性对照(SAC)，或外源性对照如样品处理对照(SPC)互补的部分。在一些实施方案中，探针包含与靶基因的区域至少90％，至少95％，或100％同一的部分。在一些这样的实施方案中，与靶基因至少90％，至少95％，或100％互补的探针与靶基因的足够部分互补，从而其在使用的特定测定条件下选择性与靶基因杂交。在一些实施方案中，与靶基因互补的探针包含与靶基因的至少8，至少9，至少10，至少11，至少12，至少13，至少14，至少15，至少16，至少17，至少18，至少19，至少20，至少21，至少22，至少23，至少24，至少25，至少26，

至少27,至少28,至少29,或至少30个连续核苷酸至少90%,至少95%,或100%互补的区域。非限制性示例性的探针显示在表1中。与靶基因至少90%,至少95%,或100%互补的探针还可以包含不与靶基因互补的部分或区域。在一些实施方案中,与靶基因至少90%,至少95%,或100%互补的探针区域是连续的,从而探针的任何不与靶基因互补的区域不破坏互补区域。

[0149] 在一些实施方案中,探针包含与靶基因,如TV 40S核糖体蛋白(Tv40Srp)基因,或内源性对照如样品充分性对照(SAC),或外源性对照如样品处理对照(SPC)的区域至少90%,至少95%,或100%同一的部分。在一些这样的实施方案中,包含靶基因的区域至少90%,至少95%,或100%同一的区域的探针能够选择性与通过扩增靶基因产生的扩增子杂交。在一些实施方案中,探针与扩增子的足够部分至少90%,至少95%,或100%互补,从而其在使用的特定测定条件下选择性与扩增子杂交。在一些实施方案中,与扩增子互补的探针包含与扩增子的至少8,至少9,至少10,至少11,至少12,至少13,至少14,至少15,至少16,至少17,至少18,至少19,至少20,至少21,至少22,至少23,至少24,至少25,至少26,至少27,至少28,至少29,或至少30个连续核苷酸至少90%,至少95%,或100%互补的区域。与扩增子至少90%,至少95%,或100%互补的探针还可以包含不与扩增子互补的部分或区域。在一些实施方案中,与扩增子至少90%,至少95%,或100%互补的探针区域是连续的,从而探针的任何不与扩增子互补的区域不破坏互补区域。

[0150] 在一些实施方案中,检测一种以上靶基因的方法包括:(a)扩增靶基因的区域;和(b)使用实时PCR和检测探针检测扩增的区域(其可以与扩增步骤(a)同时)。

[0151] 如上文所述的,在一些实施方案中,实时PCR检测可以使用FRET探针进行,其包括,但不限于,TaqMan®探针,分子信标探针和Scorpion探针。在一些实施方案中,实时PCR检测利用TaqMan®探针进行,所述TaqMan®探针即,通常在DNA的一个末端共价结合荧光染料并且别处(如在DNA的另一末端)共价结合猝灭剂分子的线性探针。FRET探针包含与扩增子的区域互补的序列,从而,当FRET探针与扩增子杂交时,染料荧光猝灭,并且当探针在扩增扩增子期间消化时,染料从探针释放并且产生荧光信号。在一些实施方案中,样品中靶基因的量与扩增期间测量的荧光的量成比例。

[0152] TaqMan®探针通常包含具有与靶基因的区域至少90%,至少95%,或100%同一或互补的序列的连续核苷酸区域,从而探针可选择性与靶基因的区域的PCR扩增子杂交。在一些实施方案中,探针包含具有与靶基因的区域完全互补或同一存在于靶基因的区域中的序列的至少6个连续核苷酸的区域。在一些实施方案中,探针包含与要检测的靶基因的至少8个连续核苷酸,至少10个连续核苷酸,至少12个连续核苷酸,至少14个连续核苷酸,或至少16个连续核苷酸至少90%,至少95%,或100%同一或互补的区域。

[0153] 在一些实施方案中,具有与TaqMan®探针序列至少90%,至少95%,或100%互补的序列的扩增子区域在或接近扩增子分子的中间。在一些实施方案中,在互补性区域的5'-末端和3'-末端独立地具有扩增子的至少2个核苷酸,如至少3个核苷酸,如至少4个核苷酸,如至少5个核苷酸。

[0154] 在一些实施方案中,分子信标可以用于检测PCR产物。像TaqMan®探针一样,分子信标通过探针的末端连接有荧光染料和猝灭剂的探针使用FRET检测PCR产物。不同于

TaqMan®探针，在PCR循环期间分子信标保持完整。分子信标探针当游离在溶液中时形成茎环结构，从而允许染料和猝灭剂足够接近以引起荧光猝灭。当分子信标与靶标杂交时，茎环结构消除，从而染料和猝灭剂空间上分开并且染料发荧光。分子信标可以例如，从Gene Link™获得(参见www.genelink.com/newsite/products/mbintro.asp)。

[0155] 在一些实施方案中，Scorpion探针可以用作序列-特异性引物和用于PCR产物检测。像分子信标一样，在未与靶核酸杂交时，Scorpion探针形成茎环结构。然而，不同于分子信标，Scorpion探针实现序列-特异性引发(priming)和PCR产物检测。荧光染料分子连接于Scorpion探针的5'-末端，并且猝灭剂连接在别处，如3'-末端。探针的3'部分与PCR引物的延伸产物互补，并且该互补部分通过不可扩增部分连接于探针的5'-末端。Scorpion引物延伸后，探针的靶标特异性序列结合延伸的扩增子内的其互补物，由此打开茎环结构并允许5'-末端的染料发荧光并且产生信号。Scorpion探针可以从例如Premier Biosoft International获得(参见www.premierbiosoft.com/tech_notes/Scorpion.html)。

[0156] 在一些实施方案中，可以在FRET探针上使用的标记包括比色法和荧光染料如Alexa Fluor染料，BODIPY染料，如BODIPY FL;Cascade Blue;Cascade Yellow;香豆素和其衍生物，如7-氨基-4-甲基香豆素，氨基香豆素和羟基香豆素;花青染料，如Cy3和Cy5;曙红和赤藓红;荧光素和其衍生物，如异硫氰酸荧光素;镧系离子的大环螯合物，如Quantum Dye™;Marina Blue;Oregon Green;罗丹明染料，如罗丹明红，四甲基罗丹明和罗丹明6G;Texas Red;荧光能量转移染料，如噻唑橙-乙锭异二聚体;和，TOTAB。

[0157] 染料的具体实例包括，但不限于，上述那些和以下的:Alexa Fluor 350，Alexa Fluor 405，Alexa Fluor 430，Alexa Fluor 488，Alexa Fluor 500。Alexa Fluor 514，Alexa Fluor 532，Alexa Fluor 546，Alexa Fluor 555，Alexa Fluor 568，Alexa Fluor 594，Alexa Fluor 610，Alexa Fluor 633，Alexa Fluor 647，Alexa Fluor 660，Alexa Fluor 680，Alexa Fluor 700，和，Alexa Fluor 750;胺反应性BODIPY染料，如BODIPY 493/503，BODIPY 530/550，BODIPY 558/568，BODIPY 564/570，BODIPY 576/589，BODIPY 581/591，BODIPY 630/650，BODIPY 650/655，BODIPY FL，BODIPY R6G，BODIPY TMR，和，BODIPY-TR;Cy3，Cy5，6-FAM，异硫氰酸荧光素，HEX，6-JOE，Oregon Green 488，Oregon Green 500，Oregon Green 514，Pacific Blue，REG，Rhodamine Green，Rhodamine Red，Renographin，ROX，SYPRO，TAMRA，2'，4'，5'，7'-四溴砜荧光素，和TET。

