

(19) **DANMARK**

(10) **DK/EP 4166678 T3**



(12) **Oversættelse af
europæisk patentskrift**

Patent- og
Varemærkestyrelsen

-
- (51) Int.Cl.: **C 12 Q 1/689 (2018.01)**
- (45) Oversættelsen bekendtgjort den: **2024-08-12**
- (80) Dato for Den Europæiske Patentmyndigheds bekendtgørelse om meddelelse af patentet: **2024-05-29**
- (86) Europæisk ansøgning nr.: **22200785.8**
- (86) Europæisk indleveringsdag: **2016-04-20**
- (87) Den europæiske ansøgnings publiceringsdag: **2023-04-19**
- (30) Prioritet: **2015-04-24 US 201562152754 P** **2016-01-15 US 201662279220 P**
- (62) Stamansøgningsnr: **16783760.8**
- (84) Designerede stater: **AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT RO RS SE SI SK SM TR**
- (73) Patenthaver: **Becton, Dickinson and Company, 1 Becton Drive, Franklin Lakes, NJ 07417, USA**
- (72) Opfinder: **PAQUETTE, Nancy, , San Diego, CA 92121, USA**
TREMBLAY, Marie-Helene, , San Diego, CA 92121, USA
TREMBLAY, Simon, , San Diego, CA 92121, USA
THERRIEN, Roseline, , San Diego, CA 92121, USA
FORTIN, Marie-Christine, , San Diego, CA 92121, USA
BELLEY-MONTFORT, Lucile, , San Diego, CA 92121, USA
CANTIN, Dany, , San Diego, CA 92121, USA
ROGER-DALBERT, Celine, , San Diego, CA 92121, USA
- (74) Fuldmægtig i Danmark: **Budde Schou A/S, Dronningens Tværgade 30, 1302 København K, Danmark**
- (54) Benævnelse: **MULTIPLEX-DETEKTION AF VULVOVAGINAL CANDIASIS, TRICHOMONIASIS OG BAKTERIEL VAGINOSIS**
- (56) Fremdragne publikationer:
WO-A1-2010/083274
MAHMOUDI RAD MAHNAZ ET AL: "Identification of Candida Species Associated with Vulvovaginal Candidiasis by Multiplex PCR", INFECTIOUS DISEASES IN OBSTETRICS AND GYNECOLOGY, vol. 2012, 1 January 2012 (2012-01-01), pages 1-5, XP093028127, US ISSN: 1064-7449, DOI: 10.1155/2012/872169 Retrieved from the Internet: URL:https://downloads.hindawi.com/journals /idog/2012/872169.pdf>
SAVOCHKINA YU A: "THE DEVELOPMENT OF TECHNIQUE OF DIAGNOSTIC OF VUULVOVAGINAL CANDIDIASIS BASED ON QUANTITATIVE MULTIPLEX POLYMERASE CHAIN REACTION", KLIN LAB DIAGN ., vol. 60, no. 4, 1 April 2015 (2015-04-01), pages 56-62, XP093028125, Retrieved from the Internet: URL:http://www.ncbi.nlm.nih.gov/pubmed/261 89293>

DESCRIPTION

Description

SEQUENCE LISTING

[0001] The present application is being filed along with a Sequence Listing in electronic format. The Sequence Listing is provided as a file entitled SEQLISTING_GENOM. 143WO.TXT, created April 20, 2016, which is 36Kb in size.

BACKGROUND

Field

[0002] The present disclosure relates to methods and compositions for the detection of vaginal disorders, for example vulvovaginal candidiasis (VVC), trichomoniasis, and bacterial vaginosis (BV). More specifically, the present disclosure relates to the detection of VVC-associated *Candida* species, *Trichomonas vaginalis* (*T. vaginalis*) and a plurality of BV-related bacteria in biological examples, such as vaginal swab samples from women with clinical symptoms of vaginitis and/or vaginosis, by nucleic acid-based test methods.

Description of the Related Art

[0003] *Candida* is a genus of yeast and is the most common cause of fungal infections worldwide. Many *Candida* species are found as a harmless commensal, part of a normal flora of a host and can be endosymbionts of hosts including humans. However, in the case of an imbalance or an immunocompromisation of a host, *Candida* is known to invade and cause disease. Some *Candida* species, such as *C. albicans*, *C. dubliniensis*, *C. tropicalis*, *C. parapsilosis*, *C. krusei*, and *C. glabrata*, are known to be associated with vulvovaginal candidiasis (VVC). *Trichomonas vaginalis* is an anaerobic, flagellated protozoan parasite, which is the causative agent of trichomoniasis. Bacterial vaginosis (BV) is an infection of vagina caused by alteration in normal balance of bacteria in the vagina.

[0004] To date, standard tests for diagnosing VVC, trichomoniasis, and BV rely on multiple subjective methods that are interpretive methods. These tests typically involve microscopic examination of wet mount preparation of patient samples (e.g., vaginal discharge), including observation of fungal hyphae or budding yeast for VVC and observation of motile trichomonads for trichomoniasis. The Nugent Score and Amsel's criteria are the most commonly used tests for diagnosing BV. The Nugent Score is a Gram stain scoring system by calculated by assessing for the presence of large Gram-positive rods (*Lactobacillus* morphotypes), small Gram-variable rods (*Gardnerella vaginalis* morphotypes), and curved Gram-variable rods (*Mobiluncus* spp. morphotypes). Amsel's criteria requires at least three of the four following criteria to be present for a confirmed diagnosis: (1) thin, white, yellow, homogeneous discharge, (2) clue cells on microscopy, (3) pH of vaginal fluid > 4.5, and (4) release of a fishy odor on adding alkali-10% potassium hydroxide (KOH) solution. These standard tests can be expensive, labor intensive and time consuming, for

example, *Candida* needs to be cultured for 48 hours on chromogenic media or up to 7 days on less selective media before a diagnose can be made. Mahmoudi Rad et al. (Infectious diseases in obstetrics and gynecology 2012) discloses a multiplex PCR for detecting vulvovaginal candidiasis by targeting the 18S rDNA and 28S rDNA of different *Candida* species. WO 2010/083274 teaches a primer to detect a region of the *Trichomonas vaginalis* AP65-1 gene.

[0005] However, there is still a need for developing more efficient and faster methods for detecting vulvovaginal candidiasis, trichomoniasis and bacterial vaginosis, for example a method allowing detecting of the three vaginal disorders in a single assay, in order to effectively deliver proper treatments to patients.

SUMMARY

[0006] The present invention is defined by the enclosed claims. The invention relates to a method to detect vulvovaginal candidiasis (VVC)-associated *Candida* species and *Trichomonas vaginalis* in a biological sample, wherein the VVC-associated *Candida* species comprises *Candida glabrata*, *Candida albicans*, *Candida tropicalis*, *C. dubliniensis*, *C. parapsilosis*, *Candida krusei*, comprising:

contacting said biological sample with a plurality of pairs of primers, wherein the plurality of pairs of primer comprises:

at least one pair of primers capable of hybridizing to the *tefl* gene of *Candida glabrata*, wherein each primer in said at least one pair of primers comprises a sequence of SEQ ID NO: 20 or SEQ ID NO: 21 or a sequence having one mismatch or two mismatches relative to SEQ ID NO: 20 or SEQ ID NO: 21;

a plurality of primers capable of hybridizing to the *tefl* gene of at least one of *Candida albicans*, *Candida tropicalis*, *C. dubliniensis*, and *C. parapsilosis*, wherein each primer in said at least one pair of primers comprises a sequence of SEQ ID NO: 23, SEQ ID NO: 24, or SEQ ID NO: 25, or a sequence having one mismatch or two mismatches relative to SEQ ID NO: 23, SEQ ID NO: 24, or SEQ ID NO: 25;

at least one pair of primers capable of hybridizing to the *tefl* gene of *Candida krusei*, wherein each primer in said at least one pair of primers comprises a sequence of SEQ ID NO: 27 or SEQ ID NO: 28, or a sequence having one mismatch or two mismatches relative to SEQ ID NO: 27 or SEQ ID NO: 28; and

at least one pair of primers capable of hybridizing to the AP-65 gene of *Trichomonas vaginalis*, wherein each primer in said at least one pair of primers comprises a sequence of SEQ ID NO: 17 or SEQ ID NO: 18, or a sequence having one mismatch or two mismatches relative to SEQ ID NO: 17 or SEQ ID NO: 18; and

generating amplicons of the *tefl* sequences of the *Candida* species and/or amplicons of the AP-65 gene sequence of *Trichomonas vaginalis* from said biological sample, if said sample comprises one or more of the VVC-associated *Candida* species and/or *Trichomonas vaginalis*;

determining the presence or amount of one or more amplified products as an indication of the presence of VVC-associated *Candida* species and *Trichomonas vaginalis* in said biological sample.

[0007] The present invention also relates to a composition for the detection of vulvovaginal candidiasis (VVC)-associated *Candida* species and *Trichomonas vaginalis*, wherein the VVC-associated *Candida* species comprises *Candida glabrata*, *Candida albicans*, *Candida tropicalis*, *C. dubliniensis*, *C. parapsilosis*, *Candida krusei*, comprising:

at least one pair of primers capable of hybridizing to the *tefl* gene of *Candida glabrata*, wherein each primer

in said at least one pair of primers comprises a sequence of SEQ ID NO: 20 or SEQ ID NO: 21 or a sequence having one mismatch or two mismatches relative to SEQ ID NO: 20 or SEQ ID NO: 21;

a plurality of primers capable of hybridizing to the *tefl* gene of at least one of *Candida albicans*, *Candida tropicalis*, *C. dubliniensis*, and *C. parapsilosis*, wherein each primer in said at least one pair of primers comprises a sequence of SEQ ID NO: 23, SEQ ID NO: 24, or SEQ ID NO: 25, or a sequence having one mismatch or two mismatches relative to SEQ ID NO: 23, SEQ ID NO: 24, or SEQ ID NO: 25;

at least one pair of primers capable of hybridizing to the *tefl* gene of *Candida krusei*, wherein each primer in said at least one pair of primers comprises a sequence of SEQ ID NO: 27 or SEQ ID NO: 28, or a sequence having one mismatch or two mismatches relative to SEQ ID NO: 27 or SEQ ID NO: 28; and

at least one pair of primers capable of hybridizing to the AP-65 gene of *Trichomonas vaginalis*, wherein each primer in said at least one pair of primers comprises a sequence of SEQ ID NO: 17 or SEQ ID NO: 18, or a sequence having one mismatch or two mismatches relative to SEQ ID NO: 17 or SEQ ID NO: 18.

[0008] The present invention further relates to a reaction mixture comprising the composition of the present invention, wherein the reaction mixture further comprises template DNA, DNA polymerase, deoxynucleotides (dNTPs), buffer solution, bivalent cations, monovalent cations, or any combination thereof.

DETAILED DESCRIPTION

[0009] The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described in any way. In the event that one or more of the cited literature and similar materials defines or uses a term in such a way that it contradicts that term's definition in this application, this application controls. While the present teachings are described in conjunction with various embodiments, it is not intended that the present teachings be limited to such embodiments. On the contrary, the present teachings encompass various alternatives, modifications, and equivalents, as will be appreciated by those of skill in the art.

[0010] Provided herein are methods and compositions for the detection of vulvovaginal candidiasis (VVC) and trichomoniasis. For example, primers and probes that can bind to specific genes of *Candida* species associated with VVC and *Trichomonas vaginalis* (*T. vaginalis*) are provided to determine the presence or absence of the VVC-associated *Candida* species and *T. vaginalis* in a sample, such as a biological sample. In some embodiments, multiplex nucleic acid amplification can be performed to allow the detection of VVC-associated *Candida* species and *T. vaginalis* in a single assay.

Definitions

[0011] As used herein, a "nucleic acid" refers to a polymeric compound comprising nucleosides or nucleoside analogs which have nitrogenous heterocyclic bases, or base analogs, linked together by nucleic acid backbone linkages (e.g., phosphodiester bonds) to form a polynucleotide. Non-limiting examples of nucleic acid include RNA, DNA, and analogs thereof. The nucleic acid backbone can include a variety of linkages, for example, one or more of sugar-phosphodiester linkages, peptide-nucleic acid bonds, phosphorothioate or methylphosphonate linkages or mixtures of such linkages in a single oligonucleotide. Sugar moieties in the nucleic acid can be either ribose or deoxyribose, or similar compounds with known substitutions. Conventional nitrogenous bases (e.g., A, G, C, T, U), known base analogs (e.g., inosine),

derivatives of purine or pyrimidine bases and "abasic" residues (i.e., no nitrogenous base for one or more backbone positions) are included in the term nucleic acid. That is, a nucleic acid can include only conventional sugars, bases and linkages found in RNA and DNA, or include both conventional components and substitutions (e.g., conventional bases and analogs linked via a methoxy backbone, or conventional bases and one or more base analogs linked via an RNA or DNA backbone).

[0012] As used herein, the term "isolate nucleic acids" refers to the purification of nucleic acids from one or more cellular components. One of skill in the art will appreciate that samples processed to "isolate nucleic acids" therefrom can include components and impurities other than nucleic acids. Samples that comprise isolated nucleic acids can be prepared from specimens using any acceptable method known in the art. For example, cells can be lysed using known lysis agents, and nucleic acids can be purified or partially purified from other cellular components. Suitable reagents and protocols for DNA and RNA extractions can be found in, for example, U.S. Patent Application Publication Nos. US 2010-0009351, and US 2009-0131650, respectively. In nucleic acid testing (e.g., amplification and hybridization methods discussed in further detail below), the extracted nucleic acid solution can be added directly to a reagents (e.g., either in liquid, bound to a substrate, in lyophilized form, or the like, as discussed in further detail below), required to perform a test according to the embodiments disclosed herein.

[0013] As used herein, "template" refers to all or part of a polynucleotide containing at least one target nucleotide sequence.

[0014] As used herein, a "primer" refers to a polynucleotide that can serve to initiate a nucleic acid chain extension reaction. The length of a primer can vary, for example, from about 5 to about 100 nucleotides, from about 10 to about 50 nucleotides, from about 15 to about 40 nucleotides, or from about 20 to about 30 nucleotides. The length of a primer can be about 10 nucleotides, about 20 nucleotides, about 25 nucleotides, about 30 nucleotides, about 35 nucleotides, about 40 nucleotides, about 50 nucleotides, about 75 nucleotides, about 100 nucleotides, or a range between any two of these values. In some embodiments, the primer has a length of 10 to about 50 nucleotides, *i.e.*, 10, 11, 12, 13, 14, 15, 16,, 17, 18, 19, 20, 21, 22, 23, 24, 25 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, or more nucleotides. In some embodiments, the primer has a length of 18 to 32 nucleotides.

[0015] As used herein, a "probe" refers to a polynucleotide that can hybridize (e.g., specifically) to a target sequence in a nucleic acid, under conditions that allow hybridization, thereby allowing detection of the target sequence or amplified nucleic acid. A probe's "target" generally refers to a sequence within or a subset of an amplified nucleic acid sequence which hybridizes specifically to at least a portion of a probe oligomer by standard hydrogen bonding (i.e., base pairing). A probe may comprise target-specific sequences and other sequences that contribute to three-dimensional conformation of the probe. Sequences are "sufficiently complementary" if they allow stable hybridization in appropriate hybridization conditions of a probe oligomer to a target sequence that is not completely complementary to the probe's target-specific sequence. The length of a probe can vary, for example, from about 5 to about 100 nucleotides, from about 10 to about 50 nucleotides, from about 15 to about 40 nucleotides, or from about 20 to about 30 nucleotides. The length of a probe can be about 10 nucleotides, about 20 nucleotides, about 25 nucleotides, about 30 nucleotides, about 35 nucleotides, about 40 nucleotides, about 50 nucleotides, about 100 nucleotides, or a range between any two of these values. In some embodiments, the probe has a length of 10 to about 50 nucleotides. For example, the primers and or probes can be at least 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, or more nucleotides. In some embodiments, the probe can be non-sequence specific.

[0016] Preferably, the primers and/or probes can be between 8 and 45 nucleotides in length. For example, the primers and or probes can be at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25,

26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, or more nucleotides in length. The primer and probe can be modified to contain additional nucleotides at the 5' or the 3' terminus, or both. One of skill in the art will appreciate that additional bases to the 3' terminus of amplification primers (not necessarily probes) are generally complementary to the template sequence. The primer and probe sequences can also be modified to remove nucleotides at the 5' or the 3' terminus. One of skill in the art will appreciate that in order to function for amplification, the primers or probes will be of a minimum length and annealing temperature as disclosed herein.

[0017] Primers and probes can bind to their targets at an annealing temperature, which is a temperature less than the melting temperature (T_m). As used herein, " T_m " and "melting temperature" are interchangeable terms which refer to the temperature at which 50% of a population of double-stranded polynucleotide molecules becomes dissociated into single strands. The formulae for calculating the T_m of polynucleotides are well known in the art. For example, the T_m may be calculated by the following equation: $T_m = 69.3 + 0.41 \times (G+C)\% - 50/L$, wherein L is the length of the probe in nucleotides. The T_m of a hybrid polynucleotide may also be estimated using a formula adopted from hybridization assays in 1 M salt, and commonly used for calculating T_m for PCR primers: $[(\text{number of A+T}) \times 2^\circ\text{C} + (\text{number of G+C}) \times 4^\circ\text{C}]$. See, e.g., C. R. Newton et al. PCR, 2nd ed., Springer-Verlag (New York: 1997), p.24. Other more sophisticated computations exist in the art, which take structural as well as sequence characteristics into account for the calculation of T_m . The melting temperature of an oligonucleotide can depend on complementarity between the oligonucleotide primer or probe and the binding sequence, and on salt conditions. In some embodiments, an oligonucleotide primer or probe provided herein has a T_m of less than about 90°C in 50mM KCl, 10 mM Tris-HCl buffer, for example about 89°C , 88, 87, 86, 85, 84, 83, 82, 81, 80, 79, 78, 77, 76, 75, 74, 73, 72, 71, 70, 69, 68, 67, 66, 65, 64, 63, 62, 61, 60, 59, 58, 57, 56, 55, 54, 53, 52, 50, 49, 48, 47, 46, 45, 44, 43, 42, 41, 40, 39°C , or less, including ranges between any two of the listed values.

