CLAIMS

1. Method for the diagnosis of fungal sepsis in a subject, the method comprising:

i) contacting a sample that has been obtained from the subject, with a combination of three or more oligonucleotide amplification primer sets under conditions that allow hybridization of oligonucleotide amplification primers to three or more target nucleic acids of fungal pathogens involved in fungal sepsis, wherein the three or more oligonucleotide amplification primer sets are directed to different target nucleic acids of at least three different fungal pathogen species involved in fungal sepsis and are independently selected from the group consisting of:

(1) a *Candida albicans* primer set targeting a nucleotide sequence in the ITS2 region of the rDNA of *Candida albicans*;

(2) a *Candida tropicalis* primer set targeting a nucleotide sequence in the ITS2 region of the rDNA of *Candida tropicalis*;

(3) a *Candida glabrata* primer set targeting a nucleotide sequence in the ITS1 region of the rDNA of *Candida glabrata*;

(4) a *Candida parapsilosis* primer set targeting a nucleotide sequence in the ITS2 region of the rDNA of *Candida parapsilosis*;

(5) a *Candida krusei* primer set targeting a nucleotide sequence in the ITS2 region of the rDNA of *Candida krusei*;

(6) a *Candida guilliermondii* primer set targeting a nucleotide sequence in the ITS2 region of the rDNA of *Candida guilliermondii*;

(7) a *Candida auris* primer set targeting a nucleotide sequence in the ITS2 region of the rDNA of *Candida auris*;

(8) a *Cryptococcus neoformans* primer set targeting a nucleotide sequence in the IGS1 region of the rDNA of *Cryptococcus neoformans*;

(9) a *Cryptococcus deneoformans* primer set targeting a nucleotide sequence in the IGS1 region of the rDNA of *Cryptococcus deneoformans*;

(10) a *Cryptococcus gattii* primer set targeting a nucleotide sequence in the IGS1 region of the rDNA of *Cryptococcus gattii*;

(11) a *Fusarium spp.* primer set targeting a nucleotide sequence in the 26S region of the rDNA of *Fusarium spp.*;

(12) a *Trichosporon spp.* primer set targeting a nucleotide sequence in the 18S region of the rDNA of *Trichosporon spp.*;

(13) a *Schizosaccharomyces pombe* primer set targeting a nucleotide sequence in the Cytochrome B gene region of the genomic DNA of *Schizosaccharomyces pombe*; and

(14) a *Candida spp.* primer set targeting a nucleotide sequence in the 18S region of the rDNA of *Candida spp.*; and

ii) amplifying the target nucleic acid(s) hybridized to any one or more of the oligonucleotide primers by PCR to produce a PCR amplified sample; and

iii) detecting the presence and/or amount of the target nucleic acid(s) in the PCR amplified sample

by subsequently contacting the PCR amplified sample of step ii) with a probe mixture comprising a combination of oligonucleotide detection probes comprising at least one probe specific for each of the amplicons produced by the amplification of the target nucleic acids in step ii) under conditions that allow the hybridization of the probes to their specific target nucleic acid sequences and determining the presence and/or amount of probe:target hybrids, wherein the combination of oligonucleotide detection probes comprises three or more probes selected from

(1) a *Candida albicans* probe comprising a nucleotide sequence of SEQ ID NO: 24 or a nucleotide sequence having at least 90% sequence identity to SEQ ID NO: 24 over its entire length, or a complement thereof;

(2) a *Candida tropicalis* probe comprising a nucleotide sequence of SEQ ID NO: 25 or a nucleotide sequence having at least 90% sequence identity to SEQ ID NO: 25 over its entire length, or a complement thereof;

(3) a *Candida glabrata* probe comprising a nucleotide sequence of SEQ ID NO: 30 or a nucleotide sequence having at least 90% sequence identity to SEQ ID NO: 30 over its entire length, or a complement thereof;

(4) a *Candida parapsilosis* probe comprising a nucleotide sequence of SEQ ID NO: 26 or a nucleotide sequence having at least 90% sequence identity to SEQ ID NO: 26 over its entire length, or a complement thereof;