[0158] 染料猝灭剂对(即，供体/受体对)的实例包括，但不限于，荧光素/tetra甲基罗丹明;IAEDANS/荧光素;EDANS/dabcyl;荧光素/荧光素;BODIPY FL/BODIPY FL;荧光素/QSY 7或QSY 9染料。当供体和受体相同时，在一些实施方案中，FRET可以通过荧光去极化检测。染料/猝灭剂对(即，供体/受体对)的某些特定实例包括，但不限于，Alexa Fluor 350/AlexaFluor488;Alexa Fluor 488/Alexa Fluor 546;Alexa Fluor 488/Alexa Fluor 555;Alexa Fluor 488/Alexa Fluor 568;Alexa Fluor 488/Alexa Fluor 594;AlexaFluor 488/Alexa Fluor 647;Alexa Fluor 546/Alexa Fluor 568;Alexa Fluor 546/Alexa Fluor 594;Alexa Fluor 546/Alexa Fluor 647;Alexa Fluor 555/Alexa Fluor 594;Alexa Fluor 555/Alexa Fluor 647;Alexa Fluor 568/Alexa Fluor 647;Alexa Fluor 594/Alexa Fluor 647;Alexa Fluor 350/QSY35;Alexa Fluor 350/dabcyl;Alexa Fluor 488/QSY 35;Alexa Fluor 488/dabcyl;Alexa Fluor 488/QSY 7或QSY 9;

Alexa Fluor 555/QSY 7或QSY9;Alexa Fluor 568/QSY 7或QSY 9;Alexa Fluor 568/QSY 21;Alexa Fluor 594/QSY 21;和Alexa Fluor 647/QSY 21。在一些情况中,相同猝灭剂可以用于多种染料,例如,宽谱猝灭剂,如Iowa Black® 猝灭剂(Integrated DNA Technologies,Coralville,IA)或Black Hole猝灭剂™(BHQ™;Sigma-Aldrich,St.Louis,MO)。

[0159] 在一些实施方案中,例如,在其中两种以上部分(如扩增子)同时检测的多重反应中,每种探针包含检测上不同的染料,从而在同一反应中同时检测时可以区分染料。本领域技术人员可以选择一组检测上不同的染料用于多重反应。

[0160] 可用于制备用于本文所述的方法的一些实施方案中的PCR探针的荧光标记的核糖核苷酸的具体实例可从Molecular Probes(Invitrogen)获得,并且这些包括,Alexa Fluor 488-5-UTP,荧光素-12-UTP,BODIPY FL-14-UTP,BODIPY TMR-14-UTP,四甲基罗丹明-6-UTP,Alexa Fluor 546-14-UTP,Texas Red-5-UTP,和BODIPY TR-14-UTP。其他荧光核糖核苷酸可从Amersham Biosciences(GE Healthcare)获得,如Cy3-UTP和Cy5-UTP。

[0161] 用于制备用于本文所述的方法的PCR探针的荧光标记的脱氧核糖核苷酸的实例包括二硝基苯基(DNP)-1'-dUTP,Cascade Blue-7-dUTP,Alexa Fluor 488-5-dUTP,荧光素-12-dUTP,Oregon Green 488-5-dUTP,BODIPY FL-14-dUTP,罗丹明绿-5-dUTP,Alexa Fluor 532-5-dUTP,BODIPY TMR-14-dUTP,四甲基罗丹明-6-dUTP,Alexa Fluor 546-14-dUTP,Alexa Fluor 568-5-dUTP,Texas Red-12-dUTP,Texas Red-5-dUTP,BODIPY TR-14-dUTP,Alexa Fluor 594-5-dUTP,BODIPY 630/650-14-dUTP,BODIPY 650/665-14-dUTP;Alexa Fluor 488-7-OBEA-dCTP,Alexa Fluor 546-16-OBEA-dCTP,Alexa Fluor 594-7-OBEA-dCTP,Alexa Fluor 647-12-OBEA-dCTP。荧光标记的核苷酸可商购并且可以从例如,Invitrogen购买。

[0162] 在一些实施方案中,通过修饰的核苷酸将染料和其他部分,如猝灭剂,引入本文所述的方法中使用的多核苷酸,如FRET探针中。"修饰的核苷酸"是指已经化学修饰,但仍然作为核苷酸行使功能的核苷酸。在一些实施方案中,修饰的核苷酸具有共价连接的化学部分,如染料或猝灭剂,并且可以例如,通过多核苷酸的固相合成方式引入多核苷酸。在其他实施方案中,修饰的核苷酸包括一种以上可以在将修饰的核苷酸引入核酸之前、期间或之后与染料或猝灭剂反应的反应性基团。在具体实施方案中,修饰的核苷酸是胺-修饰的核苷酸,即,修饰以具有反应性胺基团的核苷酸。在一些实施方案中,修饰的核苷酸包含修饰的碱基部分,如尿苷,腺苷,鸟苷,和/或胞嘧啶。在具体实施方案中,胺-修饰的核苷酸选自5-(3-氨基烯丙基)-UTP;8-[(4-氨基)丁基]-氨基-ATP和8-[(6-氨基)丁基]-氨基-ATP;N6-(4-氨基)丁基-ATP,N6-(6-氨基)丁基-ATP,N4-[2,2-氧基-二-(乙基胺)]-CTP;N6-(6-氨基)己基-ATP;8-[(6-氨基)己基]-氨基-ATP;5-炔丙基氨基-CTP,5-炔丙基氨基-UTP。在一些实施方案中,具有不同核碱基部分的核苷酸类似修饰,例如,5-(3-氨基烯丙基)-GTP替代5-(3-氨基烯丙基)-UTP。很多胺修饰的核苷酸可购自,例如,Applied Biosystems,Sigma,Jena Bioscience和TriLink。

[0163] 示例性的可检测部分还包括,但不限于,结合对的成员。在一些这样的实施方案中,结合对的第一成员连接多核苷酸。结合对的第二成员连接可检测标记,如荧光标记。当将连接于结合对的第一成员的多核苷酸与结合对的连接于可检测标记的第二成员孵育时,

结合对的第一和第二成员关联并且可以检测到多核苷酸。示例性的结合对包括，但不限于，生物素和链霉亲和素，抗体和抗原等。

[0164] 在一些实施方案中，在单个多重反应中检测多个靶基因。在一些这样的实施方案中，当从探针释放时，每种靶向独特扩增子的探针可在光谱上区分，在该情况下每种靶基因通过独特的荧光信号检测。在一些实施方案中，使用相同的荧光信号检测两种以上靶基因，在此情况下，该信号检测表明存在一种靶基因或两种。