[0018] In some embodiments, the primers disclosed herein, e.g., amplification primers, can be provided as an amplification primer pair, e.g., comprising a forward primer and a reverse primer (first amplification primer and second amplification primer). Preferably, the forward and reverse primers have T_m 's that do not differ by more than 10°C , e.g., that differ by less than 10°C , less than 9°C , less than 8°C , less than 7°C , less than 6°C , less than 5°C , less than 4°C , less than 3°C , less than 2°C , or less than 1°C .

[0019] The primer and probe sequences may be modified by having nucleotide substitutions (relative to the target sequence) within the oligonucleotide sequence, provided that the oligonucleotide contains enough complementarity to hybridize specifically to the target nucleic acid sequence. In this manner, at least 1, 2, 3, 4, or up to about 5 nucleotides can be substituted. As used herein, the term "complementary" refers to sequence complementarity between regions of two polynucleotide strands or between two regions of the same polynucleotide strand. A first region of a polynucleotide is complementary to a second region of the same or a different polynucleotide if, when the two regions are arranged in an antiparallel fashion, at least one nucleotide of the first region is capable of base pairing with a base of the second region. Therefore, it is not required for two complementary polynucleotides to base pair at every nucleotide position. "Fully complementary" refers to a first polynucleotide that is 100% or "fully" complementary to a second polynucleotide and thus forms a base pair at every nucleotide position. "Partially complementary" also refers to a first polynucleotide that is not 100% complementary (e.g., 90%, or 80% or 70% complementary) and contains mismatched nucleotides at one or more nucleotide positions. In some embodiments, an oligonucleotide includes a universal base.

[0020] As used herein, an "exogenous nucleotide sequence" refers to a sequence introduced by primers or probes used for amplification, such that amplification products will contain exogenous nucleotide sequence and target nucleotide sequence in an arrangement not found in the original template from which the target

nucleotide sequence was copied.

[0021] As used herein, "sequence identity" or "percent identical" as applied to nucleic acid molecules is the percentage of nucleic acid residues in a candidate nucleic acid molecule sequence that are identical with a subject nucleic acid molecule sequence, after aligning the sequences to achieve the maximum percent identity, and not considering any nucleic acid residue substitutions as part of the sequence identity. Nucleic acid sequence identity can be determined using any method known in the art, for example CLUSTALW, T-COFFEE, BLASTN.

[0022] As used herein, the term "sufficiently complementary" refers to a contiguous nucleic acid base sequence that is capable of hybridizing to another base sequence by hydrogen bonding between a series of complementary bases. Complementary base sequences can be complementary at each position in the oligomer sequence by using standard base pairing (e.g., G:C, A:T or A:U) or can contain one or more residues that are not complementary (including abasic positions), but in which the entire complementary base sequence is capable of specifically hybridizing with another base sequence in appropriate hybridization conditions. Contiguous bases can be at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 99%, or 100% complementary to a sequence to which an oligomer is intended to hybridize. Substantially complementary sequences can refer to sequences ranging in percent identity from 100, 99, 98, 97, 96, 95, 94, 93, 92, 91, 90, 89, 88, 87, 86, 85, 84, 83, 82, 81, 80, 75, 70 or less, or any number in between, compared to the reference sequence. A skilled artisan can readily choose appropriate hybridization conditions which can be predicted based on base sequence composition, or be determined by using routine testing (see e.g., Green and Sambrook, *Molecular Cloning, A Laboratory Manual*, 4th ed. (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 2012)).

[0023] As used herein, the term "multiplex PCR" refers to a type of PCR where more than one set of primers is included in a reaction allowing one single target, or two or more different targets to be amplified in a single reaction tube. The multiplex PCR can be, for example, a real-time PCR.

Oligonucleotides and compositions containing thereof

[0024] As described herein, nucleic acid amplifications can be performed to determine the presence, absence and/or level of *Candida* species and *T. vaginalis* in a sample. Some *Candida* species are known to be associated with VVC, including but not limited to *C. albicans*, *C. dubliniensis*, *C. tropicalis*, *C. parapsilosis*, *C. krusei*, and *C. glabrata*. In some embodiments, the presence, absence and/or level of VVC-associated *Candida* species and *T. vaginalis* is determined by detecting one or more target genes of each of the target organisms using methods known in the art, such as DNA amplifications. In some embodiments, a multiplex PCR can be performed to detect the presence, absence or level for each of the target *Candida* species and *T. vaginalis*. In some embodiments, a multiplex PCR is performed to detect the presence, absence and/or level for each of target VVC-associated *Candida* species and *T. vaginalis*. In some embodiments, the WC-associated *Candida* species are *C. albicans*, *C. dubliniensis*, *C. tropicalis*, *C. parapsilosis*, *C. krusei*, and *C. glabrata*.

[0025] Each of the target VVC-associated *Candida* species and *T. vaginalis* can be detected using separate channels in DNA amplifications. In some cases, it can be desirable to use a single fluorescence channel for detecting the presence, absence, and/or level of two or more of the VVC-associated *Candida* species (including *Candida glabrata*, *Candida albicans*, *Candida tropicalis*, *C. dubliniensis*, *C. parapsilosis*, *Candida krusei*) and *T. vaginalis*.

[0026] Oligonucleotides (for example amplification primers and probes) that are capable of specifically

hybridizing (e.g., under standard nucleic acid amplification conditions, e.g., standard PCR conditions, and/or stringent hybridization conditions) to a target gene region, or complement thereof, in VVC-associated *Candida* species and *T. vaginalis* are provided. Amplification of the target gene region of an organism in a sample (e.g., a vaginal swab sample) can, in some embodiments, be indicative of the presence, absence, and/or level of the organism in the sample.

[0027] Protein AP65 is a 65KDa protein by the parasitic organism *T. vaginalis*, which upon iron repletion acts as a surface adhesin that mediates cytoadherence of the parasite to vaginal epithelial cells. In some embodiments disclosed herein, oligonucleotides (e.g., amplification primers and probes) that are capable of specifically hybridizing (e.g., under standard nucleic acid amplification conditions, e.g., standard PCR conditions, and/or stringent hybridization conditions) to a gene region encoding AP65 in *T. vaginalis* are provided. In some embodiments, AP65 gene is used as the target gene for the DNA amplification to detect the presence, absence and/or level of *T. vaginalis* in the sample. Examples of oligonucleotides capable of specifically hybridizing to the AP65 gene region in *T. vaginalis* include, but are not limited, SEQ ID NOs: 17-19 as provided in Table 1 and sequences having one mismatch or two mismatches relative to a sequence selected from the group consisting of SEQ ID NOs: 17-19.

[0028] The elongation factor 1 alpha (*tef1*) gene found in *Candida* species encodes for protein synthesis factor EF, which is involved in the translational process during protein synthesis. As known in the art, *tef1* gene is often referred to as *tef1* gene or *tuf* gene as well. In some embodiments disclosed herein, oligonucleotides (e.g., amplification primers and probes) that are capable of specifically hybridizing (e.g., under standard nucleic acid amplification conditions, e.g., standard PCR conditions, and/or stringent hybridization conditions) to a gene region encoding *tef1* in *Candida* species are provided. In some embodiments, *tef1* gene is used as the target gene for the DNA amplification to detect the presence, absence and/or level of VVC-associated *Candida* species in the sample. In some embodiments, the VVC-associated *Candida* species comprises *C. albicans*, *C. dubliniensis*, *C. tropicalis*, *C. parapsilosis*, *C. krusei*, and *C. glabrata*. In some embodiments, the WC-associated *Candida* species is *Candida krusei*. In some embodiments, the VVC-associated *Candida* species is *Candida glabrata*. In some embodiments, the VVC-associated *Candida* species is *C. albicans*, *C. dubliniensis*, *C. tropicalis*, *C. parapsilosis*, or a combination thereof. Examples of oligonucleotides capable of specifically hybridizing to the *tef1* gene region in *C. glabrata* include, but are not limited, SEQ ID NOs: 20-22 as provided in Table 1 and sequences having one mismatch or two mismatches relative to a sequence selected from the group consisting of SEQ ID NOs: 20-22. Examples of oligonucleotides capable of specifically hybridizing to the *tef1* gene region in *C. albicans*, *C. dubliniensis*, *C. tropicalis*, and *C. parapsilosis* include, but are not limited, SEQ ID NOs: 23-26 as provided in Table 1 and sequences having one mismatch or two mismatches relative to a sequence selected from the group consisting of SEQ ID NOs: 23-26. Examples of oligonucleotides capable of specifically hybridizing to the *tef1* gene region in *C. krusei* include, but are not limited, SEQ ID NOs: 27-29 as provided in Table 1 and sequences having one mismatch or two mismatches relative to a sequence selected from the group consisting of SEQ ID NOs: 27-29.

Table 1. Primer and probes for detection of VVC-associated *Candida* species and *T. vaginalis* of the present invention are listed in the table below. Besides the claimed primer and probes, other primer and probes for detection of BV-related species which are not encompassed by the claims are also listed in the table below.

Target Organism	Targeted gene	Prime/Probe Name	Prime/Probe Sequences (5'-3')
<i>Atopobium vaginae</i>	16S rRNA	MenAv248fw	CCCTATCCGCTCCTGATACC (SEQ ID NO: 1)
	16S rRNA	MenAv334rv	CCAAATATCTGCGCATTTC (SEQ ID NO: 2)
	16S rRNA	MCF-Av-T4	TCCCCTACCAGACTCAAGCCTGC (SEQ ID NO: 3)
			(5' fluorophore: FAM, 3' fluorophore: BHQ1)

Target Organism	Targeted gene	Prime/Probe Name	Prime/Probe Sequences (5'-3')
BVAB2	16S rRNA	585F_BVAB2	GCGGCTAGATAAGTGTGATGTTT (SEQ ID NO: 4)
	16S rRNA	666R_BVAB2	CTCTCCAGCACTCAAGCTAAA (SEQ ID NO: 5)
	16S rRNA	BVAB2_613_641	CAAGGCTTAACCTTGGGGTTCATTACAA (SEQ ID NO: 6) (5' fluorophore: CFO, 3' fluorophore: BHQ1)
Megasphaera type 1	16S rRNA	456F_MegaE	GATGCCAACAGTATCCGTCCG (SEQ ID NO: 7)
	16S rRNA	667R_MegaE	CCTCTCCGACACTCAAGTTCGA (SEQ ID NO: 8)
	16S rRNA	Mega485-506-T	TACCGTAAGAGAAAGCCACGG (SEQ ID NO: 9) (5' fluorophore: CFO, 3' fluorophore: BHQ1)
Gardnerella vaginalis	vly	GVvlyfw2	GCCAACGATGATCGCGTAT (SEQ ID NO: 10)
	vly	GVvlyfw2amod	GCCAATAATGACCGCGTAT (SEQ ID NO: 11)
	vly	GVvlyrv1	AGCCGTTCACTGCGGAAGT (SEQ ID NO: 12)
	vly	MCF-Gv-T3	ACAGCACTTTCGCCGCC (SEQ ID NO: 13) (5' fluorophore: Quasar670, 3' fluorophore: BHQ2)
Lactobacillus crispatus and Lactobacillus jensenii	16S rRNA	MCF-Lj_Lc-F8	TTAAAAGGCGGCGTAAGC (SEQ ID NO: 14)
	16S rRNA	MCF-Lsp-R6	GCCAGTTACTACCTCTATC (SEQ ID NO: 15)
	16S rRNA	MCF-Lsp-T11	AAGTCTGATGGAGCAACGCC (SEQ ID NO: 16) (5' fluorophore: ROX, 3' fluorophore: BHQ2)
Trichomonas vaginalis	AP-65	TV.MAX.FP1	GAAGATTCTGGCAAGATCAAGGA (SEQ ID NO: 17)
	AP-65	TV.MAX.RP1	ACGACAATGCAGCGGATGT (SEQ ID NO: 18)
	AP-65	TV.MAX.D1-T	ATCCTCCGCAACTACCCACGCCA (SEQ ID NO: 19) (5' fluorophore: FAM, 3' fluorophore: BHQ1)
Candida glabrata	tef1	SiT-Cgla-F8	CGAACAATTGACTGAAGGTTTG (SEQ ID NO: 20)
	tef1	RT-Cgla-R7	CGGACTTCAAGAACTTTGGAGA (SEQ ID NO: 21)
	tef1	RT-Cgla-T7	CTTGTAAGTTCGAAGAATTGTTGGA (SEQ ID NO: 22) (5' fluorophore: CFO, 3' fluorophore: BHQ1)
Candida genus*	tef1	RT-Ca-Cd-Ct-F1	CCACCAAAGGGTTGTGAC (SEQ ID NO: 23)
	tef1	RT-Ca-Ct-R3	CAGCATCACCGGATTTGAC (SEQ ID NO: 24)
	tef1	RT-Cpar-R6	CGGACTTGATGAATTTGGTTCA (SEQ ID NO: 25)
	tef1	RT-Ca-Cd-T3	TGCTTGTAATTCGACACTTTGGTTG (SEQ ID NO: 26)

Target Organism	Targeted gene	Prime/Probe Name	Prime/Probe Sequences (5'-3')
			(5' fluorophore: ROX, 3' fluorophore: BHQ2)
<i>Candida krusei</i>	tef1	RT-Ckru-F7	GCAGCTTCCTTCAATGCTCAA (SEQ ID NO: 27)
	tef1	SiT-Ckru-R10a	ATCACCAGACTTGACAG (SEQ ID NO: 28)
	tef1	RT-Ckru-T9	CATGTAAGTTTCGACGAATTAATCGA (SEQ ID NO: 29)
			(5' fluorophore: Quasar670, 3' fluorophore: BHQ2)
Controls	DrosScaff2	DrosScaff2-LP	GGCATGGAGGTTGTCCCATTTGTG (SEQ ID NO: 30)
	DrosScaff2	DrosScaff2-UP	GGATCTAGCCGTGTGCCCGCT (SEQ ID NO: 31)
	DrosScaff2	Sign-T1	TTGATGCCTCTTCACATTGCTCCACCTTTCCT (SEQ ID NO: 32)
			(5' fluorophore: Quasar705, 3' fluorophore: BHQ3)
* <i>C. albicans</i> , <i>C. dubliniensis</i> , <i>C. tropicalis</i> , or <i>C. parapsilosis</i>			

[0029] Also provided herein are oligonucleotides (for example amplification primers or probes) containing 1 or 2 mismatches or universal nucleotides relative to SEQ ID NOs: 17-32 or the complement thereof. In some embodiments, the oligonucleotide comprises a sequence selected from SEQ ID NO: 17-32. In some embodiments, the oligonucleotide comprises a sequence having one mismatch or two mismatches relative to a sequence selected from SEQ ID NO: 17-32. In some embodiments, the oligonucleotide consists of a sequence selected from SEQ ID NO: 17-32. In some embodiments, the oligonucleotide consists of a sequence having one mismatch or two mismatches relative to a sequence selected from SEQ ID NO: 17-32.

[0030] The nucleic acids provided herein can be in various forms. For example, in some embodiments, the nucleic acids are dissolved (either alone or in combination with various other nucleic acids) in solution, for example buffer. In some embodiments, nucleic acids are provided, either alone or in combination with other isolated nucleic acids, as a salt. In some embodiments, nucleic acids are provided in a lyophilized form that can be reconstituted. For example, in some embodiments, the isolated nucleic acids disclosed herein can be provided in a lyophilized pellet alone, or in a lyophilized pellet with other isolated nucleic acids. In some embodiments, nucleic acids are provided affixed to a solid substance, such as a bead, a membrane, or the like. In some embodiments, nucleic acids are provided in a host cell, for example a cell line carrying a plasmid, or a cell line carrying a stably integrated sequence.

[0031] Compositions, reaction mixture, and kits that comprise the oligonucleotides (e.g., amplification primers and/or probes) that are capable of specifically hybridizing to the sequence of the AP-65 gene of *T. vaginalis*, or a complement thereof, are provided. The composition, reaction mixture, and kit comprise one or more pairs of amplification primers capable of specifically hybridizing to the sequence of the AP-65 gene sequence of *T. vaginalis*, or a complement thereof. In some embodiments, the primer comprises a sequence of SEQ ID NO: 17 or 18. In some embodiments, the primer comprises a sequence having one mismatch or two mismatches relative to a sequence of SEQ ID NO: 17 or 18. In some embodiments, the primer consists of a sequence of SEQ ID NO: 17 or 18. In some embodiments, the composition, reaction mixture, and kit comprise one or more probes capable of specifically hybridizing to the sequence of AP-65 gene of *T. vaginalis*, or complement thereof. In some embodiments, the probe comprises a sequence of SEQ ID NO:

19. In some embodiments, the probe comprises a sequence having one mismatch or two mismatches relative to a sequence of SEQ ID NO: 19. In some embodiments, the probe consists of a sequence of SEQ ID NO: 19. In some embodiments, the probe consists of a sequence having one mismatch or two mismatches relative to a sequence of SEQ ID NO: 19.