(5) a *Candida krusei* probe comprising a nucleotide sequence of SEQ ID NO: 27 or a nucleotide sequence having at least 90% sequence identity to SEQ ID NO: 27 over its entire length, or a complement thereof;

(6) a *Candida guilliermondii* probe comprising a nucleotide sequence of SEQ ID NO: 28 or a nucleotide sequence having at least 90% sequence identity to SEQ ID NO: 28 over its entire length, or a complement thereof;

(7) a *Candida auris* probe comprising a nucleotide sequence of SEQ ID NO: 29 or a nucleotide sequence having at least 90% sequence identity to SEQ ID NO: 29 over its entire length, or a complement thereof;

(8) a *Cryptococcus neoformans* probe comprising a nucleotide sequence of SEQ ID NO: 32 or a nucleotide sequence having at least 90% sequence identity to SEQ ID NO: 32 over its entire length, or a complement thereof;

(9) a *Cryptococcus deneoformans* probe comprising a nucleotide sequence of SEQ ID NO: 33 or a nucleotide sequence having at least 90% sequence identity to SEQ ID NO: 33 over its entire length, or a complement thereof;

(10) a *Cryptococcus gattii* comprising a nucleotide sequence of SEQ ID NO: 34 or a nucleotide sequence having at least 90% sequence identity to SEQ ID NO: 34 over its entire length, or a complement thereof;

(11) a *Fusarium spp.* probe comprising a nucleotide sequence of SEQ ID NO: 35 or a nucleotide sequence having at least 90% sequence identity to SEQ ID NO: 35 over its entire length, or a complement thereof;

(12) a *Trichosporon spp.* probe comprising a nucleotide sequence of SEQ ID NO: 36 or a

nucleotide sequence having at least 90% sequence identity to SEQ ID NO: 36 over its entire length, or a complement thereof;

(13) a *Schizosaccharomyces pombe* probe comprising a nucleotide sequence of SEQ ID NO: 37 or a nucleotide sequence having at least 90% sequence identity to SEQ ID NO: 37 over its entire length, or a complement thereof; and

(14) a *Candida spp.* probe comprising a nucleotide sequence of SEQ ID NO: 31 or a nucleotide sequence having at least 90% sequence identity to SEQ ID NO: 31 over its entire length, or a complement thereof.

2. The method according to Claim 1, wherein the combination of primer sets comprises three or more oligonucleotide amplification primer sets directed to different target nucleic acids of at least three different fungal pathogen genera involved in fungal sepsis, and the combination of probes comprises a probe for each of the amplicons produced by the amplification of the target nucleic acids, and wherein the at least three different fungal pathogen genera are selected from the group consisting of *Candida, Cryptococcus, Fusarium, Trichosporon* and *Schizosaccharomyces*.

3. The method according to Claim 1 or 2, wherein:
at least one primer of the *Candida albicans* primer set, the *Candida tropicalis* primer set, the *Candida parapsilosis* primer set, the *Candida krusei* primer set, the *Candida guilliermondii* primer set, and the *Candida auris* primer set comprises the nucleotide sequence of SEQ ID NO: 1 or a nucleotide sequence having at least 90% sequence identity to SEQ ID NO: 1 over its entire length, or a complement thereof.

4. The method according to any one of Claims 1-3, wherein:
(1) at least one primer of the *Candida albicans* primer set comprises the nucleotide sequence of SEQ ID NO: 2 or a nucleotide sequence having at least 90% sequence identity to SEQ ID NO: 2 over its entire length, or a complement thereof;
(2) at least one primer of the *Candida tropicalis* primer set comprises the nucleotide sequence of SEQ ID NO: 3 or a nucleotide sequence having at least 90% sequence identity to SEQ ID NO: 3 over its entire length, or a complement thereof;
(3) at least one primer of the *Candida parapsilosis* primer set comprises the nucleotide sequence of SEQ ID NO: 4 or a nucleotide sequence having at least 90% sequence identity to SEQ ID NO: 4 over its entire length, or a complement thereof;
(4) at least one primer of the *Candida krusei* primer set comprises the nucleotide sequence of SEQ ID NO: 5 or a nucleotide sequence having at least 90% sequence identity to SEQ ID NO: 5 over its entire length, or a complement thereof;
(5) at least one primer of the *Candida guilliermondii* primer set comprises the nucleotide sequence of SEQ ID NO: 6 or a nucleotide sequence having at least 90% sequence identity to SEQ ID NO: 6 over its entire length, or a complement thereof; and
(6) at least one primer of the *Candida auris* primer set comprises the nucleotide sequence of SEQ ID NO: 7 or a nucleotide sequence having at least 90% sequence identity to SEQ ID NO: 7 over its