[0165] 本领域技术人员可以为选择的测定选择合适的检测方法，例如，实时PCR测定。选择的检测方法不需要是上述方法，并且可以是任意方法。

[0166] 4.3示例性的组合物和试剂盒

[0167] 在另一方面，提供组合物。在一些实施方案中，提供组合物用于本文所述的方法。

[0168] 在一些实施方案中，提供包含至少一种靶基因-特异的引物的组合物。术语“靶基因-特异的引物”包括具有以下各项的引物：具有(i)与靶基因的区域至少90％，至少95％，或100％同一，或(ii)与靶基因中发现的连续核苷酸的区域的序列至少90％，至少95％，或100％互补的序列的连续核苷酸区域。在一些实施方案中，提供包含至少一对靶基因-特异的引物的组合物。术语“靶基因-特异的引物对”包括适于扩增限定的靶基因区域的引物对。靶基因-特异的引物对通常包含第一引物和第二引物，所述第一引物包含与靶基因的区域的序列至少90％，至少95％，或100％同一的序列，并且所述第二引物包含与靶基因的区域至少90％，至少95％，或100％互补的序列。引物对通常适于扩增50至1500个核苷酸长度，50至1000个核苷酸长度，50至750个核苷酸长度，50至500个核苷酸长度，50至400个核苷酸长度，50至300个核苷酸长度，50至200个核苷酸长度，50至150个核苷酸长度，100至300个核苷酸长度，100至200个核苷酸长度，或100至150个核苷酸长度的靶基因区域。非限制性示例性的引物，和引物对显示在表1中。

[0169] 在一些实施方案中，组合物包含至少一对靶基因-特异的引物。在一些实施方案中，组合物另外包含一对用于扩增内源性对照(如SAC)的靶基因-特异的引物和/或一对用于扩增外源性对照(如SPC)的靶基因-特异的引物。

[0170] 在一些实施方案中，组合物包含至少一个靶基因-特异的探针。术语“靶基因-特异的探针”包括具有以下各项的探针：具有(i)与靶基因的区域至少90％，至少95％，或100％同一，或(ii)与靶基因中发现的连续核苷酸区域的序列至少90％，至少95％，或100％互补的序列的连续核苷酸区域。非限制性示例性的靶-特异的探针显示在表1中。

[0171] 在一些实施方案中，组合物(包括包含一对以上靶基因-特异的引物的上述组合物)包含一种以上用于检测靶基因的探针。在一些实施方案中，组合物包含用于检测内源性对照(如SAC)的探针和/或用于检测外源性对照(如SPC)的探针。

[0172] 在一些实施方案中，组合物是水性组合物。在一些实施方案中，水性组合物包含缓冲成分，如磷酸盐，tris，HEPES等，和/或其他成分，如下文讨论的。在一些实施方案中，组合物是干燥的，例如，冻干的，并且适于通过加入流体恢复。干燥组合物可以包括一种以上缓冲成分和/或其他成分。

[0173] 在一些实施方案中，组合物还包括一种以上其他成分。其他成分包括，但不限于，盐，如NaCl、KCl和$MgCl_2$；聚合酶，包括热稳定的聚合酶如Taq；dNTPs；牛血清白蛋白(BSA)等；还原剂，如β-巯基乙醇；EDTA等等。本领域技术人员可以根据组合物的预期用途选择合

适的组合物成分。

[0174] 在一些实施方案中，提供包含至少一种用于检测至少一种靶基因的多核苷酸的组合物。在一些实施方案中，多核苷酸用作用于反转录酶反应的引物。在一些实施方案中，多核苷酸用作用于扩增的引物。在一些实施方案中，多核苷酸用作用于PCR的引物。在一些实施方案中，多核苷酸用作检测至少一种靶基因的探针。在一些实施方案中，多核苷酸被可检测标记。在一些实施方案中，多核苷酸是FRET探针。在一些实施方案中，多核苷酸是TaqMan®探针、分子信标、或Scorpion探针。

[0175] 在一些实施方案中，组合物包含至少一种FRET探针，所述探针具有与TV 40S核糖体蛋白(Tv40Srp)基因的区域至少90％，至少95％，或100％同一，或至少90％，至少95％，或100％互补的序列。在一些实施方案中，FRET探针用供体/受体对标记，从而当探针在PCR反应期间被消化时，其产生与特异的靶基因相关的独特荧光发射。在一些实施方案中，当组合物包含多个FRET探针时，每个探针被不同供体/受体对标记，从而当探针在PCR反应期间被消化时，每一个产生与特异的探针序列和/或靶基因相关的独特的荧光发射。在一些实施方案中，FRET探针的序列与靶基因的靶区域互补。在其他实施方案中，当与靶基因的最佳匹配靶区域的序列相比时，FRET探针具有包含一种以上碱基错配的序列。

[0176] 在一些实施方案中，组合物包含由至少8，至少9，至少10，至少11，至少13，至少14，至少15，至少16，至少17，至少18，至少19，至少20，至少21，至少22，至少23，至少24，或至少25个核苷酸组成的FRET探针，其中序列的至少一部分与TV 40S核糖体蛋白(Tv40Srp)基因的区域至少90％，至少95％，或100％同一，或至少90％，至少95％，或100％互补。在一些实施方案中，FRET探针的至少8，至少9，至少10，至少11，至少13，至少14，至少15，至少16，至少17，至少18，至少19，至少20，至少21，至少22，至少23，至少24，或至少25个核苷酸同一地存在于TV 40S核糖体蛋白(Tv40Srp)基因的区域中，或与TV 40S核糖体蛋白(Tv40Srp)基因的区域互补。在一些实施方案中，当与TV 40S核糖体蛋白(Tv40Srp)基因的序列或互补物时，FRET探针包括具有一个、两个或三个错配的序列。

[0177] 在一些实施方案中，试剂盒包含上文讨论的多核苷酸。在一些实施方案中，试剂盒包含上文讨论的至少一种引物和/或探针。在一些实施方案中，试剂盒包含至少一种聚合酶，如热稳定的聚合酶。在一些实施方案中，试剂盒包含dNTP。在一些实施方案中，用于本文所述的实时PCR方法的试剂盒包含一种以上靶基因-特异的FRET探针和/或一种以上用于扩增靶基因的引物。

[0178] 在一些实施方案中，一种以上引物和/或探针是“线性的”。“线性”引物是指是单链分子的，并且通常不包含例如，至少3，4或5个连续核苷酸的短区域的多核苷酸，其与相同多核苷酸内的另一区域互补，从而引物形成内双链体。在一些实施方案中，用于反转录的引物在靶基因的3’-末端包含至少4，如至少5，如至少6，如至少7或更多个连续核苷酸的区域，其具有与在靶基因的5’-末端的至少4，如至少5，如至少6，如至少7或更多个连续核苷酸的区域互补的序列。

[0179] 在一些实施方案中，试剂盒包含一对以上用于扩增靶基因的线性引物(“正向引物”和“反向引物”)。因此，在一些实施方案中，第一引物包含至少8，至少9，至少10，至少11，至少12，至少13，至少14，至少15，至少16，至少17，至少18，至少19，至少20，至少21，至少22，至少23，至少24，或至少25个连续核苷酸的区域，该区域具有与靶基因的第一位置处的至少