[0032] Compositions, reaction mixtures, and kits that comprise the oligonucleotides (e.g., amplification primers and/or probes) that are capable of specifically hybridizing to the sequence of *tefl* gene of one or more *Candida* species, or complement thereof, are provided. In some embodiments, the composition, reaction mixture, and kit comprise one or more pairs of amplification primers capable of specifically hybridizing to the sequence of *tefl* gene sequence of *Candida glabrata*, or complement thereof. In some embodiments, the primer comprises a sequence of SEQ ID NO: 20 or 21. In some embodiments, the primer comprises a sequence having one mismatch or two mismatches relative to a sequence of SEQ ID NO: 20 or 21. In some embodiments, the primer consists of a sequence of SEQ ID NO: 20 or 21. In some embodiments, the primer consists of a sequence having one mismatch or two mismatches relative to a sequence of SEQ ID NO: 20 or 21. In some embodiments, the composition, reaction mixture, and kit comprise one or more probes capable of specifically hybridizing to the sequence of *tefl* gene *Candida glabrata*, or complement thereof. In some embodiments, the probe comprises a sequence of SEQ ID NO: 22, 26 or 29. In some embodiments, the probe comprises a sequence having one mismatch or two mismatches relative to a sequence of SEQ ID NO: 22. In some embodiments, the probe consists of a sequence of SEQ ID NO: 22. In some embodiments, the probe consists of a sequence having one mismatch or two mismatches relative to a sequence of SEQ ID NO: 22.

[0033] In some embodiments, the composition, reaction mixture, and kit comprise one or more pairs of amplification primers capable of specifically hybridizing to the sequence of *tefl* gene sequence of one or more *Candida* species, or complement thereof, wherein the *Candida* species comprises *C. albicans*, *C. dubliniensis*, *C. tropicalis*, and *C. parapsilosis*. In some embodiments, the primer comprises a sequence of SEQ ID NO: 20, 21, 23, 24, 25, 27, or 28. In some embodiments, the primer comprises a sequence having one mismatch or two mismatches relative to a sequence of SEQ ID NO: 23, 24 or 25. In some embodiments, the primer consists of a sequence of SEQ ID NO: 23, 24 or 25. In some embodiments, the primer consists of a sequence having one mismatch or two mismatches relative to a sequence of SEQ ID NO: 23, 24 or 25. In some embodiments, the composition, reaction mixture, and kit comprise one or more probes capable of specifically hybridizing to the sequence of *tefl* gene of one or more *Candida* species, or complement thereof, wherein the *Candida* species comprises *C. albicans*, *C. dubliniensis*, *C. tropicalis*, and *C. parapsi*. In some embodiments, the probe comprises a sequence of SEQ ID NO: 26. In some embodiments, the probe comprises a sequence having one mismatch or two mismatches relative to a sequence of SEQ ID NO: 26. In some embodiments, the probe consists of a sequence of SEQ ID NO: 26. In some embodiments, the probe consists of a sequence having one mismatch or two mismatches relative to a sequence of SEQ ID NO: 26.

[0034] In some embodiments, the composition, reaction mixture, and kit comprise one or more pairs of amplification primers capable of specifically hybridizing to the sequence of *tefl* gene sequence of *Candida krusei*, or complement thereof. In some embodiments, the primer comprises a sequence of SEQ ID NO: 27 or 28. In some embodiments, the primer comprises a sequence having one mismatch or two mismatches relative to a sequence of SEQ ID NO: 27 or 28. In some embodiments, the primer consists of a sequence of SEQ ID NO: 27 or 28. In some embodiments, the primer consists of a sequence having one mismatch or two mismatches relative to a sequence of SEQ ID NO: 27 or 28. In some embodiments, the composition, reaction mixture, and kit comprise one or more probes capable of specifically hybridizing to the sequence of *tefl* gene of *Candida krusei*, or complement thereof. In some embodiments, the probe comprises a sequence of SEQ ID NO: 29. In some embodiments, the probe comprises a sequence having one mismatch or two mismatches relative to a sequence of SEQ ID NO: 29. In some embodiments, the probe consists of a sequence of SEQ ID NO: 29. In some embodiments, the probe consists of a sequence having one mismatch

or two mismatches relative to a sequence of SEQ ID NO: 29.

[0035] Oligonucleotide probes can, in some embodiments, include a detectable moiety. For example, the oligonucleotide probes disclosed herein can comprise a radioactive label. Non-limiting examples of radioactive labels include ^3H , ^{14}C , ^{32}P , and ^{35}S . In some embodiments, oligonucleotide probes can include one or more non-radioactive detectable markers or moieties, including but not limited to ligands, fluorophores, chemiluminescent agents, enzymes, and antibodies. Other detectable markers for use with probes, which can enable an increase in sensitivity of the method of the invention, include biotin and radionucleotides. It will become evident to the person of ordinary skill that the choice of a particular label dictates the manner in which it is bound to the probe. For example, oligonucleotide probes labeled with one or more dyes, such that upon hybridization to a template nucleic acid, a detectable change in fluorescence is generated. While non-specific dyes may be desirable for some applications, sequence-specific probes can provide more accurate measurements of amplification. One configuration of sequence-specific probe can include one end of the probe tethered to a fluorophore, and the other end of the probe tethered to a quencher. When the probe is unhybridized, it can maintain a stem-loop configuration, in which the fluorophore is quenched by the quencher, thus preventing the fluorophore from fluorescing. When the probe is hybridized to a template nucleic sequence, it is linearized, distancing the fluorophore from the quencher, and thus permitting the fluorophore to fluoresce. Another configuration of sequence-specific probe can include a first probe tethered to a first fluorophore of a FRET pair, and a second probe tethered to a second fluorophore of a FRET pair. The first probe and second probe can be configured to hybridize to sequences of an amplicon that are within sufficient proximity to permit energy transfer by FRET when the first probe and second probe are hybridized to the same amplicon.

[0036] In some embodiments, the sequence specific probe comprises an oligonucleotide as disclosed herein conjugated to a fluorophore. In some embodiments, the probe is conjugated to two or more fluorophores. Examples of fluorophores include: xanthene dyes, e.g., fluorescein and rhodamine dyes, such as fluorescein isothiocyanate (FITC), 2-[ethylamino]-3-(ethylimino)-2,7-dimethyl-3H-xanthen-9-yl]benzoic acid ethyl ester monohydrochloride (R6G)(emits a response radiation in the wavelength that ranges from about 500 to 560 nm), 1,1,3,3,3',3'-Hexamethylindodicarbocyanine iodide (HIDC) (emits a response radiation in the wavelength that ranged from about 600 to 660 nm), 6-carboxyfluorescein (commonly known by the abbreviations FAM and F), 6-carboxy-2',4',7',4,7-hexachlorofluorescein (HEX), 6-carboxy-4',5'-dichloro-2',7'-dimethoxyfluorescein (JOE or J), N,N,N',N'-tetramethyl-6-carboxyrhodamine (TAMRA or T), 6-carboxy-X-rhodamine (ROX or R), 5-carboxyrhodamine-6G (R6G5 or G5), 6-carboxyrhodamine-6G (R6G6 or G6), and rhodamine 110; cyanine dyes, e.g. Cy3, Cy5 and Cy7 dyes; coumarins, e.g., umbelliferone; benzimide dyes, e.g. Hoechst 33258; phenanthridine dyes, e.g. Texas Red; ethidium dyes; acridine dyes; carbazole dyes; phenoxazine dyes; porphyrin dyes; polymethine dyes, e.g. cyanine dyes such as Cy3 (emits a response radiation in the wavelength that ranges from about 540 to 580 nm), Cy5 (emits a response radiation in the wavelength that ranges from about 640 to 680 nm), etc; BODIPY dyes and quinoline dyes. Specific fluorophores of interest include: Pyrene, Coumarin, Diethylaminocoumarin, FAM, Fluorescein Chlorotriazinyl, Fluorescein, R110, Eosin, JOE, R6G, HIDC, Tetramethylrhodamine, TAMRA, Lissamine, ROX, Naphthofluorescein, Texas Red, Naphthofluorescein, Cy3, and Cy5, CAL fluor orange, and the like.

[0037] In some embodiments, the probe is conjugated to a quencher. A quencher can absorb electromagnetic radiation and dissipate it as heat, thus remaining dark. Example quenchers include Dabcyl, NFQ's, such as BHQ-1 or BHQ-2 (Biosearch), IOWA BLACK FQ (IDT), and IOWA BLACK RQ (IDT). In some embodiments, the quencher is selected to pair with a fluorophore so as to absorb electromagnetic radiation emitted by the fluorophore. Fluorophore/quencher pairs useful in the compositions and methods disclosed herein are well-known in the art, and can be found, e.g., described in Marras, "Selection of Fluorophore and Quencher Pairs for Fluorescent Nucleic Acid Hybridization Probes" available at www.molecular-beacons.org/download/marras,mmb06%2833%205%293.pdf.

[0038] In some embodiments, a fluorophore is attached to a first end of the probe, and a quencher is attached to a second end of the probe. Attachment can include covalent bonding, and can optionally include at least one linker molecule positioned between the probe and the fluorophore or quencher. In some embodiments, a fluorophore is attached to a 5' end of a probe, and a quencher is attached to a 3' end of a probe. In some embodiments, a fluorophore is attached to a 3' end of a probe, and a quencher is attached to a 5' end of a probe. Examples of probes that can be used in quantitative nucleic acid amplification include molecular beacons, SCORPION™ probes (Sigma), TAQMAN™ probes (Life Technologies) and the like. Other nucleic acid detection technologies that are useful in the embodiments disclosed herein include, but are not limited to nanoparticle probe technology (See, Elghanian, et al. (1997) Science 277:1078-1081.) and Amplifluor probe technology (See, U.S. Pat. Nos: 5,866,366; 6,090,592; 6,117,635; and 6,117,986).

[0039] The present invention relates to a composition for the detection of VVC-associated *Candida* species and *T. vaginalis* in a biological sample, wherein the composition comprises: primers capable of hybridizing to the *tefl* gene of *Ca. glabrata*, wherein each primer comprises a sequence of SEQ ID NO: 20 or SEQ ID NO: 21 or a sequence having one mismatch or two mismatches relative to SEQ ID NO: 20 or SEQ ID NO: 21; primers capable of hybridizing to the *tefl* gene of at least one of *C. albicans*, *C. tropicalis*, *C. dubliniensis*, and *C. parapsilosis*, wherein each primer comprises a sequence of SEQ ID NO: 23, SEQ ID NO: 24, or SEQ ID NO: 25, or a sequence having one mismatch or two mismatches relative to SEQ ID NO: 23, SEQ ID NO: 24, or SEQ ID NO: 25; primers capable of hybridizing to the *tefl* gene of *C. krusei*, wherein each primer comprises a sequence of SEQ ID NO: 27 or SEQ ID NO: 28, or a sequence having one mismatch or two mismatches relative to SEQ ID NO: 27 or SEQ ID NO: 28; and primers capable of hybridizing to the AP-65 gene of *T. vaginalis*, wherein each primer comprises a sequence of SEQ ID NO: 17 or SEQ ID NO: 18, or a sequence having one mismatch or two mismatches relative to SEQ ID NO: 17 or SEQ ID NO: 18.

[0040] In some embodiments, the primers capable of hybridizing to the *tefl* gene of *C. glabrata* comprise, or consist of, a primer comprising the sequence of SEQ ID NO: 20 and a primer comprising the sequence of SEQ ID NO: 21; the primers capable of hybridizing to the *tefl* gene of at least one of *C. albicans*, *C. tropicalis*, *C. dubliniensis*, and *C. parapsilosis* comprise, or consist of, a primer comprising the sequence of SEQ ID NO: 23, a primer comprising the sequence of SEQ ID NO: 24, and a primer comprising the sequence of SEQ ID NO: 25; the primers capable of hybridizing to the *tefl* gene of *C. krusei* comprise, or consist of, a primer comprising the sequence of SEQ ID NO: 27 and a primer comprising the sequence of SEQ ID NO: 28; and the primers capable of hybridizing to the AP-65 gene of *T. vaginalis* comprise, or consist of a primer comprising the sequence of SEQ ID NO: 17 and a primer comprising the sequence of SEQ ID NO: 18.

[0041] The composition can, in some embodiments, further comprises a plurality of oligonucleotide probes, wherein each of the plurality of oligonucleotide probes comprises a sequence selected from the group consisting of SEQ ID NOs: 22, 26, 29, and 19, or a sequence having one mismatch or two mismatches relative to a sequence selected from the group consisting of SEQ ID NOs: 22, 26, 29, and 19. In some embodiments, each of the plurality of oligonucleotide probes comprises, or consists of, a sequence selected from the group consisting of SEQ ID NOs: 22, 26, 29, and 19.

[0042] Any probes described herein can comprise a fluorescence emitter moiety and a fluorescence quencher moiety.

[0043] As disclosed herein, a reaction mixture can comprise one or more of the primers disclosed herein, one or more of the probes disclosed herein (e.g., the fluorophore-containing probes), or any combination thereof. In some embodiments, the reaction mixture comprises one or more of the primer and/or probe-containing composition disclosed herein. The reaction mixture can also comprise various additional components. Examples of the additional components in the reaction mixture include, but are not limited to, template DNA, DNA polymerase (e.g., Taq DNA polymerase), deoxynucleotides (dNTPs), buffer solution,

bivalent cations, monovalent cation potassium ions, and any combination thereof. In some embodiments, the reaction mixture is a master mix for real-time PCR.

Samples

[0044] The methods and compositions disclosed herein are suitable for detecting vaginal disorders, in particular VVC and trichomoniasis, in a wide variety of samples. As used herein, a "sample" refers to any type of material of biological origin taken from one or more number of subjects that are suspected of suffering from WC, and/or trichomoniasis. The sample can comprise, for example, fluid, tissue or cell. The sample can comprise a biological material taken directly from a subject, or cultured cell or tissues, or any fraction or products produced from or derived from biological materials. A sample can be purified, partially purified, unpurified, enriched, or amplified.

[0045] The sample can be a biological sample, for example a clinical sample. In some embodiments, the sample is taken from a biological source, such as vagina, urethra, penis, anus, throat, cervix, fermentation broths, cell cultures, and the like. The sample can comprise, for example, fluid and cells from vagina. The biological sample can be used (i) directly as obtained from the subject or source, or (ii) following a pre-treatment to modify the character of the sample. Thus, the test sample can be pre-treated prior to use, for example, by disrupting cells or viral particles, preparing liquids from solid materials, diluting viscous fluids, filtering liquids, concentrating liquids, inactivating interfering components, adding reagents, purifying nucleic acids, and the like. Accordingly, a "biological sample" as used herein includes nucleic acids (DNA, RNA or total nucleic acids) extracted from a clinical or biological specimen. Sample preparation can also include using a solution that contains buffers, salts, detergents, and/or the like which are used to prepare the sample for analysis. In some embodiments, the sample is processed before molecular testing. In some embodiments, the sample is analyzed directly, and is not pre-processed prior to testing. The sample can be, for example, a vaginal sample, such as a single vaginal swab sample. In some embodiments, the sample is a vaginal swab sample from a female with clinical symptoms of vaginitis and/or vaginosis.

[0046] Vaginal or urine samples are often infected with multiple organisms. The disclosed primers and probes are tolerant to mixed infections of the vaginal or urine matrix.

[0047] In some embodiments, a sample to be tested is processed prior to performing the methods disclosed herein. For example, in some embodiments, the sample can be isolated, concentrated, or subjected to various other processing steps prior to performing the methods disclosed herein. For example, in some embodiments, the sample can be processed to isolate nucleic acids from the sample prior to contacting the sample with the oligonucleotides, as disclosed herein. In some embodiments, the methods disclosed herein are performed on the sample without culturing the sample *in vitro*. In some embodiments, the methods disclosed herein are performed on the sample without isolating nucleic acids from the sample prior to contacting the sample with oligonucleotides as disclosed herein.

Sample Extraction

[0048] In typical sample extractions, cells are lysed by mechanical shearing with glass beads as described in US Patent No. 7,494,771 to lyse the target organisms. As disclosed in WO03/008636, such a generic method of cell lysis is efficient for a wide variety of target organisms and specimen matrices. There are also other less universal lysis methods that are designed specifically to target a certain species or group of organisms, or which exploit specific enzymatic or chemical activities. For example, ACP enzyme is commonly used to lyse of Gram-positive organisms (Ezaki et al., J. Clin. Microbiol., 16(5):844-846 (1982); Paule et al.,

J. Mol. Diagn., 6(3): 191-196 (2004); US Patent No. 3,649,454) and mycobacteria (US Patent No. 5,185,242) but is generally considered to be less efficacious with respect to lysis of Gram-negative species such as *E. coli* and *Pseudomonas aeruginosa* (US Patent No. 3,649,454).

[0049] Inventors of the present disclosure was surprised to find that neither ACP nor Proteinase K can efficiently lyse *Candida* cells walls, and lyticase described in patent US Patent No. 3,716,452 can effectively lyse cell walls of *Candida* species. Cell lysis can be performed under various temperatures, for example between 18°C to 75°C, for example, 37 °C and 50 °C. It is advantageous to lyse the cells at 37°C to achieve higher lysis efficiency as compared to 50°C (LND490E38). In some embodiments, lyticase is used to lyse *Candida* species, including but not limited to *C. albicans*, *C. krusei*, *C. parapsilosis*, *C. tropicalis*, and *C. glabrata*. The time required to achieve desired lysis efficiency for the sample is not particularly limited. In some embodiments, it requires about 10 minute to achieve desired lysis efficiency of the sample.