entire length, or a complement thereof.

5. The method according to any one of Claims 1-4, wherein

(1) the *Candida glabrata* primer set comprises a primer having the nucleotide sequence of SEQ ID NO: 8 or a nucleotide sequence having at least 90% sequence identity to SEQ ID NO: 8 over its entire length, or a complement thereof, and a primer having the nucleotide sequence of SEQ ID NO: 9 or a nucleotide sequence having at least 90% sequence identity to SEQ ID NO: 9 over its entire length, or a complement thereof;

(2) the *Cryptococcus neoformans* primer set comprises a primer having the nucleotide sequence of SEQ ID NO: 10 or a nucleotide sequence having at least 90% sequence identity to SEQ ID NO: 10 over its entire length, or a complement thereof, and a primer having the nucleotide sequence of SEQ ID NO: 11 or a nucleotide sequence having at least 90% sequence identity to SEQ ID NO: 11 over its entire length, or a complement thereof;

(3) the *Cryptococcus deneoformans* primer set comprises a primer having a nucleotide sequence of SEQ ID NO: 12 or the nucleotide sequence having at least 90% sequence identity to SEQ ID NO: 12 over its entire length, or a complement thereof, and a primer having the nucleotide sequence of SEQ ID NO: 13 or a nucleotide sequence having at least 90% sequence identity to SEQ ID NO: 13 over its entire length, or a complement thereof;

(4) the *Cryptococcus gattii* primer set comprises a primer having the nucleotide sequence of SEQ ID NO: 14 or a nucleotide sequence having at least 90% sequence identity to SEQ ID NO: 14 over its entire length, or a complement thereof, and a primer having the nucleotide sequence of SEQ ID NO: 15 or a nucleotide sequence having at least 90% sequence identity to SEQ ID NO: 15 over its entire length, or a complement thereof;

(5) the *Fusarium spp*. primer set comprises a primer having the nucleotide sequence of SEQ ID NO: 16 or a nucleotide sequence having at least 90% sequence identity to SEQ ID NO: 16 over its entire length, or a complement thereof, and a primer having the nucleotide sequence of SEQ ID NO: 17 or a nucleotide sequence having at least 90% sequence identity to SEQ ID NO: 17 over its entire length, or a complement thereof;

(6) the *Trichosporon spp*. primer set comprises a primer having the nucleotide sequence of SEQ ID NO: 18 or a nucleotide sequence having at least 90% sequence identity to SEQ ID NO: 18 over its entire length, or a complement thereof, and a primer having the nucleotide sequence of SEQ ID NO: 19 or a nucleotide sequence having at least 90% sequence identity to SEQ ID NO: 19 over its entire length, or a complement thereof;

(7) the *Schizosaccharomyces pombe* primer set comprises a primer having the nucleotide sequence of SEQ ID NO: 20 or a nucleotide sequence having at least 90% sequence identity to SEQ ID NO: 20 over its entire length, or a complement thereof, and a primer having the nucleotide sequence of SEQ ID NO: 21 or a nucleotide sequence having at least 90% sequence identity to SEQ ID NO: 21 over its entire length, or a complement thereof; and

(8) the *Candida spp.* primer set comprises a primer having the nucleotide sequence of SEQ ID NO: 22 or a nucleotide sequence having at least 90% sequence identity to SEQ ID NO: 22 over its entire

length, or a complement thereof, and a primer having the nucleotide sequence of SEQ ID NO: 23 or a nucleotide sequence having at least 90% sequence identity to SEQ ID NO: 23 over its entire length, or a complement thereof.