8,至少9,至少10,至少11,至少12,至少13,至少14,至少15,至少16,至少17,至少18,至少19,至少20,至少21,至少22,至少23,至少24,或至少25个连续核苷酸的区域的序列至少90%,至少95%,或100%同一的序列。此外,在一些实施方案中,第二引物包含至少8,至少9,至少10,至少11,至少12,至少13,至少14,至少15,至少16,至少17,至少18,至少19,至少20,至少21,至少22,至少23,至少24,或至少25个连续核苷酸的区域,该区域具有与在靶基因的第二位置处的至少8,至少9,至少10,至少11,至少12,至少13,至少14,至少15,至少16,至少17,至少18,至少19,至少20,至少21,至少22,至少23,至少24,或至少25个连续核苷酸的区域的序列至少90%,至少95%,或100%互补的序列,从而使用两条引物的PCR反应造成扩增子从靶基因的第一位置向靶基因的第二位置延伸。

[0180] 在一些实施方案中,所述试剂盒包含至少两组,至少三组,或至少四组引物,其各自用于扩增不同靶基因,如内源性对照和/或外源性对照。

[0181] 在一些实施方案中,用于本文所述的组合物的探针和/或引物包含脱氧核糖核苷酸。在一些实施方案中,用于本文所述的组合物的探针和/或引物包含脱氧核糖核苷酸和一种以上核苷酸类似物,如LNA类似物或上文所述的其他稳定双链体的核苷酸类似物。在一些实施方案中,用于本文所述的组合物的探针和/或引物包含所有核苷酸类似物。在一些实施方案中,探针和/或引物在互补性区域包含一种以上稳定双链体的核苷酸类似物,如LNA类似物。

[0182] 在一些实施方案中,用于本文所述的实时PCR方法的试剂盒还包括用于反转录和扩增反应的试剂。在一些实施方案中,试剂盒包含酶如热稳定DNA聚合酶,如Taq聚合酶。在一些实施方案中,试剂盒还包括三磷酸脱氧核糖核苷酸(dNTP)用于扩增。在其他实施方案中,试剂盒包含对于探针和引物特异的杂交优化的缓冲液。

[0183] 试剂盒一般包括具有一个以上容纳试剂的容器的包装,作为一种以上分开的组合物或,任选地,作为混合物(其中允许试剂相容)。所述试剂盒还可以包括从用户的角度可能需要的其他一种或多种材料,如一种或多种缓冲液、一种或多种稀释剂、一种或多种标准物,和/或用于样品处理、洗涤、或进行测定的任何其他步骤的任何其他材料。

[0184] 试剂盒优选包括进行本文所述的一种以上方法的使用说明。试剂盒中包括的使用说明可以附加于包装材料或可以作为药品说明书包括在内。使用说明通常是手写或打印的材料,但它们不限于此。本发明考虑任何能够储存该使用说明并它们传达给终端用户的介质。这样的介质包括,但不限于,电子储存介质(例如,磁盘、磁带、磁筒、磁芯片),光学介质(例如,CD ROM)等。如本文中使用的,术语"使用说明"可以包括提供使用说明的网站地址。

[0185] 在一些实施方案中,所述试剂盒可以包含一种以上GeneXpert®样品筒中提供的上述试剂。这些筒允许提取、扩增和检测在该自带的"筒中实验室"中进行(参见例如,美国专利5,958,349,6,403,037,6,440,725,6,783,736,6,818,185;其各自以其整体通过引用结合于本文)。测量基因组拷贝数水平和检测病原的试剂可以在试剂盒内分开的筒中提供或这些试剂(适于多重检测)可以在单个筒中提供。

[0186] 在一些实施方案中,本文所述的任意试剂盒可以包括用于收集尿道拭子样品、阴道拭子样品、或子宫颈内拭子样品的尿样品和/或拭子容器。

[0187] 以下实施例仅为了说明的目的,并且不意在以任何方式限制。

[0188] 5.实施例

[0189] 5.1.实施例1:阴道毛滴虫的检测

[0190] 设计测定以通过PCR,使用表1中所示引物和探针检测阴道毛滴虫(TV)40S核糖体蛋白(Tv40Srp)的基因。除了TV-特异的引物和探针,包括检测用作样品充分性对照(SAC)靶标的单拷贝人基因的引物和探针。还包括检测细菌基因的引物和探针,所述细菌基因作为样品处理对照(SPC)靶标包括在多重反应中。

[0191] 表1:引物和探针序列

[0192]

寡聚物名称	靶	序列	SEQ ID NO	扩增子 SEQ ID NO
TV 正向	Tv40Srp 基因	GTAACAACCTTGGAGTTCTTCTTAAG	1	5
TV 反向	Tv40Srp 基因	ACATCAATCTACAAGACACCACTTGA	2	
TV 探针	Tv40Srp 基因	F1-AGTTTGGCTGCTTAGCTTCGAC-Q1	3	

[0193] 多重测定的最终引物和探针组合物显示在表2中。

[0194] 表2:引物和探针浓度

[0195]

靶标	标记	目的	终浓度 正向引物	终浓度 反向引物	终浓度 探针
Tv40Srp	F1	TV 检测	0.3μM	0.3μM	0.5 μM
单拷贝人基因	F2	SAC	0.35 μM	0.35 μM	0.35 μM
细菌基因	F2	SPC	0.4 μM	0.4 μM	0.2 μM

[0196] F1和F2是检测上不同的染料,其可以同时在多重反应中检测和区分。每个探针还包含猝灭剂(例如,Q1,上述)。

[0197] 每个反应含有42-58mM KCl,3.5-5.0mM $MgCl_2$,250-350μM dNTPs,50mM Tris,pH 8.6,和0.01%叠氮钠。AptaTaq(0.27-0.37单位/μl;Roche)用于扩增。

[0198] 对于每个要测试的样品,将约7mL的首次,新排出的(voided)尿加入至1mL缓冲液中(优选样品收集2小时内)。将医生收集的子宫颈内拭子或自己收集的(在临床环境)阴道拭子立即置于2.5mL缓冲液中。

[0199] 将500μL的缓冲的尿或拭子样品加载于GeneXpert®筒上用于分析。将样品与裂解试剂混合以释放核酸。裂解后,将从样品释放的核酸捕获在DNA-结合基材上。将核酸从基材上洗脱并用于恢复用于实时PCR的试剂(上述的)。使用的反应循环是:在GeneXpert®系统中使用GeneXpert®筒,95℃1分钟,接着92.5℃5秒的40个循环,在68℃20秒。

[0200] 测定结果如在表3中解释的。TV、SAC、和SPC靶的Ct值的有效范围是9-39.9Ct。

[0201] 表3:Xpert TV测定结果和解释

[0202]

结果	解释
检测到 TV	检测到毛滴虫靶 DNA。 ● 毛滴虫靶在有效范围内具有 Ct 和阈值设定以上的荧光终点。 ● SPC-不可用。忽略 SPC，因为毛滴虫靶扩增可以与此对照竞争。 ● SAC-不可用。忽略 SAC，因为毛滴虫靶扩增可以与此对照竞争。 ● PCC-合格。所有探针检查结果合格。
未检测到 TV	未检测到毛滴虫靶 DNA。SPC 满足接受标准。 ● 未检测到毛滴虫靶 DNA。 ● SPC-合格。SPC 在有效范围内具有 Ct 和阈值设定以上的荧光终点。 ● SAC-合格。SAC 在有效范围内具有 Ct 和阈值设定以上的荧光终点。 ● PCC-合格。所有探针检查结果合格。
无效	不能确定存在或不存在毛滴虫靶 DNA。根据 11.2 节中的说明重复测试，重测试步骤。 ● SPC-失败。SPC Ct 不在有效范围内并且荧光终点低于阈值设定。