Nucleic acid testing

[0050] The methods described herein can include, for example, nucleic acid testing. For example, the test can include testing for target nucleic acid sequences in a sample. Various forms of nucleic acid testing can be used in the embodiments disclosed herein, including but not limited to, testing that involves nucleic acid amplification.

[0051] As used herein, nucleic acid amplification refers to any known procedure for obtaining multiple copies of a target nucleic acid sequence or its complement or fragments thereof, using sequence-specific methods. Examples of known amplification methods include, but are not limited to, polymerase chain reaction (PCR), ligase chain reaction (LCR), loop-mediated isothermal amplification (LAMP), strand displacement amplification (SDA) (e.g., multiple displacement amplification (MDA)), replicase-mediated amplification, immuno-amplification, nucleic acid sequence based amplification (NASBA), self-sustained sequence replication (3SR), rolling circle amplification, and transcription-mediated amplification (TMA). See, e.g., Mullis, "Process for Amplifying, Detecting, and/or Cloning Nucleic Acid Sequences," U.S. Pat. No. 4,683,195; Walker, "Strand Displacement Amplification," U.S. Pat. No. 5,455,166; Dean et al, "Multiple displacement amplification," U.S. Pat. No. 6,977,148; Notomi et al., "Process for Synthesizing Nucleic Acid," U.S. Pat. No. 6,410,278; Landegren et al. U.S. Pat. No. 4,988,617 "Method of detecting a nucleotide change in nucleic acids"; Birkenmeyer, "Amplification of Target Nucleic Acids Using Gap Filling Ligase Chain Reaction," U.S. Pat. No. 5,427,930; Cashman, "Blocked-Polymerase Polynucleotide Immunoassay Method and Kit," U.S. Pat. No. 5,849,478; Kacian et al., "Nucleic Acid Sequence Amplification Methods," U.S. Pat. No. 5,399,491; Malek et al., "Enhanced Nucleic Acid Amplification Process," U.S. Pat. No. 5,130,238; Lizardi et al., *BioTechnology*, 6:1197 (1988); Lizardi et al., U.S. Pat. No. 5,854,033 "Rolling circle replication reporter systems." In some embodiments, two or more of the aforementioned nucleic acid amplification methods can be performed, for example sequentially.

[0052] For example, LCR amplification uses at least four separate oligonucleotides to amplify a target and its complementary strand by using multiple cycles of hybridization, ligation, and denaturation (EP Patent No. 0 320 308). SDA amplifies by using a primer that contains a recognition site for a restriction endonuclease which nicks one strand of a hemimodified DNA duplex that includes the target sequence, followed by amplification in a series of primer extension and strand displacement steps (U.S. Pat. No. 5,422,252 to Walker et al.).

[0053] PCR is a method well-known in the art for amplification of nucleic acids. PCR involves amplification of a target sequence using two or more extendable sequence-specific oligonucleotide primers that flank the target sequence. The nucleic acid containing the target sequence of interest is subjected to a program of

multiple rounds of thermal cycling (denaturation, annealing and extension) in the presence of the primers, a thermostable DNA polymerase (e.g., Taq polymerase) and various dNTPs, resulting in amplification of the target sequence. PCR uses multiple rounds of primer extension reactions in which complementary strands of a defined region of a DNA molecule are simultaneously synthesized by a thermostable DNA polymerase. At the end of each cycle, each newly synthesized DNA molecule acts as a template for the next cycle. During repeated rounds of these reactions, the number of newly synthesized DNA strands increases exponentially such that after 20 to 30 reaction cycles, the initial template DNA will have been replicated several thousand-fold or million-fold. Methods for carrying out different types and modes of PCR are thoroughly described in the literature, for example in "PCR Primer: A Laboratory Manual" Dieffenbach and Dveksler, eds. Cold Spring Harbor Laboratory Press, 1995, and by Mullis et al. in patents (e.g., U.S. Patent Nos. 4,683,195, 4,683,202 and 4,800,159) and scientific publications (e.g. Mullis et al. 1987, Methods in Enzymology, 155:335-350).

[0054] PCR can generate double-stranded amplification products suitable for post-amplification processing. If desired, amplification products can be detected by visualization with agarose gel electrophoresis, by an enzyme immunoassay format using probe-based colorimetric detection, by fluorescence emission technology, or by other detection means known to one of skill in the art.

[0055] A wide variety of PCR methods have been described in many sources, for example, Ausubel et al. (eds.), Current Protocols in Molecular Biology, Section 15, John Wiley & Sons, Inc., New York (1994). Examples of PCR method include, but not limited to, Real-Time PCR, End-Point PCR, Amplified fragment length polymorphism PCR (AFLP-PCR), Alu-PCR, Asymmetric PCR, Colony PCR, DD-PCR, Degenerate PCR, Hot-start PCR, In situ PCR, Inverse PCR Long-PCR, Multiplex PCR, Nested PCR, PCR-ELISA, PCR-RFLP, PCR-single strand conformation polymorphism (PCR-SSCP), quantitative competitive PCR (QC-PCR), rapid amplification of cDNA ends-PCR (RACE-PCR), Random Amplification of Polymorphic DNA-PCR (RAPD-PCR), Real-Time PCR, Repetitive extragenic palindromic-PCR (Rep-PCR), reverse transcriptase PCR (RT-PCR), TAIL-PCR, Touchdown PCR and Vectorette PCR.

[0056] Real-time PCR, also called quantitative real time polymerase chain reaction (QRT-PCR), can be used to simultaneously quantify and amplify a specific part of a given nucleic acid molecule. It can be used to determine whether a specific sequence is present in the sample; and if it is present, the number of copies of the sequence that are present. The term "real-time" refers to periodic monitoring during PCR. Certain systems such as the ABI 7700 and 7900HT Sequence Detection Systems (Applied Biosystems, Foster City, Calif.) conduct monitoring during each thermal cycle at a pre-determined or userdefined point. Real-time analysis of PCR with fluorescence resonance energy transfer (FRET) probes measures fluorescent dye signal changes from cycle-to-cycle, preferably minus any internal control signals. The real-time procedure follows the general pattern of PCR, but the nucleic acid is quantified after each round of amplification. Two examples of method of quantification are the use of fluorescent dyes (e.g., SYBRGreen) that intercalate into double-stranded DNA, and modified DNA oligonucleotide probes that fluoresce when hybridized with a complementary DNA. Intercalating agents have a relatively low fluorescence when unbound, and a relatively high fluorescence upon binding to double-stranded nucleic acids. As such, intercalating agents can be used to monitor the accumulation of double stranded nucleic acids during a nucleic acid amplification reaction. Examples of such non-specific dyes useful in the embodiments disclosed herein include intercalating agents such as SYBR Green I (Molecular Probes), propidium iodide, ethidium bromide, and the like.

[0057] Vaginal samples are often infected with multiple organisms. The disclosed primers and probes are tolerant to mixed infections of the vaginal matrix. Because of the specific target sequences, primers and probes, the methods and compositions disclosed herein can be used to detect the presence/absence or level of VVC-associated *Candida* species and/or *T. vaginalis* in a sample with high sensitivity, specificity and accuracy.

[0058] The primers disclosed herein can be paired with additional PCR systems using a uniform chemistry and thermal PCR profile to provide a panel of assays for the detection of vaginal organisms, to improve overall assay sensitivity and robustness.

[0059] In some embodiments, multiplex PCR is performed to amplify and detect, e.g., by direct or indirect means, the presence or absence of VVC-associated *Candida* species and *T. vaginalis* to allow diagnose of VVC and Trichomoniasis using one test. In the multiplex PCR, the presence or absence of VVC-associated *Candida* species can be determined by amplifying and detecting the presence or absence of tefl gene of *C. albicans*, *C. dubliniensis*, *C. tropicalis*, *C. parapsilosis*, *C. krusei*, and *C. glabrata*; the presence or absence of *T. vaginalis* can be determined by amplifying and detecting the presence or absence of AP-65 gene of *T. vaginalis*.

[0060] Accordingly, some embodiments for the detection and/or identification of VVC-associated *Candida* species and *T. vaginalis* in a sample include the steps of providing a test sample; and contacting the sample with oligonucleotide primers as described above that can specifically hybridize and amplify (1) tefl genes of *C. albicans*, *C. dubliniensis*, *C. tropicalis*, *C. parapsilosis*, *C. krusei*, and *C. glabrata*, and (2) AP-65 gene of *T. vaginalis*, and oligonucleotide probes that can specifically hybridizes to (1) tefl gene regions of *C. albicans*, *C. dubliniensis*, *C. tropicalis*, *C. parapsilosis*, *C. krusei*, and *C. glabrata* and (2) AP-65 gene region of *T. vaginalis* under standard nucleic acid amplification conditions and/or stringent hybridization conditions. As described herein, the sample can be contacted with all of the primers and probes at once, or can be contacted with some of the primers and probes first and subsequently contacted by the remainder of the primers and probes. In some embodiments, the sample is contacted with the primers that can specifically hybridize and amplify (1) tefl genes of *C. albicans*, *C. dubliniensis*, *C. tropicalis*, *C. parapsilosis*, *C. krusei*, and *C. glabrata*, and (2) AP-65 gene of *Trichomonas vaginalis*, and the probes that can specifically hybridizes to (1) tefl gene regions of *C. albicans*, *C. dubliniensis*, *C. tropicalis*, *C. parapsilosis*, *C. krusei*, and *C. glabrata*, and (2) AP-65 gene region of *T. vaginalis*.

[0061] The oligonucleotide probe can be, for example, between about 10 and about 45 nucleotides in length, and comprises a detectable moiety. In some embodiments, the contacting is performed under conditions allowing for the specific hybridization of the primers to the corresponding targeted gene region if the target organism is present in the sample. The presence and/or amount of probe that is specifically bound to the corresponding targeted gene region (if present in the sample being tested) can be determined, wherein bound probe is indicative of the presence of the corresponding target organism in the sample. In some embodiments, the amount of bound probe is used to determine the amount of the corresponding target organism in the sample.

[0062] The determining step can be achieved using any methods known to those skilled in the art, including but not limited to, *in situ* hybridization, following the contacting step. The detection of hybrid duplexes (*i.e.*, of a probe specifically bound to the targeted gene region) can be carried out by a number of methods. Typically, hybridization duplexes are separated from unhybridized nucleic acids and the labels bound to the duplexes are then detected. Such labels refer to radioactive, fluorescent, biological or enzymatic tags or labels of standard use in the art. A label can be conjugated to either the oligonucleotide probes or the nucleic acids derived from the biological sample. Those of skill in the art will appreciate that wash steps may be employed to wash away excess sample/target nucleic acids or oligonucleotide probe (as well as unbound conjugate, where applicable). Further, standard heterogeneous assay formats are suitable for detecting the hybrids using the labels present on the oligonucleotide primers and probes.

[0063] Provided is a method to detect VVC-associated *Candida* species (including *Candida glabrata*, *Candida albicans*, *Candida tropicalis*, *C. dubliniensis*, *C. parapsilosis*, *Candida krusei*) and *T. vaginalis* in a biological sample, wherein the method comprises: contacting the biological sample with a plurality of pairs of

primers, wherein the plurality of pairs of primer comprises: primers capable of hybridizing to the *tefl* gene of *C. glabrata*, wherein each primer comprises a sequence of SEQ ID NO: 20 or SEQ ID NO: 21 or a sequence having one mismatch or two mismatches relative to SEQ ID NO: 20 or SEQ ID NO: 21; primers capable of hybridizing to the *tefl* gene of at least one of *C. albicans*, *C. tropicalis*, *C. dubliniensis*, and *C. parapsilosis*, wherein each primer comprises a sequence of SEQ ID NO: 23, SEQ ID NO: 24, or SEQ ID NO: 25, or a sequence having one mismatch or two mismatches relative to SEQ ID NO: 23, SEQ ID NO: 24, or SEQ ID NO: 25; primers capable of hybridizing to the *tefl* gene of *C. krusei*, wherein each primer comprises a sequence of SEQ ID NO: 27 or SEQ ID NO: 28, or a sequence having one mismatch or two mismatches relative to SEQ ID NO: 27 or SEQ ID NO: 28; and primers capable of hybridizing to the AP-65 gene of *T. vaginalis*, wherein each primer comprises a sequence of SEQ ID NO: 17 or SEQ ID NO: 18, or a sequence having one mismatch or two mismatches relative to SEQ ID NO: 17 or SEQ ID NO: 18; and generating amplicons of the *tefl* sequences of the *Candida* species and/or amplicons of the AP-65 gene sequence of *T. vaginalis* from said biological sample, if said sample comprises one or more of the VVC-associated *Candida* species and/or *T. vaginalis*; determining the presence or amount of one or more amplified products as an indication of the presence of VVC-associated *Candida* species and *T. vaginalis* in said biological sample.

[0064] In some embodiments, the plurality of pairs of primers comprises a primer comprising the sequence of SEQ ID NO: 20, a primer comprising the sequence of SEQ ID NO: 21, a primer comprising the sequence of SEQ ID NO: 23, a primer comprising the sequence of SEQ ID NO: 24, a primer comprising the sequence of SEQ ID NO: 25, a primer comprising the sequence of SEQ ID NO: 27, a primer comprising the sequence of SEQ ID NO: 28, a primer comprising the sequence of SEQ ID NO: 17, and a primer comprising the sequence of SEQ ID NO: 18.

[0065] In some embodiments, the primers capable of hybridizing to the *tefl* gene of *C. glabrata* comprise SEQ ID NOs: 20 and 21; the primers capable of hybridizing to the *tefl* gene of at least one of *C. albicans*, *C. tropicalis*, *C. dubliniensis*, and *C. parapsilosis* comprise: (a) SEQ ID NOs: 23 and 24, (b) SEQ ID NOs: 23 and 25, or (c) a combination thereof; the primers capable of hybridizing to the *tefl* gene of *C. krusei* comprise of SEQ ID NOs: 27 and 28; and the primers capable of hybridizing to the AP-65 gene of *T. vaginalis* comprise SEQ ID NOs: 17 and 18.

[0066] As described herein, the amplification can be carried out by real-time PCR, for example, quantitative real-time PCR (QRT-PCR). The primers suitable for use in the methods and compositions described herein can comprise exogenous nucleotide sequence which allows post-amplification manipulation of amplification products without a significant effect on amplification itself. In some embodiments, the primer can be flanked by complementary sequences comprising a fluorophore at the 5' end, and a fluorescence quencher at the 3' end.

[0067] The oligonucleotide probes disclosed herein can comprise a fluorescence emitter moiety and a fluorescence quencher moiety

[0068] The methods disclosed herein are amendable to automation, thereby providing a high-throughput option for the detection and/or quantification of WC-associated *Candida* species (including *Candida glabrata*, *Candida albicans*, *Candida tropicalis*, *C. dubliniensis*, *C. parapsilosis*, *Candida krusei*) and *T. vaginalis* in a sample. Various multiplex PCR platforms, e.g., BD MAX™, Viper™, or Viper™ LT platforms, can be used to perform one or more steps of the disclosed methods. The methods can be performed in a multiplex fashion. For example, the nucleic acid amplification and/or detection, in some embodiments, comprise performing multiplex PCR.

EXAMPLES

[0069] The following examples are provided to demonstrate particular situations and settings in which this technology may be applied and are not intended to restrict the scope of the invention and the claims included in this disclosure. In the following examples the detection of bacterial vaginosis (BV) is only disclosed for reference purposes and the corresponding embodiments do not belong to the claimed invention.

Example 1

Detection of WC, trichomoniasis and BV in vaginal swab samples

[0070] The study described in this example shows the detection of *Candida* species associated with VVC, trichomoniasis and BV using an automated qualitative *in vitro* diagnostic test in vaginal swab samples. The test utilizes real time PCR for the amplification of DNA targets and fluorogenic hybridization probes for the detection and identification of target organisms.

[0071] Vaginal swabs were collected from women with clinical symptoms of vaginitis/vaginosis. Vaginal specimens were characterized by In Pouch™ TV for *T. vaginalis* while culture followed by BD Phoenix™ identification was used for *Candida* species and the Nugent score (Nugen et al., J. Clin. Microbiol. 29(2):297-301 (1991)), as reference method for BV. Amsel's criteria (Amsel et al., Am. J. Med. 74(1): 14-22 (1983)) were used only in determination of BV statuses for specimens with intermediate Nugent score (Nugent's score 4-6). Three swabs were for test on the BD MAX™ System (Becton, Dickinson and Company, New Jersey) for detection of trichomoniasis, *Candida* species associated with VVC, and BV using a Receiver Operating Characteristic (ROC) curve analysis. The diagnosis of BV was determined using an algorithm based on PCR parameters for the detection of BV-related bacteria, including *Lactobacillus* species, *G. vaginalis*, *Atopobium vaginae*, *BVAB-2*, and *Megasphaera-1*.

[0072] Real-time PCR for the amplification of DNA targets was performed using the primers provided in Table 1 and fluorogenic hybridization probes provided in Table 1 were used to detect *Candida* species associated with WC, *T. vaginalis*, and BV-related bacteria *L. crispatus*, *L. jensenii*, *G. vaginalis*, *Atopobium vaginae*, *Megasphaera Type 1*, and *BVAB2* in each of the vaginal swab samples.