6. The method according to any one of Claims 1-5, wherein:

the combination of primer sets comprises the *Candida albicans* primer set, *Candida tropicalis* primer set, *Candida glabrata* primer set, *Candida parapsilosis* primer set, *Candida krusei* primer set, *Candida guilliermondii* primer set, *Candida auris* primer set, *Cryptococcus neoformans* primer set, *Cryptococcus deneoformans* primer set, *Cryptococcus gattii* primer set, *Fusarium spp.* primer set and *Trichosporon spp.* primer set; and

the combination of probes comprises the *Candida albicans* probe, *Candida tropicalis* probe, *Candida glabrata* probe, *Candida parapsilosis* probe, *Candida krusei* probe, *Candida guilliermondii* probe, *Candida auris* probe, *Cryptococcus neoformans* probe, *Cryptococcus deneoformans* probe, *Cryptococcus gattii* probe, *Fusarium spp.* probe and *Trichosporon spp.* probe..

7. The method according to any one of Claims 1-5, wherein:

the combination of primer sets comprises the *Candida albicans* primer set, *Candida tropicalis* primer set, *Candida glabrata* primer set, *Candida parapsilosis* primer set, *Candida krusei* primer set, *Candida guilliermondii* primer set and *Candida auris* primer set; and

the combination of probes comprises the *Candida albicans* probe, *Candida tropicalis* probe, *Candida glabrata* probe, *Candida parapsilosis* probe, *Candida krusei* probe, *Candida guilliermondii* probe and *Candida auris* probe.

8. The method according to any one of Claims 1-5, wherein:

the combination of primer sets comprises the *Candida albicans* primer set, *Candida tropicalis* primer set, *Candida glabrata* primer set, *Candida parapsilosis* primer set and *Candida krusei* primer set; and

the combination of probes comprises the *Candida albicans* probe, *Candida tropicalis* probe, *Candida glabrata* probe, *Candida parapsilosis* probe and *Candida krusei* probe.

9. The method according to any one of Claims 1-5, wherein:

the combination of primer sets comprisesthe *Candida albicans* primer set, *Candida tropicalis* primer set, *Candida glabrata* primer set, *Candida parapsilosis* primer set, *Candida krusei* primer set, *Candida auris* primer set, *Cryptococcus neoformans* primer set, *Cryptococcus deneoformans* primer set, and *Cryptococcus gattii* primer set; and

the combination of probes comprises the *Candida albicans* probe, *Candida tropicalis* probe, *Candida glabrata* probe, *Candida parapsilosis* probe, *Candida krusei* probe, *Candida auris* probe, *Cryptococcus neoformans* probe, *Cryptococcus deneoformans* probe, and *Cryptococcus gattii* probe.

10. The method according to any one of Claims 1 to 5, wherein:

the combination of primer sets comprises the *Cryptococcus neoformans* primer set, *Cryptococcus deneoformans* primer set, *Cryptococcus gattii* primer set, *Fusarium spp.* primer set and *Trichosporon spp.* primer set; and

the combination of probes comprises the *Cryptococcus neoformans* probe, *Cryptococcus deneoformans* probe, *Cryptococcus gattii* probe, *Fusarium spp.* probe and *Trichosporon spp.* probe.

11. The method according to any one of Claims 1 to 10, wherein the combination of primer sets comprises at least 14 different primer sets, and the combination of probes comprises at least 14 different probes.

12. The method according to any one of Claims 1 to 5, wherein the combination of primer sets and combination of probes comprises:

(1) the *Candida albicans* primer set and *Candida albicans* probe, *Candida tropicalis* primer set and *Candida tropicalis* probe and *Candida glabrata* primer set and *Candida glabrata* probe; or

(2) the *Candida albicans* primer set and *Candida albicans* probe and, *Candida tropicalis* primer set and *Candida tropicalis* probe; or

(3) the *Cryptococcus neoformans* primer set and *Cryptococcus neoformans* probe, *Cryptococcus deneoformans* primer set and *Cryptococcus deneoformans* probe and *Cryptococcus gattii* primer set and *Cryptococcus gattii* probe.