[0203]

	● SAC-合格。SAC 在有效范围内具有 Ct 和阈值设定以上的荧光终点。 ● PCC-合格。所有探针检查结果合格。 或 ● SPC-合格。SPC 在有效范围内具有 Ct 和阈值设定以上的荧光终点。 ● SAC-失败。SAC Ct 不在有效范围内并且荧光终点低于阈值设定。 ● PCC-合格。所有探针检查结果合格。 或 ● SPC-失败。SPC Ct 不在有效范围内并且荧光终点低于阈值设定。 ● SAC-失败。SAC Ct 不在有效范围内并且荧光终点低于阈值设定。 ● PCC-合格。所有探针检查结果合格。
错误	不能确定存在或不存在毛滴虫靶 DNA。根据 11.2 节中的说明重复测试，重测试步骤。 ● TRICHOMONAS-无结果 ● SPC-无结果 ● SAC-无结果 ● PCC-失败。*所有探针或一种探针检测结果失败。 *如果探针检查合格，由最大压力限制超过可接受范围或由系统成分失败引起错误。
无结果	不能确定存在或不存在毛滴虫靶 DNA。根据 11.2 节中的说明重复测试，重测试步骤。无结果表明收集的数据不充足。例如操作者终止了进行中的测试或发生电力故障。 ● TRICHOMONAS-无结果 ● SPC-无结果 ● SAC-无结果

[0204]

	● PCC-不可用

[0205] 5.2实施例2:临床性能

[0206] Xpert TV测定的性能特征在美国的13个机构进行评估。由于阴道毛滴虫的低流行程度和难以从男性受试者获得新鲜阴道毛滴虫-阳性样本，用于本研究的样本群体利用人为的男性尿样本提供。

[0207] 受试者包括知情同意的无症状和有症状的、性活跃的男性和女性，他们在包括，但不限于的位置观察：OB/GYN、性传播疾病(STD)、青少年、公共卫生设施和计划生育诊所。

[0208] 研究样本由预期收集的男性尿液、女性尿液、子宫颈的拭子和患者-收集的阴道拭子(在临床环境收集)组成。包括人为的男性尿样本以补充男性样品尺寸。

[0209] 将Xpert TV测定性能与使用转录介导的测定(APTIMA®阴道毛滴虫测定，GenProbe Hologic，San Diego，USA)检测阴道毛滴虫的核糖体RNA的FDA-明确的体外定量核酸扩增比较仪测定相比较。将在Xpert TV测定和比较仪测定之间具有差异结果的样品利用分开的重复的基因组DNA序列的双向测序进行分析。参见Bandea，等人，Jornal of Clinical Microbiology.2013，51(4)：1298-1300。

[0210] 利用合格的样品进行的Xpert TV测定运行中，这些样本中的97.3％(5327/5474)对于第一次尝试中成功的。剩余的147个对于第一次尝试给出不明确的结果(91个错误，44个无效并且12个无结果)。在单个重测后，147个样本中的119个得到有效结果；样本中的17个对于第二次尝试不明确并且11个样本未重测。总体测定成功率是99.5％(5446/5474)。

[0211] 将来自Xpert TV测定的结果与比较仪测定想比较，并对有差异的双向测序。性别、样品类型和症状状态的灵敏度和特异性在表4中提供。

[0212]

表 4: Xpert TV 测定相对参考 NAAT 测试加上测序

样本		Sx 状态	n	TP	FP	TN	FN	Prev %	灵敏度% (95 CI)	特异性% (95 CI)	PPV % (95 CI)	NPV % (95 CI)
女性	PC-VS	有症状的	717	63	0	649	5	9.4	92.6 (83.7-97.6)	100 (99.4-100)	100 (94.3-100)	99.2 (98.1-99.8)
		无症状的	857	50	0	806	1	6.1	98.0 (89.6-100)	100 (99.5-100)	100 (92.9-100)	99.9 (99.3-100)
		全部	1574	113	0	1455	6	7.6	95.0 (89.3-98.1)	100 (99.7-100)	100 (96.8-100)	99.6 (99.1-99.8)
	ES	有症状的	714	59	0	651	4	8.8	93.7 (84.5-98.2)	100 (99.4-100)	100 (93.9-100)	99.4 (98.4-99.8)
		无症状的	859	49	0	809	1	5.8	98.0 (89.4-99.9)	100 (99.5-100)	100 (92.7-100)	99.9 (99.3-100)
		全部	1573	108	0	1460	5	7.2	95.6 (90.0-98.5)	100 (99.7-100)	100 (96.6-100)	99.7 (99.2-99.9)
	UR	有症状的	713	60	0	651	2	8.7	96.8 (88.8-99.6)	100 (99.4-100)	100 (94.0-100)	99.7 (98.9-100)
		无症状的	856	48	0	806	2	5.8	96.0 (86.3-99.5)	100 (99.5-100)	100 (92.6-100)	99.8 (99.1-100)
		全部	1569	108	0	1457	4	7.1	96.4 (91.1-99.0)	100 (99.7-100)	100 (96.6-100)	99.7 (99.3-99.9)
男性	UR	有症状的	125	1	0	124	0	0.8	100 (2.5-100)	100 (97.1-100)	100 (2.5-100)	100 (97.1-100)
		无症状的	411	13	0	398	0	3.2	100 (75.3-100)	100 (99.1-100)	100 (75.3-100)	100 (99.1-100)
		CS	183	62	19	99	3	NA	95.4 (87.1-99.0)	83.9 (76.0-90.0)	NA	NA
		全部	719	76	19	621	3	NA	96.2 (89.3-99.2)	97.0 (95.4-98.2)	NA	NA

a. TP=真阳性，FP=假阳性，TN=真阴性，FN=假阴性，PC-VS=患者收集的阴道拭子，ES=子宫颈内拭子，CS=人为样本，UR=尿

[0213] 5.3实施例3:检测极限

[0214] 分析Xpert TV测定的灵敏度或检测极限(LoD)使用两个阴道毛滴虫株评估,一个甲硝哒唑易感的(阴道毛滴虫ATCC®30001™),和一个甲硝哒唑抗性的(阴道毛滴虫ATCC®30238™)。两个株都在与缓冲液混合的阴道毛滴虫-阴性汇集男性尿液(MU)和缓冲液中的阴道毛滴虫-阴性汇集的阴道拭子(VS)中测试。

[0215] 经三天以对于每个株和样品类型最少五个浓度测试20个重复评估检测极限(LoD)。通过逻辑回归评估LoD。LoD定义为可以重复以95％置信度与阴性样品相区别的最低细胞数/mL或20个重复中的19个是阳性的最低浓度。利用两个不同批次的Xpert TV试剂进行研究，并且对于每株所提出的LoD是两次确定中较高的那个(表5)。对于缓冲液中拭子样品，检测极限是5个细胞/mL。对于缓冲液中的尿样品，检测极限是6个细胞/mL。提出的LoD通过分析稀释至估计的LoD浓度的至少20个重复来验证。

[0216] 表5：使用Xpert TV的阴道毛滴虫检测极限

[0217]