[0073] An inclusivity study was performed with cultivable strains originating from 12 countries. The inclusivity study analysis was based on positive/negative status of each individual target according to established PCR parameter thresholds. As shown in Table 2, the assay is capable of detecting a large diversity of strains belonging to species involved in VVC, trichomoniasis and BV. The level of detection of specific organisms in mixtures demonstrated a high level of analytical sensitivity, indicating that clinicians can be able to obtain a clear identification of the pathogen(s) involved in vaginal infection and select the treatment using only one vaginal specimen.

Table 2. Inclusivity Study

Microorganism (Load/swab)	Strain ^a	Origin	Status
<i>Candida albicans</i> (5.4 x10 ⁵ CFU/swab)	ATCC 18804	Uruguay	POS
	ATCC 36232	ND	POS
	ATCC 60193	USA	POS
	ATCC 32032	South Africa	POS
	CCUG 44014	Sweden	POS

Microorganism (Load/swab)	Strain ^a	Origin	Status
<i>Atopobium vaginas</i> (1.1x10 ³ CFU/swab)	CCUG 43049	Sweden	POS
	CCUG 44156	Sweden	POS
	CCUG 55226	Belgium	POS
	CCUG 44258	Sweden	POS
	CCUG 48515	Sweden	POS
<i>Trichomonas vaginalis</i> (1.4x 10 ³ Cells/swab)	ATCC 30001	NO	POS
	ATCC 30092	USA	POS
	ATCC 30185	USA	POS
	ATCC 30184	USA	POS
	ATCC 30237	USA	POS
<i>Candida glabrata</i> (2.8x 10 ⁴ CFU/swab)	ATCC 2001	NO	POS
	ATCC 15545	NO	POS
	ATCC 90876	Germany	POS
	YST-192 ^b	USA	POS
	ATCC MYA-276	Scotland	POS
<i>Candida krusei</i> (3.4x 10 ⁴ CFU/swab)	ATCC 6258	Sri Lanka	POS
	ATCC 28870	Italy	POS
	ATCC 32672	New Zeland	POS
	ATCC 44507	England	POS
	YST-367 ^b	USA	POS
^a One strain from each microorganism tested in each mix			
^b Strain from BD collection.			
ND: not determined			
Microorganism	Strain ^a	Origin	Status
<i>Gardnerella vaginalis</i> (3.4x10 ⁴ CFU/swab)	ATCC 14018	USA	POS
	ATCC 14019	NO	POS
	CCUG 44111	Sweden	POS
	CCUG 44159	Sweden	POS
	CCUG 60143 A	Sweden	POS
	ATCC 49145	ND	POS
	CCUG 44280	Sweden	POS
<i>Lactobacillus crispatus</i> (1.4x10 ⁴ CFU/swab)	ATCC 33820	NO	POS
	CCUG 44073	Sweden	POS
	CCUG 42898	NO	POS
	ATCC 33197	NO	POS
	ATCC 53545	NO	POS
<i>Lactobacillus jensenii</i> (2.1x10 ³ CFU/swab)	ATCC 25258	NO	POS
	CCUG 44492	South Africa	POS
	CCUG 44003	Sweden	POS

Microorganism	Strain ^a	Origin	Status
<i>Candida parapsilosis</i> (5.4 x10 ⁵ CFU/swab)	CCUG 44122	Sweden	POS
	CCUG 44495	South Africa	POS
	ATCC 22019	Puerto Rico	POS
	ATCC 28475	Norway	POS
	YST-100 ^b	Germany	POS
	CCUG 37233	Sweden	POS
<i>Candida tropicalis</i> (5.4 x10 ⁵ CFU/swab)	YST-194	USA	POS
	ATCC 750	ND	POS
	ATCC 1369	NO	POS
	ATCC 9968	former USSR	POS
	YST-1051 ^b	USA	POS
	CCUG 21298	Sweden	POS

[0074] In simulated co-infection studies, low load of *T. vaginalis* or a low load of *C. glabrata* and *C. krusei* were tested in presence of high loads of *C. albicans*; and low load of *T. vaginalis* was tested in presence of a high load of *C. glabrata*. For each study above, simulated matrix was used rather than vaginal matrix due to the presence of some targets in vaginal flora from asymptomatic/symptomatic women. The results of the simulated co-infection studies are shown in Table 3.

Table 3. Stimulated co-infection study of the vaginal panel

High load (Organisms/swab)	<i>Candida albicans</i> (2.8 E+6)			<i>Candida glabrata</i> (2.8 E+6)
Low load (Organisms/swab)	<i>Candida krusei</i> (3.4 E+4)	<i>Trichomonas vaginalis</i> (1.4 E+3)	<i>Candida glabrata</i> (4.2 E+3)	<i>Trichomonas vaginalis</i> (1.4 E+3)
% of conforming Assay results	95%	100%	100%	100%

[0075] Clinical specimens were defined as positive/negative sample for *Candida* species and *T. vaginalis*. The results of the performance study shown in Table 4 demonstrate that the vaginal panel disclosed herein can be used to detect *T. vaginalis* and *Candida* species with high sensitivity and specificity.

Table 4. Performance study for TV and *Candida* species

Vaginal panel assay	Reference method	Performance			
		Sensitivity/Specificity ^a	Fraction ^b	%	[2-sided 95 CI] ^e
<i>T. vaginalis</i>	Inpouch TV TM	Sensitivity	34/36	94.4	[81.3 - 99.3]
		Specificity	729/729	100.0	[99.6 - 100.0]
<i>Candida</i> species ^{c,d}	BD Phoenix TM	Sensitivity	171/197	86.8	[81.3 - 91.2]
		Specificity	544/568	95.8	[93.8 - 97.3]

Vaginal panel assay	Reference method	Performance			
		Sensitivity/Specificity ^a	Fraction ^b	%	[2-sided 95 CI] ^e
^a Sensitivity = True POS/Total POS from reference method and Specificity= True NEG/Total NEG from reference method					
^b «Unresolved» non-reportable results were excluded from Sensitivity/Specificity calculation «1 %»					
^c <i>Candida</i> species: <i>C. albicans</i> , <i>C. dubliniensis</i> , <i>C. guilliermondii</i> , <i>C. tropicalis</i> , and <i>C. parapsilosis</i>					
^d <i>Candida glabrata</i> and <i>Candida krusei</i> are detected in two distinct channels: eight <i>C. glabrata</i> positive specimens for PCR and reference method, one positive for peR and two positive for reference method separately were obtained. One <i>C. krusei</i> positive specimen for PCR and reference method and one positive for each method separately were obtained.					
^e 2-sided 95% CI was calculated using the Clopper-Pearson method					

[0076] Assay performance for detection of BV was established using a Receiver Operating Characteristic (ROC) curve analysis. Using PCR metrics from the amplification and detection of *Lactobacillus* species, *L. vaginalis*, *Atopobium vaginae*, *BVAB-2* and *Megasphaera-1*, a logistic regression model-based algorithm was built to estimate BV positive probability and give a single BV positive or BV negative call. Patients were considered to have BV if their estimated probability exceeds a threshold determined by ROC curve analysis.

[0077] As shown in Table 5, preliminary assay performance results (sensitivity/specificity) based on analysis of 771 total characterized clinical samples was 91.9%(sensitivity)/86.2% (specificity) or increased to 95.4(sensitivity)/92.5%(specificity) when intermediate Nugent Score and Amsel's criteria results were not considered.

Table 5. Performance study for BV detection

New BV Assay	Reference Method	Performance			
		Sensitivity/Specificity ^a	Fraction ^b	%	[2-sided 95% CI]
BV	Nugent score/Amsel's criteria	Sensitivity	350/381	91.9	[88.7 - 94.4]
		Specificity	330/383	86.2	[82.3 - 89.5]
BV	Nugent score/Amsel's criteria	Sensitivity	311/326 ^c	95.4	[92.5 - 97.4]
		Specificity	297/321 ^d	92.5	[89.1 - 95.2]
^a Sensitivity = True POS/Total POS from reference method and Specificity= True NEG/Total NEG from reference method					
^b «Unresolved» non-reportable results were excluded from Sensitivity/Specificity calculation (<1%)					
^c 55 specimens with intermediate Nugent Score and classified as POS by Amsel's criteria were excluded.					
^d 62 specimens with intermediate Nugent Score and classified as NEG by Amsel's criteria were excluded.					

[0078] This example demonstrates that the compositions and methods disclosed herein can be used to detect organisms related to VVC, TV, and BV with high specificity and sensitivity.

Example 2**Selection of primers and probes for multiplex detection of WC. trichomoniasis and BV in vaginal samples**

[0079] Various primers and probes have been designed and tested for their performance in amplification and detection of VVC-associated *Candida* species, *T. vaginalis*, and BV individually or in a multiplex fashion. Table 6a and 6b provide various primers, primer pairs, and probes that were not selected because of a number of undesired properties, including weak signal, lack of amplification, large size of amplicon, false positive signal, non-specific detection, sensitivity to temperature variation, failure to detect large number of variant strains, limitations in multiplex assay, selective of partner primers/probes, interaction with other primers/probes. Surprisingly, as described in Example 1, a number of primers and probes were found to perform well in the amplification and detection of VVC-associated *Candida* species, *T. vaginalis*, and BV individually or in a multiplex fashion. The superior properties of those primers, probes and some combination thereof were unpredicted. Moreover, the ability of the oligonucleotides of SEQ ID NOs: 1-16 to effectively perform (i.e., specifically amplify and detect target DNA) in a multiplex real-time PCR reaction was not predicted. Similarly, the ability of the oligonucleotides of SEQ ID NOs: 17-29 to effectively perform (i.e., specifically amplify and detect target DNA) in a multiplex real-time PCR reaction also was not predicted.

Table 6a. Non-selected primers and probes for detection of BV

Analyte (target organism)	Targeted gene	Non-selected primers, primer pairs and probes	Primer and probe sequences (5'-3')
<i>Atopobium vaginae</i>	16S rRNA	HINAVFW	GTTAGGTCAGGAGTTAAATCTG (SEQ ID NO: 33)
		HINAVRV	TCATGGCCCAGAAGACC (SEQ ID NO: 34)
		HINAV-RVA	TCGTGGCCCAGAAGGCC (SEQ ID NO: 35)
		AVFP-BV1	CCCTGGTAGTCCTAGCT (SEQ ID NO: 36)
		AVFP-BV1A*	CCCTGGTAGTCCTAGCC (SEQ ID NO: 37)
		AVRP-BV1	CGGCACGGAAAGTATAATCT (SEQ ID NO: 38)
		<u>Forward primer (FW):</u> ATOVAGRT3FW	GGTGAAGCAGTGGAACACT (SEQ ID NO: 39)
		<u>Reverse primer (RV):</u> MCF-AV-R2	GCAGCCCAGGACATAAGG (SEQ ID NO: 41)
		<u>RV:</u> ATOVAGRT3REV*	ATTCGCTTCTGCTCGCGCA (SEQ ID NO: 42)
		<u>FW :</u> ATOP-442F	GCAGGGACGAGGCCGCAA (SEQ ID NO: 43)
		<u>RV:</u> HINAVRV	TCATGGCCCAGAAGACC (SEQ ID NO: 44)
<u>FW:</u> MCF-AV-F1, and	CGGATTCATTGGGCGTAAA (SEQ ID NO: 45)		

Analyte (target organism)	Targeted gene	Non-selected primers, primer pairs and probes	Primer and probe sequences (5'-3')
		<u>RV</u> : MCF-AV-R3	CGCCTCAGCGTCAGT (SEQ ID NO: 46)
		<u>FW</u> : MCF-AV-F1, and	CGGATTCATTGGGCGTAAA (SEQ ID NO: 47)
		<u>RV</u> : MCF-AV-R4	ACACCTAGTGTCCATCGTTTA (SEQ ID NO: 48)
		<u>FW</u> : MCF-AV-F2, and	CCTTCGGGTTGTAAACCG (SEQ ID NO: 49)
		<u>RV</u> : MCF-AV-R3	CGCCTCAGCGTCAGT (SEQ ID NO: 50)
<i>BVAB2</i>	16S	<u>FW</u> : HINBVAB2FW, and	AGGCGGCTAGATAAGTGTGA (SEQ ID NO: 51)
		<u>RV</u> : HINB VAB2RV	TCCTCTCCAGCACTCAAGCTAA (SEQ ID NO: 52)
		<u>FW</u> : BVAB2-619F, and	TTAACCTTGGGGTTCATTACAA (SEQ ID NO: 53)
		<u>RV</u> : BVAB2-1024R	AATTCAGTCTCCTGAATCGTCAGA (SEQ ID NO: 54)
		<u>FW</u> : BVAB2 585FA	GCGGCTAGATAAGTGTGATGTTT (SEQ ID NO: 4)
		<u>FW</u> : HINBVAB2FW	AGGCGGCTAGATAAGTGTGA (SEQ ID NO: 55)
		<u>FW</u> : BVABFP-BV2	CGTGTAGGCGGCTAGATAAGTG (SEQ ID NO: 56)
		<u>RV</u> : BVAB2_879R	GAATACTTATTGTGTTAACTGCGC (SEQ ID NO: 57)
<i>Megasphaera type 1</i>	16S	<u>FW</u> : HINMGSTYP1FW, and	GACGGATGCCAACAGTATCCGTCCG (SEQ ID NO: 7)
		<u>RV</u> : HINMGSTYP1RV	AAGTTCGACAGTTTCCGTCCCCTC (SEQ ID NO: 58)
<i>Gardnerella vaginalis</i>	vaginolysin (vly)	<u>FW</u> : GWLYFW1, and	GCGGCGAAAGTGCTGTA (SEQ ID NO: 59)
		<u>RV</u> : GVLYRV1	AGCCGTTCACTGCGGAAGT (SEQ ID NO: 12)
		<u>FW</u> : GWLYFW2, and	GCCAACGATGATCGCGTAT (SEQ ID NO: 10)
		<u>RV</u> : GVLYRV2A	CAAGCTCGGCATGTTATCCAT (SEQ ID NO: 60)
		<u>FW</u> : MCF-GV-F6	CCAGAATTTGATGGATAACATGCC (SEQ ID NO: 65)
		<u>FW</u> : MCF-GV-F7	ATGGACAATATGCCAAGCCT (SEQ ID NO: 66)
		<u>RV</u> : MCF-GV-R2	TTCACTGCGGAAGTTACAGA (SEQ ID NO: 67)
		<u>RV</u> : MCF-GV-R3	TTAACTGCGGAAGTAACGGA (SEQ ID NO: 68)
		<u>RV</u> : MCF-GV-R4	TTAACTGCTGAAGTAACGGA (SEQ ID

Analyte (target organism)	Targeted gene	Non-selected primers, primer pairs and probes	Primer and probe sequences (5'-3')
			NO: 69)
		Probes (5' fluorophore: Cy5: 3' fluorophore: BHQ2):	
		MCF-GV-T3-CY5-B2	ACAGCACTTTCGCCGCC (SEQ ID NO: 13)
		MCF-GV-T4-CY5-B2	ACAGCACTCTCGGCCGCC (SEQ ID NO: 70)
<i>Gardnerella vaginalis</i>	16S rRNA	<u>FW</u> : HINGVFW, and	GGAAACGGGTGGTAATGCTGG (SEQ ID NO: 61)
		<u>RV</u> : HINGVRV	CGAAGCCTAGGTGGGCCATT (SEQ ID NO: 62)
		<u>FW</u> : GV1FW, and	TTACTGGTGTATCACTGTAAGG (SEQ ID NO: 63)
		<u>RV</u> : GV3RV	CCGTCACAGGCTGAACAGT (SEQ ID NO: 64)
<i>Lactobacillus crispatus</i>	16S rRNA	<u>FW</u> : L.crisp-452F, and	GATAGAGGTAGTAACTGGCCTTTA (SEQ ID NO: 71)
		<u>RV</u> : L.crisp-1023R	CTTTGTATCTCTACAAATGGCACTA (SEQ ID NO: 72)
		<u>FW</u> : HIN LG FW, and	CGAGCTTGCCTAGATGAATTTG (SEQ ID NO: 73)
		<u>RV</u> : HIN LG RV	CTCTAGACATGCGTCTAGTG (SEQ ID NO: 74)
		<u>FW</u> : HIN LC FW, and	GATTTACTTCGGTAATGACGTTAGGA (SEQ ID NO: 75)
		<u>RV</u> : HIN LC RV	AGCTGATCATGCGATCTGCTTTC (SEQ ID NO: 76)
		<u>FW</u> : HIN LJ FW	GCCTATAGAAATTCTTCGGAATGGACA (SEQ ID NO: 77)
		<u>RV</u> : HIN LJ RV	CAAATGGTATCCCAGACTTAAGGG (SEQ ID NO: 78)
		<u>FW</u> : MEG-LG_LJ-F6	GTCGAGCGAGCTTGCCCTA (SEQ ID NO: 79)
		<u>FW</u> : MCF-LC-F4	GAACTAACAGATTTACTTCGGTAATG (SEQ ID NO: 80)
		<u>RV</u> : MCF-LG-R3	AAACTCTAGACATGCGTCTAGT (SEQ ID NO: 81)
		<u>RV</u> : MCF-LJ_LC-R1	GTTTCCAAATGGTATCCCAGA (SEQ ID NO: 82)
		Probes:	
		MCF-Lj-Lc-T1_ROX-B2	Probes:
		MCF-Lg-T5_ROX-B2	CGGCGGATGGGTGAGTAAC (SEQ ID NO: 103)
		MCF-Lj-T7_ROX-B2	CCAAGAGACTGGGATAACACCTG (SEQ