阴道毛滴虫株和基质	LoD 评估(逻辑回归)(下和上 95%置信区间)(细胞/mL)		验证的 LoD (细胞/mL)	验证 (阳性数/20)	LoD 提出 (细胞/mL)
	批次 1	批次 2			
阴道拭子中的 ATCC 30001	3.9 (3.0–6.0)	4.2 (3.3–6.3)	4.2	20/20	5
阴道拭子中的 ATCC 30238	4.4 (3.5–6.5)	3.7 (2.9–5.5)	4.4	19/20	5
男性尿液中的 ATCC 30001	5.8 (4.7–7.9)	3.2 (2.6–4.8)	5.8	20/20	6
男性尿液中的 ATCC 30238	4.9 (4.0–6.6)	4.3 (3.4–6.2)	4.9	19/20	5

[0218] 5.4实施例4：测定重复性

[0219] 具有不同浓度的阴道毛滴虫一组八个样本在12不同日，由两个不同操作者，在三个地点的每个测试(8个样本x1次/天x 12天x2个操作者x3个地点)。在3个测试地点的每个使用三批Xpert TV测定。根据Xpert TV测定程序进行Xpert TV测定。结果总结在表6中。

[0220] 还关于对于各个检测的靶标以Ct值表达的荧光信号评价Xpert TV测定的重复性。地点间的、批次间的、日间的、操作者之间的和对于每个组成员的测定内的平均值、标准偏差(SD)和变异系数(CV)在表7中提供。

[0221]

表 6: 重复性结果总结

样品	地点 1			地点 2			地点 3			%样品的总一致率
	Op 1	Op 2	地点	Op 1	Op 2	地点	Op 1	Op 2	地点	
FS-Neg	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	100% (24/24)	100% (48/48)	100% (144/144)
FS-Mod Pos	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	100% (24/24)	100% (48/48)	100% (144/144)
FS-Low Pos	62.5% (15/24)	75.0% (18/24)	68.8% (33/48)	70.8% (17/24)	83.3% (20/24)	77.1% (37/48)	90.7% (17/24)	87.5% (21/24)	79.2% (38/48)	75.0% (108/144)
FS-LoD	91.7% (22/24)	100% (24/24)	95.8% (46/48)	95.8% (23/24)	95.8% (23/24)	95.8% (46/48)	95.8% (23/24)	100% (24/24)	97.9% (47/48)	96.5% (139/144)
UR-Neg	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	100% (24/24)	100% (48/48)	100% (144/144)
UR-Mod Pos	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	100% (24/24)	100% (48/48)	100% (144/144)
UR-Low Pos	87.5% (31/24)	45.8% (11/24)	66.7% (32/48)	70.8% (17/24)	70.8% (17/24)	70.8% (34/48)	79.2% (19/24)	66.7% (16/24)	72.9% (35/48)	70.1% (101/144)
UR-LoD	91.7% (22/24)	100% (24/24)	95.8% (46/48)	95.8% (23/24)	91.7% (22/24)	93.8% (45/48)	100% (24/24)	91.7% (22/24)	95.8% (46/48)	95.1% (137/144)

a. FS=女性拭子基质；UR=男性尿液基质

[0222]

表 7: 重复性数据的总结

样品	测定通道 (分析物)	N[a]	平均 Ct	地点间		批次间		日间		操作者间		测定内		总的	
				SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
FS-Neg	SAC	144	24.61	0	0	0.12	0.5	0.15	0.6	0	0	0.31	1.3	0.37	1.5
FS-Mod Pos	TV	144	35.40	0.09	0.2	0.32	0.9	0.16	0.4	0	0	0.68	1.9	0.77	2.2
FS-Low Pos	TV	108	38.18	0	0	0	0	0	0	0.52	1.4	0.86	2.3	1.01	2.6
FS-LoD	TV	139	37.14	0.16	0.4	0.36	1.0	0.21	0.6	0	0	0.92	2.5	1.02	2.7
UR-Neg	SAC	144	34.10	0.06	0.2	0.20	0.6	0	0	0.17	0.5	0.28	0.8	0.39	1.1
UR-Mod Pos	TV	144	35.40	0	0	0.37	1.0	0.13	0.4	0.18	0.5	0.65	1.8	0.78	2.2
UR-Low Pos	TV	101	37.95	0	0	0	0	0.36	1.0	0.52	1.4	0.94	2.5	1.14	3.0
UR-LoD	TV	137	37.03	0	0	0.33	0.9	0	0	0	0	0.96	2.6	1.01	2.7

a. 144 个中具有非 0Ct 值的结果

[0223] 5.5.实施例5:分析包容性

[0224] Xpert TV测定的分析包容性通过以不大于3x分析检测极限(3x LoD)的浓度一式三份测试17个阴道毛滴虫株评价。在缓冲液中的阴道毛滴虫-阴性汇集的阴道拭子(VS),和

与缓冲液混合的男性尿(MU)中测试各株。参见表8。在该研究条件下,所有株报告TV检测ED结果。Xpert TV测定在两种样品类型中显示100%包容性。

[0225] 表8:分析包容性表

分离物 ATCC#	分离来源	结果阴道拭子	结果男性尿液
30001	阴道分泌物	检测到 TV	检测到 TV
30184	阴道拭子	检测到 TV	检测到 TV
30187	子宫颈内拭子	检测到 TV	检测到 TV
30188	阴道	检测到 TV	检测到 TV
30236	子宫颈内拭子	检测到 TV	检测到 TV
30240	阴道汇集	检测到 TV	检测到 TV
30245	阴道和子宫颈内材料	检测到 TV	检测到 TV
30247	阴道	检测到 TV	检测到 TV
50138	人	检测到 TV	检测到 TV
50139	人	检测到 TV	检测到 TV
50141	人	检测到 TV	检测到 TV
50143	人	检测到 TV	检测到 TV
50147	人	检测到 TV	检测到 TV
50167	阴道	检测到 TV	检测到 TV
50183	前列腺液	检测到 TV	检测到 TV
PRA-95	阴道分泌物	检测到 TV	检测到 TV
PRA-98	人	检测到 TV	检测到 TV

[0226]

[0227] 5.6.实施例6:分析特异性

[0228] 利用Xpert TV测定测试一组47种生物(包括泌尿生殖道中常见的细菌、真菌和病毒,以及其他与毛滴虫密切相关的原生动物)。以1x 10^7cfu/mL或更高测试每个细菌或真菌株。将不产生可计数克隆的株稀释到0.5McFarland单位,大约相当于对于大肠杆菌1.5x 108cfu/mL。病毒株作为热灭活的储物从ZeptoMetrix Corp.购买并且以1x 10^6 U/mL或10^6基因组/mL测试。在生长培养基中培养原生动物,通过光学显微镜视觉计数并且以1x 10^6细胞/mL测试。测试一式三份进行。测试的生物和Xpert TV测定结果在表9中列出。

[0229] 一种生物,口腔毛滴虫(Trichomonas tenax),利用Xpert TV测定报告了TV检测结果。该该研究的条件下,Xpert TV测定的分析特异性为98%。

[0230] 表9:分析特异性表

[0231]

物种	株 ID	测试结果
鲁氏不动杆菌 (*Acinetobacter Iwoffi*)	ATCC 17925	未检测到 TV
以色列放线菌 (*Actinomyces israelii*)	ATCC 12102	未检测到 TV
阴道阿托波氏菌 (*Atopobium vaginae*)	ATCC BAA-55	未检测到 TV
脆弱拟杆菌 (*Bacteroides fragilis*)	ATCC 25285	未检测到 TV
解尿素拟杆菌 (*Bacteroides ureolyticus*)	ATCC 33387	未检测到 TV
青春双岐杆菌 (*Bifidobacterium adolescentis*)	ATCC 15703	未检测到 TV
空肠弯曲杆菌 (*Campylobacter jejuni*)	ATCC 33560	未检测到 TV
白念珠菌 (*Candida albicans*)	ATCC 14053	未检测到 TV
光滑念珠菌 (*Candida glabrata*)	ATCC 90030	未检测到 TV
近平滑念珠菌 (*Candida parapsilosis*)	ATCC 90018	未检测到 TV

[0232]

热带念珠菌*(Candida tropicalis)*	ATCC 13803	未检测到 TV
沙眼衣原体*(Chlamydia trachomatis)*	ATCC VR-885	未检测到 TV
艰难梭状芽胞杆菌*(Clostridium difficile)*	ATCC 43594	未检测到 TV
产气荚膜梭状芽胞杆菌*(Clostridium perfringens)*	ATCC 13124	未检测到 TV
生殖器棒状杆菌*(Corynebacterium genitalium)*	ATCC 33031	未检测到 TV
新型隐球菌*(Cryptococcus neoformans)*	ATCC 32045	未检测到 TV
巨细胞病毒*(Cytomegalovirus)*	ZeptoMetrix 0810003CF	未检测到 TV
产气肠杆菌*(Enterobacter aerogenes)*	ATCC 51697	未检测到 TV
粪肠球菌*(Enterococcus feacalis)*	ATCC 19433	未检测到 TV
大肠杆菌*(Escherichia coli)*	ATCC 24922	未检测到 TV
核粒梭形杆菌*(Fusobacterium nucleatum)*	ATCC 31647	未检测到 TV
阴道加德菌*(Gardnerella vaginalis)*	ATCC 49145	未检测到 TV
杜克雷氏嗜血杆菌*(Haemophilus ducreyi)*	ATCC 33940	未检测到 TV
I 型单纯疱疹病毒*(Herpes simplex virus* I*)*	ZeptoMetrix 0810005CF	未检测到 TV
II 型单纯疱疹病毒*(Herpes simplex virus* II*)*	ZeptoMetrix 0810006CF	未检测到 TV
HIV-1	ZeptoMetrix 0801032CF	未检测到 TV
HPV 16(Caski)	ZeptoMetrix 0810232	未检测到 TV
产酸克雷伯菌*(Klebsiella oxytoca)*	ATCC 43165	未检测到 TV

[0233]

嗜酸乳杆菌 *(Lactobacillus acidophilus)*	ATCC 314	未检测到 TV
詹氏乳杆菌 *(Lactobacillus jensenii)*	ATCC 25258	未检测到 TV
阴道乳杆菌 *(Lactobacillus vaginalis)*	ATCC 49540	未检测到 TV
单核细胞增多性李斯特氏菌 *(Listeria monocytogenes)*	ATCC 15313	未检测到 TV
柯氏动弯杆菌 *(Mobiluncus curtisii)*	ATCC 35241	未检测到 TV
人型支原体*(Mycoplasma hominis)*	ATCC 23114	未检测到 TV
淋病奈瑟氏菌 *(Neisseria gonorrhoeae)*	ATCC 35201	未检测到 TV
人五毛滴虫 *(Pentatrichomonas hominis)*	ATCC 30000	未检测到 TV
厌氧消化链球菌 *(Peptostreptococcus anaerobius)*	ATCC 49031	未检测到 TV
二路普雷沃氏菌*(Prevotella bivia)*	ATCC 29303	未检测到 TV
痤疮丙酸杆菌(*Propionibacterium acnes)*	ATCC 6919	未检测到 TV
奇异变形杆菌*(Proteus mirabilis)*	ATCC 25933	未检测到 TV
绿脓假单胞菌 *(Pseudomonas aeruginosa)*	ATCC 35554	未检测到 TV
金黄色酿脓葡萄球菌 *(Staphylococcus aureus)*	ATCC 700699	未检测到 TV
表皮葡萄球菌 *(Staphylococcus epidermidis)*	ATCC 14990	未检测到 TV
无乳链球菌 *(Streptococcus agalactiae)*	ATCC 13813	未检测到 TV

[0234]

酿脓链球菌 *(Streptococcus pyogenes)*	ATCC 19615	未检测到 TV
口腔毛滴虫*(Trichomonas tenax)*	ATCC 30207	检测到 TV
解脲支原体 *(Ureaplasma urealyticum)*	ATCC 27618	未检测到 TV

[0235] 5.7.实施例7:干扰物质

[0236] 在非临床研究中,利用Xpert TV测定评价可能在泌尿生殖道内并且存在于子宫颈和阴道拭子中或首次尿样品中的可能干扰内源和外源物质。

[0237] 将物质分别稀释在汇集的阴性阴道拭子基质和汇集的阴性男性尿基质。以不大于对于各个样品类型的检测极限的三倍在加有阴道毛滴虫细胞的相同基质中测试物质。利用Xpert TV测定测试每组阴性和阳性样品的八个重复并且与在没有潜在的干扰物质加入的情况下在相同样品的对照中获得结果相比较。物质和测试浓度在表10和表11中列出。

[0238] 在研究条件下,在利用稀释于阴性尿基质中的物质的测试中,未报告无效结果;如预期的,所有测试报告未检测到TV。在利用稀释在阳性尿基质中的0.75%v/v的血液和1.8mg/mL的阿奇霉素的测试中观察测定干扰。对于利用0.5%v/v的血液和1mg/mL的阿奇霉素的测试未报道假阴性结果。

[0239] 在研究的条件下,在利用稀释在汇集的阴性拭子基质的测试中,未报告无效结果;如预期的,所有测试报告未检测到TV。

[0240] 在稀释在汇集的阳性拭子基质中的物质的测试中,未报道假阴性TV结果。如预期的,利用所有物质的测试报告检测到TV。

[0241] 表10:尿样品中的可能的干扰物质

[0242]

类型/物质	活性成分	测试浓度
血液	血液	0.3% v/v
精液	精液	5.0% v/v
粘液	粘蛋白	0.8% w/v
镇痛剂和抗生素	乙酰水杨酸 500mg	8 mg/mL
	醋氨酚	3.2 mg/mL

[0243]

	阿奇霉素	1.0 mg/mL
	强力霉素	0.5 mg/mL
OTC 除臭剂和粉末	PEG-20；PEG-32；PEG-20 硬脂酸酯	0.25% w/v
	Nanoxynol-9	0.25% w/v
白蛋白	BSA	10 mg/mL
葡萄糖	葡萄糖	10 mg/mL
胆红素	胆红素	1 mg/mL
酸性尿(pH 4.0)	尿+N-乙酰基-L-半胱氨酸	pH 4.0
碱性尿(pH 9.0)	尿+柠檬酸铵	pH 9.0
白细胞	白细胞	10^6 个细胞/mL
阴道内激素	孕酮；雌二醇	7 mg/mL 孕酮 +0.07 mg/mL β雌二醇

[0244] 表11：拭子样品中的可能干扰物质

[0245]