Analyte (target organism)	Targeted gene	Non-selected primers, primer pairs and probes	Primer and probe sequences (5'-3')
			ID NO: 105)
		MCF-Lc-T3_ROX-B2	TCTTCGGAATGGACATAGATACAAGCTA (SEQ ID NO: 115)
			ATCCGCCGCTCGCTTT (SEQ ID NO: 116)
		<u>FW</u> : MCF-LG-F5	GCCTAGATGAATTTGGTGCTT (SEQ ID NO: 83)
		<u>FW</u> : MCF-LJ-F6	CGAGCTTGCCTATAGAAATTCTT (SEQ ID NO: 84)
		<u>FW</u> : MCF-LC-F4	GAACTAACAGATTTACTTCGGTAATG (SEQ ID NO: 85)
		<u>RV</u> : MCF-LG-R3	AAACTCTAGACATGCGTCTAGT (SEQ ID NO: 86)
		<u>RV</u> : MCF-LJ_LC-R1	GTTTCCAAATGGTATCCCAGA (SEQ ID NO: 87)
		Probes:	Probes:
		MCF-Lj -Lc-T1_ROX-B2	CGGCGGATGGGTGAGTAAC (SEQ ID NO: 103)
		MCF-Lg-T5_ROX-B2	CCAAGAGACTGGGATAACACCTG (SEQ ID NO: 105)
			TTAAAAGGCGGCGTAAGC (SEQ ID NO: 14)
		<u>FW</u> : MCF-LJ_LC-F8	ACTAGACGCATGTCTAGAGTTT (SEQ ID NO: 88)
		<u>FW</u> : MCF-LG-F9	GCCAGTTACTACCTCTATC (SEQ ID NO: 15)
		<u>RV</u> : MCF-LSP-R6	TGCATTAGCTAGTTGGTAAGGTAAC (SEQ ID NO: 89)
		Primers:	Primers:
		MCF-Lj_Lc-F8	TTAAAAGGCGGCGTAAGC (SEQ ID NO: 14)
		MCF-Lg-F9	ACTAGACGCATGTCTAGAGTTT (SEQ ID NO: 88)
		MCF-Lj_Lc-R7	
		<u>Probes: (5' fluorophore:</u>	GCCAGTTACTACCTCTATCCT (SEQ ID NO: 15)
		<u>ROX: 3' fluorophore:</u>	Probes:
		<u>BHQ2):</u>	
		MCF-LSP-T8_ROX-B2	AAGTCTGATGGAGCAACGCC (SEQ ID NO: 16)
		MCF-LSP-T11_ROX-B2	ACATTGGGACTGAGACACGG (SEQ ID NO: 90)
		MCF-LSP-T13_ROX-B2	AGGCTTACCAAGGCGATGAT (SEQ ID NO: 91)
		MCF-LJ LC-T15 ROX-B2	

Analyte (target organism)	Targeted gene	Non-selected primers, primer pairs and probes	Primer and probe sequences (5'-3')
		MCF-LG-T16 ROX-B2	CGGCTTACCAAGGCAATGAT (SEQ ID NO: 92)
		<u>FW</u> : HIN LG FW	CGAGCTTGCCTAGATGAATTTG (SEQ ID NO: 97)
		<u>FW</u> : HIN LJ FW	GCCTATAGAAATTCTTCGGAATGGACA (SEQ ID NO: 98)
		<u>FW</u> : HIN LC FW	GATTTACTTCGGTAATGACGTTAGGA (SEQ ID NO: 99)
		<u>RV</u> : HIN LG RV	CTCTAGACATGCGTCTAGTG (SEQ ID NO: 100)
		<u>RV</u> : HIN LJ RV	CAAATGGTATCCCAGACTTAAGGG (SEQ ID NO: 101)
		<u>RV</u> : HIN LC RV	AGCTGATCATGCGATCTGCTTTC (SEQ ID NO: 102)
		<u>Probes (5' fluorophore:</u>	<u>Probes:</u>
		<u>ROX: 3' fluorophore:</u> <u>BHQ2):</u>	CGGCGGATGGGTGAGTAAC (SEQ ID NO: 103)
		MCF-Lj -Lc-T1_ROX-B2	CCAAGAGACTGGGATAACACCTG (SEQ ID NO: 105)
		MCF-Lg-T5_ROX-B2	

Table 6b. Non-selected primers and probes for detection of VVC and trichomoniasis

Analyte (target organism)	Targeted gene	Non-selected primers and probes	Primer and probe sequences (5'-3')
<i>Candida albicans</i>	RNase P RNA 1 (RPR1)	<u>FW</u> : cand-CR1	CGGGTGGGAAATTCGGT (SEQ ID NO: 117)
		<u>RV</u> : cand-CR5	CAATGATCGGTATCGGGT (SEQ ID NO: 118)
		<u>Probes:</u>	<u>Probes:</u>
		alb-T-FAM-B1	CAGCTTGTAGTAAAGAATTACTCAC (SEQ ID NO: 119)
		cand-T-FAM-B1	TTCGCATATTGCACTAAATAG (SEQ ID NO: 120)
		cand-Ta-FAM-B1	TTCGCATATTGCACTAAACAG (SEQ ID NO: 121)
<i>Candida albicans</i>	Topoisomerase III	<u>FW</u> : MenCa1377fw	CAACGCCAACGAAGACAAG (SEQ ID NO: 122)
		<u>RV</u> : MenCa1472rv	CCAGCTTTGTTTGCATCAA (SEQ ID NO: 123)
		<u>Probe:</u>	<u>Probe:</u>
		MenCa-T-FAM-B1	AAAGCCGATGGTAGTAGAAAAGTGC (SEQ ID NO: 124)
<i>Candida albicans</i>	Topoisomerase II	<u>FW</u> : CABF59	TTGAACATCTCCAGTTTCAAAGGT (SEQ ID NO: 125)
		<u>RV</u> : CABR110	GTTGGCGTTGGCAATAGCTCTG (SEQ ID NO: 126)

Analyte (target organism)	Targeted gene	Non-selected primers and probes	Primer and probe sequences (5'-3')
<i>Candida species</i>	CHS1	<u>FW:</u> Jorprimer1Fw	CGCCTCTTGATGGTGATGAT (SEQ ID NO: 127)
		<u>RV:</u> Jorprimer2Rv	TCCGGTATCACCTGGCTC (SEQ ID NO: 128)
		<u>Probes:</u> JorCa-T-FAM-B1	Probes: CGTTCGTA TAGAGTTGTGTTGTTTTGGAT (SEQ ID NO: 129)
		JorCpara-T-FAM-	GAGGCTGTGATGTGTGCTGTTGACCAG (SEQ ID NO: 130)
		B1 JorCtro-T-FAM-B1	AGGCTTGCTCTTTGTCTGGGCGAGCGAACG (SEQ ID NO: 131)
<i>Candida species</i>	TEF cand-	<u>FW Primers:</u> ECanG278	FW Primers: CAGGTCACAGAGATTTCAATCAAG (SEQ ID NO: 132)
		CR1-NP-CaCR1-NP-Ca	GAAATTCGGTGGTACGCTCC (SEQ ID NO: 133)
		cand-CR 1-NP-CtCp	GAAATTCGGTGGTACTCTCC (SEQ ID NO: 134)
		RT-Ca_Cd-F2 RT-Ctro-F3	GTTGTGACTCTTTCAATGCCCAA (SEQ ID NO: 135)
		RT-Cpara-F4	GTTGTGACTCTTTCAACGCTCAA (SEQ ID NO: 136)
		<u>RV Primers:</u> ECanG401	GATGTGACTCCTTCAATGCTCAA (SEQ ID NO: 137)
		ECanG401a Cand-CR5-NP-CaCt	RV Primers:
		Cand-CR5-NP-Cp RT-Cdub-R4	GTAAGCCAACAAAGCGTGTCTC (SEQ ID NO: 138)
		<u>Probes:</u>	GAAAGCCAATAGAGCGTGTCTC (SEQ ID NO: 139)
		ECanG-TL1-O2-FAM-B 1	GATCGGTATCGGGTGCTTG (SEQ ID NO: 140)
		cand-T-FAM-B 1	GATCGGTATCGGGTTCTTG (SEQ ID NO: 141)
		cand-Ta-FAM-B 1	CAGCGTCACCGGATTTGAC (SEQ ID NO: 142)
		<u>Probes:</u>	
		RT-Ca_Cd_Cp-T1-FAM-B1	TGATTATTGCTGGTGG (SEQ ID NO: 143)
		RT-Ctro-T4-FAM-B1	TTCGCATATTGCACTAAATAG (SEQ ID NO: 120)
		RT-Ca_Cd-T2-FAM-B1	TTCGCATATTGCACTAAACAG (SEQ ID NO: 121)
		RT-Cpar-T6-FAM-B1	TGCTTGTAATTCGACACTTTG (SEQ ID NO: 144)
			TGTAAATTCGACACCTTGTTGA (SEQ ID NO: 145)

Analyte (target organism)	Targeted gene	Non-selected primers and probes	Primer and probe sequences (5'-3')
			145)
			TTGTAAATTCGACACTTTGGTTG (SEQ ID NO: 146)
			CGACACTTTGATTGAAAAGATTGAC (SEQ ID NO: 147)
<i>Candida species</i>	ITS2		<u>FW Primers:</u>
		<u>FW Primers:</u>	GGGTTTGCTTGAAAGACGGTA (SEQ ID NO: 148)
		ITS2-Ca-Fow	CGTGGTAACTTATTTTAAGCG (SEQ ID NO: 149)
		ITS2-Ctr-Fow	GGGTTTGGTGTGAGCGATAC (SEQ ID NO: 150)
		ITS2-Cpar-Fow	
		<u>RV Primers:</u>	<u>RV Primers:</u>
		ITS2-Ca-Rev	TTGAAGATATACGTGGTGGACGTTA (SEQ ID NO: 151)
		ITS2-Ctr-Rev	GCTTAAGTTCAGCGGGTAGTCCTA (SEQ ID NO: 152)
		ITS2-Cpar-Rev	GGAGTTTGTACCAATGAGTGGAAA (SEQ ID NO: 153)
		<u>Probes:</u>	<u>Probes</u>
		ITS2-Ca-CFO-B 1	ACCTAAGCCATTGTCAAAGCGATCCCG (SEQ ID NO: 154)
		ITS2-Ctr-CFO-B 1	TGGCCACCATTATTTTCATAACTTTGACC (SEQ ID NO: 155)
		ITS2-Cpar-CFO-B 1	CTCCGCCTTTCTTTCAAGCAAACCCAG (SEQ ID NO: 156)
		<i>Candida glabrata</i>	RNase P RNA 1 (RPR1)
gla-CR3	GGCAACGGCTGGGAAT (SEQ ID NO: 157)		
gla-CR3a	AGCAACGGCTGGGAAT (SEQ ID NO: 158)		
<u>Primer:</u>	<u>RV Primer:</u>		
<u>RV cand-CR5</u>	CAATGATCGGTATCGGGT (SEQ ID NO: 159)		
<u>Probe:</u>	<u>Probe:</u>		
gla-T-FAM-B1	TAAAGCCTCACCACGATTTTGACAC (SEQ ID NO: 160)		
<i>Candida glabrata</i>	Topoisomerase II	<u>FW Primer:</u>	CCCAAAAATGGCCGTAAGTATG (SEQ ID NO: 161)
		CGBF35	
		<u>RV Primer:</u>	CTGCTTGAAAGAAATATCGGAGAC (SEQ ID NO: 162)
		CGBR77	
<i>Candida glabrata</i>	CHS1	<u>FW:</u> Jorprimer1Fw	CGCCTCTTGATGGTGATGAT (SEQ ID NO: 127)
		<u>RV:</u>	TCCGGTATCACCTGGCTC (SEQ ID NO: 128)

Analyte (target organism)	Targeted gene	Non-selected primers and probes	Primer and probe sequences (5'-3')
		Jorprimer2Rv	
			<u>Probe:</u>
		<u>Probe:</u>	CGACTGGTTGACGATAATCAGAGGAGATGGG (SEQ ID NO: 163)
		JorCgla-T-FAM-B1	
<i>Candida glabrata</i>	TEF	<u>FW Primers:</u>	<u>Primers:</u>
		RT-Cgla-F5	ACCCACCAAAGGCTGCT (SEQ ID NO: 164)
		RT-Cgla-F6	CGACCCACCAAAGGCTGCT (SEQ ID NO: 165)
		<u>Probe:</u>	<u>Probe:</u>
		RT-Cgla-T8-FAM-B1	ACTGTCACACCGCCACATT (SEQ ID NO: 166)
<i>Candida krusei</i>	RNase P RNA 1 (RPR1)	<u>FW Primers:</u>	CGGGTGGGAAATTCGGT (SEQ ID NO: 117)
		cand-CR1	ATAGAGTAGCTCGGTCCC (SEQ ID NO: 167)
		kru-CR1-SiT	TAGTGATCGGTATCGAGTT (SEQ ID NO: 168)
		<u>RV Primers:</u>	CGGTATCGAGTTTCCATG (SEQ ID NO: 169)
		kru-CR5	
		Kru-CR5-NP2	<u>Probe:</u>
		<u>Probe:</u>	CCAAAGTTGTACAAGCAAGTACCA (SEQ ID NO: 170)
		kru-T-FAM-B1	
<i>Candida krusei</i>	Topoisomerase II (KANBE, 2002)	<u>FW:</u> CKSF35	GAGCCACGGTAAAGAATACACA (SEQ ID NO: 171)
		<u>RV:</u> CKSR57	TTTAAAGTGACCCGGATACC (SEQ ID NO: 172)
<i>Candida krusei</i>	TEF	<u>RV Primers:</u>	CTTTGGATGGTCTTCAACAGA (SEQ ID NO: 173)
		RT-Ckru-R5	173)
		SiT-Ckru-R10	ATCACCAGACTTGACGG (SEQ ID NO: 174)
		<u>Probes:</u>	<u>Probes:</u>
		SiT-Ckru-T10-CFO-B1	AGTCTGTTGAAGACCATCCA (SEQ ID NO: 175)
		SiT-Ckru-T9-CFO-B1	ATGTAAGTTCGACGAATTAATC (SEQ ID NO: 176)
TV	Ap65-1	<u>FW Primers:</u>	TCTGGCAAGATCAAGGACAT (SEQ ID NO: 177)
		NP.TV.MAX.FP1	
		SiT.TV.MAX.FP1	GAAGATTCTGGCAAGATCA (SEQ ID NO: 178)
		<u>RV Primers:</u>	CATCTGTAACGACAATGCAGC (SEQ ID NO: 179)
		NP.TV.MAX.RP1	
		SiT.TV.MAX.RP1	GACAATGCAGCGGAT (SEQ ID NO: 180)
		<u>Probes:</u>	<u>Probes:</u>
		NP.TV.MAX.D1-	AACTACCCACGCCAGGACAT (SEQ ID NO: 181)

Analyte (target organism)	Targeted gene	Non-selected primers and probes	Primer and probe sequences (5'-3')
		T-FAM-B1	181)
		SiT.TV.MAX.D1-T-FAM-B1	CCGCAACTACCCACGCCA (SEQ ID NO: 182)

[0080] Tables 7a and 7b provide a number of master mixes of primers and probes that were not selected because of a number of undesired properties, including false positive signal and failure to detect variant strains.