类型/物质	活性成分	测试浓度
血液	血液	1.0 % v/v
精液	精液	5.0% v/v
粘液	粘蛋白	0.8% w/v
非处方(OTC)阴道产品；避孕药；阴道治疗	苯坐卡因 5% 雷琐酚 2%	0.25% w/v
	克霉唑 2%	0.25% w/v
	硝酸咪康唑 2%	0.25% w/v
	噻康唑	0.25% w/v
	5% w/w 阿昔洛维	0.25% w/v
	甘油，丙二醇	0.25% w/v
	甘油；卡波姆	0.12% w/v
	甘油；羟乙基纤维素	0.25% w/v

[0246]

	白毛茛 3X HPUS 木馏油 12X HPUS	0.25% w/v
	聚维酮碘 10%	0.25% w/v
	壬苯聚醇-9 12.5%	0.25% w/v
痔疮膏	甘油 14%; 普莫卡因 HCl 1%	0.25% w/v
白细胞	白细胞	10^6 个细胞/mL
阴道内激素	孕酮；雌二醇	7 mg/mL 孕酮 +0.07 mg/mL β雌二醇

[0247] 5.8.实施例8:携带污染

[0248] 研究由在缓冲液样品中的高(10^6个细胞/mL)TV阳性阴道拭子汇集物后立即在相同GeneXpert模块内处理的缓冲液样品中的TV-阴性阴道拭子汇集物的重复测试组成。研究由在相同GeneXpert模块内处理的在缓冲液样品中的TV-阴性阴道拭子汇集物仅接着缓冲液样品中的高(10^6个细胞/mL)TV阳性阴道拭子汇集物的组成。该测试方案在两个GeneXpert模块上再重复20次,达总共82次运行,得到40个阳性和42个阴性样品。所有40个阳性样品正确地报告为检测到TV并且所有42个阴性样品正确地报告为未检测到TV。

[0249] 5.9.实施例9:测试以用于检测TV的替代引物和探针

[0250] 为了开发本文所述的TV测定,在测定中对用于检测TV 40S核糖体蛋白(Tv40Srp)基因的四种不同正向引物,两种不同反向引物,和两种不同探针测试灵敏度和特异性(例如,与其他物种的交叉反应性)。表12显示测试的引物和探针。

[0251] 表12:替代的引物和探针序列

[0252]

寡聚物名称	SEQ ID NO	序列	结果
TV 正向	1	GTAACAACCTTGGAGTTCTTCTTAAG	最终设计

[0253]

TV 反向	2	ACATCAATCTACAAGACACCACTTGA	最终设计
TV 探针	3	F1-AGTTTGGCTGCTTAGCTTCGAC-Q1	最终设计
TV 正向 ALT1	6	GAGTTCTTCTTAAGCTGAACAC	FW 设计 2
TV 正向 ALT2	7	GAGTTCTTCTTGAGCTGAACAC	FW 设计 2，在位置 12 具有不同 SNP
TV 正向 ALT3	8	AACAACCTTGGAGTTCTTCTTA	FW 设计 3
TV 反向 ALT1	9	ATCTACAAGACACCACTTGA	RV 设计 2
TV 探针 ALT1	10	F1-AGTTTGGCTGCTTGGCTTCGAC-Q1	PR 设计 2，在位置 14 具有不同 SNP

[0254] 发现，TV正向ALT1与人五毛滴虫(Pentatric homonas hominis)(Pth)交叉反应，所述人五毛滴虫(Pth)是在人肠道中发现的另一密切相关的毛滴虫。当TV正向ALT1用于利用1000拷贝的TV的测定和利用500,000拷贝Pth的测定时，检测到TV具有30.7的Ct以及检测到Pth具有26.3的Ct。TV正向ALT2没有最终设计灵敏，以31.5的更高Ct值检测TV。类似地，TV正向ALT3没有最终设计灵敏，也以更高Ct值检测TV。TV反向ALT1还导致较不灵敏的测定，以较高Ct值检测到TV。最后，TV探针ALT1不如最终设计灵敏和一致。

[0255] 像表明每个个体公开、专利、专利申请或其他文献各自为了所有目的通过引用结合一样，为了所有目的，本申请中引用的所有公开、专利、专利申请和其他文件在此通过引用以其整体结合。

[0256] 在已经说明和描述了各种具体实施方案的同时，将理解在不偏离本发明的精神和范围的情况下可以进行改变。

[0257] 某些序列的表

[0258]

SEQ ID NO	描述	序列
4	阴道毛滴虫 40S 核糖体蛋白 (Tv40Srp) 基因	ggccggcctt tctgatgggt aagtctaaag cttgcggtcg tctcgctgct cgtaaactcc gtcttgcaca caagtccaac ttgtgggctt ccaacgcata ccgccgttcc cttggtacat caatctacaa gacaccactt gagggtacat caatggcatc tggcatcgtc gtcggcaagg tcgctgtcga agccaagcag ccaaactctg ctattcgtaa agctgtccgt gttcagctta agaagaactc taaggttgtc acagctttcg ttccacgcga tggttccctc cgtcttattg atgataacga ccgtgttctt attgccggta tgggtcgttc tggccgttct gtcggtgacc ttccaggatg ccgtttcaaa gttatcaagg tcgctggttt ctccctcctt gctctttggc tcggcaagaa ggagaagccg cgcagctaaa taaatactct tgggtttacc ggtaaataaa aacatatatt acgaaataca aatattat
5	Tv40Srp 扩增子	ACATCAATCT ACAAGACACC ACTTGAAGGC ACCTCAATGG CCTCCGGCAT TGTTGTCGGC AAAGTTGCTG TCGAAGCTAA GCAGCCAAAC TCCGCTATTC GTAAAGCAGT TCGTGTTCAG CTTAAGAAGA ACTCTAAAGT TGTTAC

序列表

<110> 西菲伊德公司

<120> 检测阴道毛滴虫的方法

<130> CEPHD-33923/WO-1/PRI

<160> 10

<170> PatentIn version 3.5

<210> 1

<211> 26

<212> DNA

<213> 人工序列

<220>

<223> 合成的

<400> 1

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<210> 2

<211> 26

<212> DNA

<213> 人工序列

<220>

<223> 合成的

<400> 2

acatcaatct acaagacacc acttga 26

<210> 3

<211> 22

<212> DNA

<213> 人工序列

<220>

<223> 合成的

<400> 3

agtttggctg cttagcttcg ac 22

<210> 4

<211> 508

<212> DNA

<213> 阴道毛滴虫(Trichomonas vaginalis)

<400> 4

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gtcttgcaca caagtccaac ttgtgggctt ccaacgcata ccgccgttcc cttggtacat 120

caatctacaa gacaccactt gagggtacat caatggcatc tggcatcgtc gtcggcaagg 180

tcgctgtcga agccaagcag ccaaactctg ctattcgtaa agctgtccgt gttcagctta 240
agaagaactc taaggttgtc acagctttcg ttccacgcga tggttccctc cgtcttattg 300
atgataacga ccgtgttctt attgccggta tgggtcgttc tggccgttct gtcggtgacc 360
ttccaggatg ccgtttcaaa gttatcaagg tcgctggttt ctccctcctt gctctttggc 420
tcggcaagaa ggagaagccg cgcagctaaa taaatactct tgggtttacc ggtaaataaa 480
aacatatatt acgaaataca aatattat 508

<210> 5
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