Table 7a. Non-selected master mixes for detection of BV

Master Mix ID	Primers and Probes	Primer and Probe Sequences (5'-3')
Master Mix I	<u>Primers:</u>	GCGGCTAGATAAGTGTGATGTTT (SEQ ID NO: 4)
	BVAB2_585Fa	CTCTCCAGCACTCAAGCTAAA (SEQ ID NO: 5)
	BVAB2_666RA	TTAAAAGGCGGCGTAAGC (SEQ ID NO: 14)
	MCF-LJ_LC-F8	ACTAGACGCATGTCTAGAGTTT (SEQ ID NO: 88)
	MCF-LG-F9	GCCAGTTACTACCTCTATC (SEQ ID NO: 15)
	MCF-LSP-R6	CCCTATCCGCTCCTGATACC (SEQ ID NO: 1)
	MENAV248FW	CCAAATATCTGCGCATTTC (SEQ ID NO: 2)
	MENAV334RV	CCAGAATTTGATGGATAACATGCC (SEQ ID NO: 65)
	MCF-GV-F6	65)
	MCF-GV-F7	ATGGACAATATGCCAAGCCT (SEQ ID NO: 66)
	MCF-GV-R2	TTCACTGCGGAAGTTACAGA (SEQ ID NO: 67)
	MCF-GV-R4	TTAACTGCTGAAGTAACGGA (SEQ ID NO: 69)
	MEGAE-456F	GATGCCAACAGTATCCGTCG (SEQ ID NO: 7)
	MEGAE-667R	CCTCTCCGACACTCAAGTTCGA (SEQ ID NO: 8)
	<u>Probes:</u>	FAM-CAAGGCTTAACCTGGGGTTCATTACAA-BHQ1 (SEQ ID NO: 6)
	BVAB2_613_641_FAM-B1	ROX-AAGTCTGATGGAGCAACGCC-BHQ2 (SEQ ID NO: 16)
	MCF-LSP-T11_ROX-B2	FAM-TCCCCTACCAGACTCAAGCCTGC-BHQ1 (SEQ ID NO: 3)
	MCF-AV-T4_FAM-B1	Cy5-ACAGCACTTTCGCCGCC-BHQ2 (SEQ ID NO: 13)
	MCF-GV-T3-CY5-B2	ID NO:
	MCF-GV-T4-CY5-B2	Cy5-ACAGCACTCTCGCCGCC-BHQ2 (SEQ 70)
MEGA_485-506-T-HEX-BHQ1	HEX-GTACCGTAAGAGAAAGCCACGG-BHQ1 (SEQ ID NO: 9)	
Master Mix II	<u>Primers:</u>	GCGGCTAGATAAGTGTGATGTTT ID NO:
	BVAB2585Fa	(SEQ 4)
	_BVAB2_666Ra	CTCTCCAGCACTCAAGCTAAA (SEQ ID NO: 5)
	MCF-Lj_Lc-F8	TTAAAAGGCGGCGTAAGC (SEQ ID NO: 14)

Master Mix ID	Primers and Probes	Primer and Probe Sequences (5'-3')
	MCF-Lg-F9	ACTAGACGCATGTCTAGAGTTT (SEQ ID NO: 88)
	MCF-Lsp-R6	GCCAGTTACTACCTCTATC (SEQ ID NO: 15)
	MenAv248fw	CCCTATCCGCTCCTGATACC (SEQ ID NO: 1) CCAAATATCTGCGCATTTC (SEQ ID NO: 2)
	MenAv334rv	
	MenGV981fw,	CGCATCTGCTAAGGATGTTG (SEQ ID NO: 106) CAGCAATCTTTTCGCCAACT (SEQ ID NO: 107)
	MenGV1072rv	
	MegaE-456F	GATGCCAACAGTATCCGTCCG (SEQ ID NO: 7)
	MegaE-667R	CCTCTCCGACACTCAAGTTCGA (SEQ ID NO: 8)
	<u>Probes:</u>	FAM-CAAGGCTTAACCTTGGGGTTCATTACAA-BHQ1 (SEQ ID NO: 6)
	BVAB2_613_641_CFO-B1	ROX-AAGTCTGATGGAGCAACGCC-BHQ2 (SEQ ID NO: 16)
	MCF-Lsp-T11_ROX-B2	
	MCF-Av-T4_FAM-B1	FAM-TCCCCTACCAGACTCAAGCCTGC-BHQ1 (SEQ ID NO: 3)
	MenGV-T-ROX-B2	
	Mega_485-506-T-CFO-BHQ1	ROX-TGCAACTATTTCTGCAGCAGATCC-BHQ2 (SEQ ID NO: 108)
		CFO-GTACCGTAAGAGAAAGCCACGG-BHQ 1 (SEQ ID NO: 9)
Master Mix IIII	<u>Primers:</u>	GCGGCTAGATAAGTGTGATGTTT ID NO:
	BVAB2_585Fa	(SEQ 4)
	BVAB2_666Ra	CTCTCCAGCACTCAAGCTAAA (SEQ ID NO: 5)
	MCF-Lj_Lc-F8	TTAAAAGGCGGCGTAAGC (SEQ ID NO: 14)
	MCF-Lg-F9	ACTAGACGCATGTCTAGAGTTT (SEQ ID NO: 88) GCCAGTTACTACCTCTATC ID NO:
	MCF-Lsp-R6	(SEQ 15)
	MenAv248fw	CCCTATCCGCTCCTGATACC (SEQ ID NO: 1)
	MenAv334rv	CCAAATATCTGCGCATTTC (SEQ ID NO: 2) GCCAACGATGATCGCGTAT ID NO:
	GVvlyfw2	(SEQ 10) CAGGCTTGGCATATTGTCCAT ID NO:
	GVvlyrv2	(SEQ 109)
	GVvlyfw2a	GCCAATAATGACCGCGTAT (SEQ ID NO: 11)
	GVvlyrv2a	CAAGCTCGGCATGTTATCCAT (SEQ ID NO: 60) GATGCCAACAGTATCCGTCCG ID NO:
	MegaE-456F	(SEQ 7) CCTCTCCGACACTCAAGTTCGA ID NO:
	MegaE-667R	(SEQ 8)
	<u>Probes:</u>	FAM-CAAGGCTTAACCTTGGGGTTCATTACAA-BHQ1 (SEQ ID NO: 6)

Master Mix ID	Primers and Probes	Primer and Probe Sequences (5'-3')
		ROX-AAGTCTGATGGAGCAACGCC-BHQ2 (SEQ
	BVAB2_613_641_CFO-B1	ID NO: 16)
	MCF-Lsp-T11_ROX-B2	FAM-TCCCCTACCAGACTCAAGCCTGC-BHQ1 (SEQ ID NO: 3)
	MCF-Av-T4_FAM-B1	ROX-CCCAGGTGCTCTTTTCCGTGCTGA-BHQ2
	GVvly-T2-ROX-B2	(SEQ ID NO: 110)
	GVvly-T2a-ROX-B2	ROX-CCCAGGTGCGCTGTTCCGCGCTGA-BHQ2 (SEQ ID NO: 111)
	Mega_485-506-T-CFO-BHQ1	CFO-GTACCGTAAGAGAAAGCCACGG-BHQ 1 (SEQ ID NO: 9)
Master Mix IV	<u>Primers:</u>	
	BVAB2_585Fa	GCGGCTAGATAAGTGTGATGTTT (SEQ ID NO: 4) CTCTCCAGCACTCAAGCTAAA ID NO:
	BVAB2_666Ra	(SEQ 5)
	MCF-Lj_Lc-F8	TTAAAAGGCGGCGTAAGC (SEQ ID NO: 14)
	MCF-Lg-F9	ACTAGACGCATGTCTAGAGTTT (SEQ ID NO: 88)
	MCF-Lsp-R6	GCCAGTTACTACCTCTATC (SEQ ID NO: 15) CCCTATCCGCTCCTGATACC ID NO:
	MenAv248fw	(SEQ 1)
	MenAv334rv	CCAAATATCTGCGCATTTC (SEQ ID NO: 2)
	GVvlyfw1	GGCGGCGAAAGTGCTGTA (SEQ ID NO: 59)
	GVvlyrv1	AGCCGTTCACTGCGGAAGT (SEQ ID NO: 12) GGCGGCGAAAGTGCTGTC ID NO:
	GVvlyfw1a	(SEQ 112) GATGCCAACAGTATCCGTCCG ID NO:
	MegaE-456F	(SEQ 7) CCTCTCCGACACTCAAGTTCTGA ID NO:
	MegaE-667R	(SEQ 8)
	<u>Probes:</u>	
		FAM-CAAGGCTTAACCTTGGGGTTCATTACAA-BHQ1 (SEQ ID NO: 6)
	BVAB2_613_641_CFO-B1	ROX-AAGTCTGATGGAGCAACGCC-BHQ2 (SEQ
	MCF-Lsp-T11_ROX-B2	ID NO: 16)
	MCF-Av-T4_FAM-B1	FAM-TCCCCTACCAGACTCAAGCCTGC-BHQ1 (SEQ ID NO: 3)
	GVvly-T1-ROX-B2	
	Gwly-T1a-ROX-B2	ROX-TTCAGCGCCCAACCAAGAGCTCTGT-BHQ2 (SEQ ID NO: 113)
	Mega_485-506-T-CFO-BHQ1	ROX-TTAAGCATCCAATAAGAGCTCTGT-BHQ2 (SEQ ID NO: 114)
		CFO-GTACCGTAAGAGAAAGCCACGG-BHQ1 (SEQ ID NO: 9)

Table 7b. Non-selected master mixes for detection of VVC and trichomoniasis

Master Mix ID	Primers and Probes	Primer and Probe Sequences (5'-3')
Master Mix I	SiT-Cgla-F8	CGAACAATTGACTGAAGGTTTG (SEQ ID NO: 20)
	RT-Cgla-R7	
	RT-Cgla-T7-Fam-B1	CGGACTTCAAGAACTTTGGAGA (SEQ ID NO: 21)
	TV.MAX.FP1	
	TV.MAX.RP1	CTTGTAAGTTCTGAAGAATTGTTGGA (SEQ ID NO: 22)
	TV.MAX.D1-T-ROX-B2	
	kru-CR1-SiT	GAAGATTCTGGCAAGATCAAGGA (SEQ ID NO: 17)
	Kru-CR5-NP2	
	kruS-T-FAM-B1	ACGACAATGCAGCGGATGT (SEQ ID NO: 18)
	RT-Ca_Cd_Ct-F1	ATCCTCCGCAACTACCCACGCCA (SEQ ID NO: 19)
	RT-Cpara-F4	
	RT-Ca_Ct-R3	ATAGAGTAGCTCGGTCCC (SEQ ID NO: 167)
	RT-Cpar-R6	CGGTATCGAGTTTCCATG (SEQ ID NO: 169)
	RT-Cdub-R4	CCAAAGTTGTACAAGCAAGTACCA (SEQ ID NO: 170)
	RT-Ca_Cd_Cp-T1-FAM-B1	
	RT-Ctro-T4-FAM-B1	CCACCAAAGGTTGTGAC (SEQ ID NO: 23)
		GATGTGACTCCTTCAATGCTCAA (SEQ ID NO: 137)
		CAGCATCACCGGATTTGAC (SEQ ID NO: 24)
		CGGACTTGATGAATTTTGGTTCA (SEQ ID NO: 25)
		CAGCGTCACCGGATTTGAC (SEQ ID NO: 142)
		TGCTTGTAATTCGACACTTTG (SEQ ID NO: 144)
		TGTAAATTCGACACCTTGGTTGA (SEQ ID NO: 145)
Master Mix II	SiT-Cgla-F8	CGAACAATTGACTGAAGGTTTG (SEQ ID NO: 20)
	RT-Cgla-R7	
	RT-Cgla-T7-Fam-B1	CGGACTTCAAGAACTTTGGAGA (SEQ ID NO: 21)
	TV.MAX.FP1	
	TV.MAX.RP1	CTTGTAAGTTCTGAAGAATTGTTGGA (SEQ ID NO: 22)
	TV.MAX.D1-T-ROX-B2	
	kru-CR1-SiT	GAAGATTCTGGCAAGATCAAGGA (SEQ ID NO: 17)
	Kru-CR5-NP2	
	kruS-T-FAM-B1	ACGACAATGCAGCGGATGT (SEQ ID NO: 18)
	RT-Ca_Cd-F2	ATCCTCCGCAACTACCCACGCCA (SEQ ID NO: 19)
	RT-Ctro-F3	
	RT-Cpara-F4	ATAGAGTAGCTCGGTCCC (SEQ ID NO: 167)
	RT-Ca_Ct-R3	CGGTATCGAGTTTCCATG (SEQ ID NO: 169)
	RT-Cpar-R6	CCAAAGTTGTACAAGCAAGTACCA (SEQ ID NO: 170)

Master Mix ID	Primers and Probes	Primer and Probe Sequences (5'-3')
	RT-Cdub-R4	170)
	RT-Ca_Cd-T3-FAM-B1	GTTGTGACTCTTTCAATGCCCAA (SEQ ID
	RT-Ctro-T4-FAM-B1	NO: 135)
	RT-Cpar-T6-FAM-B1	GTTGTGACTCTTTCAACGCTCAA (SEQ ID NO: 136)
		GATGTGACTCCTTCAATGCTCAA (SEQ ID NO: 137)
		CAGCATCACCGGATTTGAC (SEQ ID NO: 24)
		CGGACTTGATGAATTTGGTTCA (SEQ ID NO: 25)
		CAGCGTCACCGGATTTGAC (SEQ ID NO: 142)
		TGCTTGTAATTCGACACTTTGGTTG (SEQ ID NO: 26)
		TGTAAATTCGACACCTTGGTTGA (SEQ ID NO: 145)
		CGACACTTTGATTGAAAAGATTGAC (SEQ ID NO: 147)
Master Mix III	SiT-Cgla-F8	CGAACAATTGACTGAAGGTTTG (SEQ ID NO: 20)
	RT-Cgla-R7	CGGACTTCAAGAACTTTGGAGA (SEQ ID NO: 21)
	RT-Cgla-T7-Fam-B1	CTTGTAAGTTCGAAGAATTGTTGGA (SEQ ID NO: 22)
	TV.MAX.FP1	GAAGATTCTGGCAAGATCAAGGA (SEQ ID NO: 17)
	TV.MAX.RP1	ACGACAATGCAGCGGATGT (SEQ ID NO: 18)
	TV.MAX.D1-T-ROX-B2	ATCCTCCGCAACTACCCACGCCA (SEQ ID NO: 19)
	RT-Ckm-F7	GCAGCTTCCTTCAATGCTCAA (SEQ ID NO: 27)
	RT-Ckru-R5	CTTTGGATGGTCTTCAACAGA (SEQ ID NO: 173)
	RT-Ckru-T9-FAM-B1	CATGTAAGTTGACGAATTAATCGA (SEQ ID NO: 29)
	RT-Ca_Cd_Ct-F1	CCACCAAAGGGTTGTGAC (SEQ ID NO: 23)
	RT-Ca_Ct-R3	CAGCATCACCGGATTTGAC (SEQ ID NO: 24)
	RT-Cpar-R6	CGGACTTGATGAATTTGGTTCA (SEQ ID NO: 25)
	RT-Ca_Cd_Cp-T1-FAM-B1	TGCTTGTAATTCGACACTTTG (SEQ ID NO: 144)
Master Mix IV	SiT-Cgla-F8	CGAACAATTGACTGAAGGTTTG (SEQ ID NO: 20)
	RT-Cgla-R7	
	RT-Cgla-T7-Fam-B1	CGGACTTCAAGAACTTTGGAGA (SEQ ID NO:

Master Mix ID	Primers and Probes	Primer and Probe Sequences (5'-3')
	TV.MAX.FP1	21)
	TV.MAX.RP1	CTTGTAAGTTCGAAGAATTGTTGGA (SEQ ID NO: 22)
	TV.MAX.D1-T-ROX-B2	GAAGATTCTGGCAAGATCAAGGA (SEQ ID NO: 17)
	RT-Ckru-F7	ACGACAATGCAGCGGATGT (SEQ ID NO: 18)
	SiT-Ckru-R10	ATCCTCCGCAACTACCCACGCCA (SEQ ID NO: 19)
	SiT-Ckru-T9-CFO-B1	GCAGCTTCCTTCAATGCTCAA (SEQ ID NO: 27)
	RT-Ca_Cd_Ct-F1	ATCACCAGACTTGACGG (SEQ ID NO: 174)
	RT-Ca_Ct-R3	ATGTAAGTTTCGACGAATTAATC (SEQ ID NO: 176)
	RT-Ca_Cd_Cp-T1-FAM-B1	CCACCAAAGGGTTGTGAC (SEQ ID NO: 23)
		CAGCATCACCGGATTTGAC (SEQ ID NO: 24)
		TGCTTGTAATTCGACACTTTG (SEQ ID NO: 144)
Master Mix V	SiT-Cgla-F8	CGAACAATTGACTGAAGGTTTG (SEQ ID NO: 20)
	RT-Cgla-R7	
	RT-Cgla-T7-Fam-B1	CGGACTTCAAGAACTTTGGAGA (SEQ ID NO: 21)
	TV.MAX.FP1	21)
	TV.MAX.RP1	CTTGTAAGTTCGAAGAATTGTTGGA (SEQ ID NO: 22)
	TV.MAX.D1-T-ROX-B2	
	RT-Ckru-F7	GAAGATTCTGGCAAGATCAAGGA (SEQ ID NO: 17)
	SiT-Ckru-R10a	
	SiT-Ckru-T10-CFO-B1	ACGACAATGCAGCGGATGT (SEQ ID NO: 18)
	RT-Ca_Cd_Ct-F1	ATCCTCCGCAACTACCCACGCCA (SEQ ID NO: 19)
	RT-Ca_Ct-R3	
	RT-Ca_Cd_Cp-T1-FAM-B1	GCAGCTTCCTTCAATGCTCAA (SEQ ID NO: 27)
		ATCACCAGACTTGACAG (SEQ ID NO: 28)
		AGTCTGTTGAAGACCATCCA (SEQ ID NO: 175)
		CCACCAAAGGGTTGTGAC (SEQ ID NO: 23)
		CAGCATCACCGGATTTGAC (SEQ ID NO: 24)
		TGCTTGTAATTCGACACTTTG (SEQ ID NO: 144)

[0081] One skilled in the art will appreciate that, for this and other processes and methods disclosed herein, the functions performed in the processes and methods can be implemented in differing order. Furthermore, the outlined steps and operations are only provided as examples, and some of the steps and operations can be optional, combined into fewer steps and operations, or expanded into additional steps and operations without detracting from the essence of the disclosed embodiments.

[0082] With respect to the use of substantially any plural and/or singular terms herein, those having skill in the art can translate from the plural to the singular and/or from the singular to the plural as is appropriate to the context and/or application. The various singular/plural permutations may be expressly set forth herein for sake of clarity.

[0083] It will be understood by those within the art that, in general, terms used herein, and especially in the appended claims (e.g., bodies of the appended claims) are generally intended as "open" terms (e.g., the term "including" should be interpreted as "including but not limited to," the term "having" should be interpreted as "having at least," the term "includes" should be interpreted as "includes but is not limited to," etc.). It will be further understood by those within the art that if a specific number of an introduced claim recitation is intended, such an intent will be explicitly recited in the claim, and in the absence of such recitation no such intent is present. For example, as an aid to understanding, the following appended claims may contain usage of the introductory phrases "at least one" and "one or more" to introduce claim recitations. However, the use of such phrases should not be construed to imply that the introduction of a claim recitation by the indefinite articles "a" or "an" limits any particular claim containing such introduced claim recitation to embodiments containing only one such recitation, even when the same claim includes the introductory phrases "one or more" or "at least one" and indefinite articles such as "a" or "an" (e.g., "a" and/or "an" should be interpreted to mean "at least one" or "one or more"); the same holds true for the use of definite articles used to introduce claim recitations. In addition, even if a specific number of an introduced claim recitation is explicitly recited, those skilled in the art will recognize that such recitation should be interpreted to mean at least the recited number (e.g., the bare recitation of "two recitations," without other modifiers, means at least two recitations, or two or more recitations). Furthermore, in those instances where a convention analogous to "at least one of A, B, and C, etc." is used, in general such a construction is intended in the sense one having skill in the art would understand the convention (e.g., "a system having at least one of A, B, and C" would include but not be limited to systems that have A alone, B alone, C alone, A and B together, A and C together, B and C together, and/or A, B, and C together, etc.). In those instances where a convention analogous to "at least one of A, B, or C, etc." is used, in general such a construction is intended in the sense one having skill in the art would understand the convention (e.g., "a system having at least one of A, B, or C" would include but not be limited to systems that have A alone, B alone, C alone, A and B together, A and C together, B and C together, and/or A, B, and C together, etc.). It will be further understood by those within the art that virtually any disjunctive word and/or phrase presenting two or more alternative terms, whether in the description, claims, or drawings, should be understood to contemplate the possibilities of including one of the terms, either of the terms, or both terms. For example, the phrase "A or B" will be understood to include the possibilities of "A" or "B" or "A and B."

[0084] In addition, where features or aspects of the disclosure are described in terms of Markush groups, those skilled in the art will recognize that the disclosure is also thereby described in terms of any individual member or subgroup of members of the Markush group.

[0085] As will be understood by one skilled in the art, for any and all purposes, such as in terms of providing a written description, all ranges disclosed herein also encompass any and all possible subranges and combinations of subranges thereof. Any listed range can be easily recognized as sufficiently describing and enabling the same range being broken down into at least equal halves, thirds, quarters, fifths, tenths, etc. As a non-limiting example, each range discussed herein can be readily broken down into a lower third, middle third and upper third, etc. As will also be understood by one skilled in the art all language such as "up to," "at least," and the like include the number recited and refer to ranges which can be subsequently broken down into subranges as discussed above.

[0086] Whenever a range of values is provided herein, the range is meant to include the starting value, the ending value, each individual value, or value range there between unless otherwise specifically stated. For

example, "from 0.2 to 0.5" means 0.2, 0.3, 0.4, 0.5; ranges there between such as 0.2-0.3, 0.3-0.4, 0.2-0.4; increments there between such as 0.25, 0.35, 0.225, 0.335, 0.49; increment ranges there between such as 0.26-0.39; and the like. As another example, a group having 1-3 cells refers to groups having 1, 2, or 3 cells. Similarly, a group having 1-5 cells refers to groups having 1, 2, 3, 4, or 5 cells, and so forth.

REFERENCES CITED IN THE DESCRIPTION

Cited references

This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.

Patent documents cited in the description

- [WO2010083274A \[0004\]](#)
- [US20100009351A \[0012\]](#)
- [US20090131650A \[0012\]](#)
- [US5866366A \[0038\]](#)
- [US6090592A \[0038\]](#)
- [US6117635A \[0038\]](#)
- [US6117986A \[0038\]](#)
- [US7494771B \[0048\]](#)
- [WO03008636A \[0048\]](#)
- [US3649454A \[0048\] \[0048\]](#)
- [US5185242A \[0048\]](#)
- [US3716452A \[0048\]](#)
- [US4683195A \[0051\] \[0053\]](#)
- [US5455166A \[0051\]](#)
- [US6977148B \[0051\]](#)
- [US6410278B \[0051\]](#)
- [US4988617A \[0051\]](#)
- [US5427930A \[0051\]](#)
- [US5849478A \[0051\]](#)
- [US5399491A \[0051\]](#)
- [US5130238A \[0051\]](#)
- [US5854033A \[0051\]](#)
- [EP0320308A \[0052\]](#)
- [US422252A \[0052\]](#)
- [US4683202A \[0053\]](#)
- [US4800159A \[0053\]](#)

Non-patent literature cited in the description

- **MAHMOUDI RAD et al.**Infectious diseases in obstetrics and gynecology, 2012, [0004]
- **C. R. NEWTON et al.**PCRSpringer-Verlag1997000024- [0017]
- **GREENSAMBROOK**Molecular Cloning, A Laboratory ManualCold Spring Harbor Laboratory Press20120000 [0022]
- **ELGHANIAN et al.**Science, 1997, vol. 277, 1078-1081 [0036]
- **EZAKI et al.**J. Clin. Microbiol., 1982, vol. 16, 5844-846 [0048]
- **PAULE et al.**J. Mol. Diagn., 2004, vol. 6, 3191-196 [0048]
- **WALKER**Strand Displacement Amplification, [0051]
- **DEAN et al.**Multiple displacement amplification, [0051]
- **NOTOMI et al.**Process for Synthesizing Nucleic Acid, [0051]
- **CASHMAN**Blocked-Polymerase Polynucleotide Immunoassay Method and Kit, [0051]
- **KACIAN et al.**Nucleic Acid Sequence Amplification Methods, [0051]
- **MALEK et al.**Enhanced Nucleic Acid Amplification Process, [0051]
- **LIZARDI et al.**BioTechnology, 1988, vol. 6, 1197- [0051]
- PCR Primer: A Laboratory ManualCold Spring Harbor Laboratory Press19950000 [0053]
- **MULLIS et al.**Methods in Enzymology, 1987, vol. 155, 335-350 [0053]
- Current Protocols in Molecular BiologyJohn Wiley & Sons, Inc.19940000 [0055]
- **NUGEN et al.**J. Clin. Microbiol., 1991, vol. 29, 2297-301 [0071]
- **AMSEL et al.**Am. J. Med., 1983, vol. 74, 114-22 [0071]

PATENTKRAV

1. Fremgangsmåde til detektering af vulvovaginal candidiasis (VVC)-associerede *Candida* arter og *Trichomonas vaginalis* i en biologisk prøve, hvor de VVC-
 5 associerede *Candida* arter omfatter *Candida glabrata*, *Candida albicans*, *Candida tropicalis*, *C. dubliniensis*, *C. parapsilosis*, *Candida krusei*, omfattende:

at bringe den biologiske prøve i kontakt med adskillige par af primere, hvor de adskillige par af primere omfatter:

- 10 i det mindste ét par af primere, som er i stand til at hybridisere til tef1-genet af *Candida glabrata*, hvor hver primer i det i det mindste ene par af primere omfatter en sekvens ifølge SEQ ID NO: 20 eller SEQ ID NO: 21, eller en sekvens med én uoverensstemmelse eller to uoverensstemmelser i forhold til SEQ ID NO: 20 eller SEQ ID NO: 21;
- 15 adskillige primere, som er i stand til at hybridisere til tef1-genet af i det mindste én af *Candida albicans*, *Candida tropicalis*, *C. dubliniensis* og *C. parapsilosis*, hvor hver primer i det idet mindste ene par af primere omfatter en sekvens ifølge SEQ ID NO: 23, SEQ ID NO: 24 eller SEQ ID NO: 25 eller en sekvens, som har en uoverensstemmelse eller har to uoverensstemmelser i forhold til SEQ ID NO: 23,
- 20 SEQ ID NO: 24 eller SEQ ID NO: 25;
- i det mindste et par af primere, som er i stand til at hybridisere til tef1-genet af *Candida krusei*, hvor hver primer i det idet mindste ene par af primere omfatter en sekvens ifølge SEQ ID NO: 27 eller SEQ ID NO: 28, eller en sekvens med én uoverensstemmelse eller to uoverensstemmelser i forhold til SEQ ID NO: 27 eller
- 25 SEQ ID NO: 28; og
- i det mindste et par af primere, som er i stand til at hybridisere til AP-65 genet af *Trichomonas vaginalis*, hvor hver primer i det idet mindste ene par af primere omfatter en sekvens ifølge SEQ ID NO: 17 eller SEQ ID NO: 18 eller en sekvens med én uoverensstemmelse eller to uoverensstemmelser i forhold til SEQ ID NO:
- 30 17 eller SEQ ID NO: 18; og
- generering af ampliconer af tef1 sekvenserne af *Candida* arterne og/eller ampliconer af AP-65 gensekvensen af *Trichomonas vaginalis* fra den biologiske prøve, hvis prøven omfatter én eller flere af de VVC-associerede *Candida* arter og/eller *Trichomonas vaginalis*;

bestemmelse af tilstedeværelsen eller mængden af ét eller flere amplificerede produkter som en indikation af tilstedeværelsen af VVC-associerede *Candida* arter og *Trichomonas vaginalis* i den biologiske prøve.

5 2. Fremgangsmåde ifølge krav 1, hvor

parret af primere, som er i stand til at hybridisere til tef1-genet af *Candida glabrata* er SEQ ID NOs: 20 og 21;

10 primerne, som er i stand til at hybridisere til tef1-genet af i det mindste én af *Candida albicans*, *Candida tropicalis*, *C. dubliniensis* og *C. parapsilosis* er:

a) SEQ ID NO: 23 og 24,

b) SEQ ID NO: 23 og 25 eller

c) en kombination deraf;

15

parret af primere, som er i stand til at hybridisere til tef1-genet af *Candida krusei* består af SEQ ID NO: 27 og 28; og

parret af primere, som er i stand til at hybridisere til AP-65 genet af *Trichomonas vaginalis* er SEQ ID NO: 17 og 18.

20

3. Fremgangsmåde ifølge krav 1 eller 2, hvor bestemmelse af tilstedeværelsen eller mængden af ét eller flere amplificerede produkter omfatter at bringe de amplificerede produkter i kontakt med adskillige oligonucleotidsonder, hvor hver af adskillige oligonucleotidsonder omfatter en sekvens valgt fra gruppen bestående af SEQ ID NO: 22, 26, 29 og 19 eller en sekvens med én uoverensstemmelse eller to uoverensstemmelser i forhold til en sekvens valgt fra gruppen bestående af SEQ ID NO: 22, 26, 29 og 19.

4. Fremgangsmåde ifølge krav 3, hvor bestemmelse af tilstedeværelsen eller mængden af ét eller flere amplificerede produkter omfatter at bringe de amplificerede produkter i kontakt med adskillige oligonucleotidsonder, som hver især består af en sekvens ifølge SEQ ID NO: 22, 26, 29 og 19 eller en sekvens med én uoverensstemmelse eller to uoverensstemmelser i forhold til en sekvens valgt fra gruppen bestående af SEQ ID NO: 22, 26, 29 og 19.

35

5. Fremgangsmåde ifølge krav 3 eller 4, hvor i det mindste én af de adskillige oligonucleotidsonder omfatter en fluorescens-emittergruppe og en fluorescens- quenchergruppe.
- 5 6. Fremgangsmåde ifølge ethvert af kravene 1-5, hvor de adskillige par af primere omfatter en første primer omfattende sekvensen ifølge SEQ ID NO: 20, en anden primer omfattende sekvensen ifølge SEQ ID NO: 21, en tredje primer omfattende sekvensen ifølge SEQ ID NO: 23, en fjerde primer omfattende sekvensen ifølge SEQ ID NO: 24, en femte primer omfattende sekvensen ifølge SEQ ID NO: 25, en sjette primer omfattende sekvensen ifølge SEQ ID NO: 27, en syvende primer omfattende sekvensen ifølge SEQ ID NO: 28, en ottende primer omfattende sekvensen ifølge SEQ ID NO: 17 og en niende primer omfattende sekvensen ifølge SEQ ID NO: 18.
- 10 7. Fremgangsmåde ifølge ethvert af kravene 1-6, hvor amplifikationen udføres under anvendelse af en fremgangsmåde valgt fra gruppen bestående af polymerase- kædereaktion (PCR), ligasekædereaktion (LCR), loop-medieret isotherm amplifikation (LAMP), strandforskydningsamplifikation (SDA), replikase-medieret amplifikation, immuno-amplifikation, nucleinsyresekvens baseret amplifikation (NASBA), selv- opretholdt sekvensreplikation (3SR), rullende cirkelamplifikation og transkriptions- medieret amplifikation (TMA).
- 15 8. Fremgangsmåde ifølge krav 7, hvor den nævnte PCR er sand-tids PCR eller kvantitativ sand-tids PCR (QRT-PCR).
- 20 9. Fremgangsmåde ifølge ethvert af kravene 1-8, hvor hver primer omfatter exo- gennucleotidsekvens, som tillader post-amplifikationsmanipulation af amplifika- tionsprodukter uden en betydelig virkning på selve amplifikationen.
- 25 10. Fremgangsmåde ifølge ethvert af kravene 1-9, hvor hver primer er flankeret af komplementære sekvenser, omfattende en fluorofor ved 5' enden og en fluorescens- quencher ved 3' enden.
- 30 11. Fremgangsmåde ifølge ethvert af kravene 1-10, hvor den biologiske prøve er en klinisk prøve eller hvor den biologiske prøve er opsamlet fra urethra, penis, anus, hals, cervix eller vagina.
- 35

12. Sammensætning til detektion af vulvovaginal candidiasis (VVC)-associerede *Candida* arter og *Trichomonas vaginalis*, hvor de VVC-associerede *Candida* arter omfatter *Candida glabrata*, *Candida albicans*, *Candida tropicalis*, *C. dubliniensis*, *C. parapsilosis*, *Candida krusei*, omfattende:

5

i det mindste et par af primere, som er i stand til at hybridisere til tef1-genet af *Candida glabrata*, hvor hver primer i det idet mindste ene par af primere omfatter en sekvens ifølge SEQ ID NO: 20 eller SEQ ID NO: 21 eller en sekvens med én uoverensstemmelse eller to uoverensstemmelser i forhold til SEQ ID NO:

10

20 eller SEQ ID NO: 21;

adskillige primere, som er i stand til at hybridisere tef1-genet af i det mindste én af *Candida albicans*, *Candida tropicalis*, *C. dubliniensis* og *C. parapsilosis*, hvor hver primer i det i det mindste ene par af primere omfatter en sekvens ifølge SEQ ID NO: 23, SEQ ID NO: 24 eller SEQ ID NO: 25 eller en sekvens som har én uoverensstemmelse eller to uoverensstemmelser i forhold til SEQ ID NO: 23, SEQ ID NO: 24 eller SEQ ID NO: 25;

15

i det mindste et par af primere, som er i stand til at hybridisere til tef1-genet af *Candida krusei*, hvor hver primer i det idet mindste ene par af primere omfatter en sekvens ifølge SEQ ID NO: 27 eller SEQ ID NO: 28 eller en sekvens med én uoverensstemmelse eller to uoverensstemmelser i forhold til SEQ ID NO: 27 eller SEQ ID NO: 28; og

20

i det mindste ét par af primere, som er i stand til at hybridisere til AP-65 genet af *Trichomonas vaginalis*, hvor hver primer i det idet mindste ene par af primere omfatter en sekvens ifølge SEQ ID NO: 17 eller SEQ ID NO: 18 eller en sekvens med én uoverensstemmelse eller to uoverensstemmelser i forhold til SEQ ID NO: 17 eller SEQ ID NO: 18.

25

13. Sammensætning ifølge krav 12, hvor

30

det i det mindste ene par af primere, som er i stand til at hybridisere tef1-genet af *Candida glabrata* omfatter en primer omfattende en sekvens ifølge SEQ ID NO: 20 og en primer omfattende en sekvens ifølge SEQ ID NO: 21;

de adskillige primere, som er i stand til at hybridisere tef1-genet af i det mindste én af *Candida albicans*, *Candida tropicalis*, *C. dubliniensis* og *C.*

35

parapsilosis, omfatter en primer omfattende sekvensen ifølge SEQ ID NO: 23, en

- primer omfattende sekvensen ifølge SEQ ID NO: 24 og en primer omfattende sekvensen ifølge SEQ ID NO: 25;
- det i det mindste ene par af primere, som er i stand til at hybridisere til tef1-genet af *Candida krusei* omfatter en primer omfattende sekvensen ifølge SEQ ID NO: 27
- 5 og en primer omfattende sekvensen ifølge SEQ ID NO: 28; og
- det i det mindste ene par af primere, som er i stand til at hybridisere til AP-65 genet af *Trichomonas vaginalis*, omfatter en primer omfattende sekvensen ifølge SEQ ID NO: 17 og en primer omfattende sekvensen ifølge SEQ ID NO: 18.
- 10 **14.** Sammensætning ifølge krav 12 eller 13, som yderligere omfatter adskillige oligonucleotidsonder, hvor hver af de adskillige oligonucleotidsonder omfatter en sekvens valgt fra gruppen bestående af SEQ ID NO: 22, 26, 29 og 19 eller en sekvens med én uoverensstemmelse eller to uoverensstemmelse i forhold til en sekvens valgt fra gruppen bestående af SEQ ID NO: 22, 26, 29 og 19; og eventuelt hvor i det
- 15 mindste én af de adskillige sonder omfatter en fluorescens-emittergruppe og/eller en fluorescens-quenchergruppe.
- 15.** Reaktionsblanding omfattende sammensætningen ifølge ethvert af kravene 12-14, hvor reaktionsblandingen yderligere omfatter skabelon DNA, DNA polymerase,
- 20 deoxynucleotider (dNTPs), bufferopløsning, bivalente kationer, monovalent kation-kaliumioner eller vilkårlige kombinationer deraf.

SEKVENSLISTE

Sekvenslisten er udeladt af skriftet og kan hentes fra det Europæiske Patent Register.

The Sequence Listing was omitted from the document and can be downloaded from the European Patent Register.