13. Kit for carrying out the method of any one of claims 1 to 12, wherein the kit comprises three or more amplification primer sets and three or more detection probes, wherein

(A) the three or more amplification primer sets are directed to different target nucleic acids of at least three different fungal pathogen species involved in fungal sepsis and are independently selected from the group consisting of:

(1) a *Candida albicans* primer set targeting a nucleotide sequence in the ITS2 region of the rDNA of *Candida albicans*;

(2) a *Candida tropicalis* primer set targeting a nucleotide sequence in the ITS2 region of the rDNA of *Candida tropicalis*;

(3) a *Candida glabrata* primer set targeting a nucleotide sequence in the ITS1 region of the rDNA of *Candida glabrata*;

(4) a *Candida parapsilosis* primer set targeting a nucleotide sequence in the ITS2 region of the rDNA of *Candida parapsilosis*;

(5) a *Candida krusei* primer set targeting a nucleotide sequence in the ITS2 region of the rDNA of *Candida krusei*;

(6) a *Candida guilliermondii* primer set targeting a nucleotide sequence in the ITS2 region of the rDNA of *Candida guilliermondii*;

(7) a *Candida auris* primer set targeting a nucleotide sequence in the ITS2 region of the rDNA of *Candida auris*;

(8) a *Cryptococcus neoformans* primer set targeting a nucleotide sequence in the IGS1 region

of the rDNA of *Cryptococcus neoformans*;

(9) a *Cryptococcus deneoformans* primer set targeting a nucleotide sequence in the IGS1 region of the rDNA of *Cryptococcus deneoformans*;

(10) a *Cryptococcus gattii* primer set targeting a nucleotide sequence in the IGS1 region of the rDNA of *Cryptococcus gattii*;

(11) a *Fusarium spp.* primer set targeting a nucleotide sequence in the 26S region of the rDNA of *Fusarium spp.*;

(12) a *Trichosporon spp.* primer set targeting a nucleotide sequence in the 18S region of the rDNA of *Trichosporon spp.*;

(13) a *Schizosaccharomyces pombe* primer set targeting a nucleotide sequence in the Cytochrome B gene region of the genomic DNA of *Schizosaccharomyces pombe*; and

(14) a *Candida spp.* primer set targeting a nucleotide sequence in the 18S region of the rDNA of *Candida spp.*; and

(B) the three or more detection probes comprise at least one probe specific for each of the amplicons produced by amplification of the target nucleic acids by each of the three or more primer pairs, wherein the three or more probes are selected from:

(1) a *Candida albicans* probe comprising a nucleotide sequence of SEQ ID NO: 24 or a nucleotide sequence having at least 90% sequence identity to SEQ ID NO: 24 over its entire length, or a complement thereof;

(2) a *Candida tropicalis* probe comprising a nucleotide sequence of SEQ ID NO: 25 or a nucleotide sequence having at least 90% sequence identity to SEQ ID NO: 25 over its entire length, or a complement thereof;

(3) a *Candida glabrata* probe comprising a nucleotide sequence of SEQ ID NO: 30 or a nucleotide sequence having at least 90% sequence identity to SEQ ID NO: 30 over its entire length, or a complement thereof;

(4) a *Candida parapsilosis* probe comprising a nucleotide sequence of SEQ ID NO: 26 or a nucleotide sequence having at least 90% sequence identity to SEQ ID NO: 26 over its entire length, or a complement thereof;

(5) a *Candida krusei* probe comprising a nucleotide sequence of SEQ ID NO: 27 or a nucleotide sequence having at least 90% sequence identity to SEQ ID NO: 27 over its entire length, or a complement thereof;

(6) a *Candida guilliermondii* probe comprising a nucleotide sequence of SEQ ID NO: 28 or a nucleotide sequence having at least 90% sequence identity to SEQ ID NO: 28 over its entire length, or a complement thereof;

(7) a *Candida auris* probe comprising a nucleotide sequence of SEQ ID NO: 29 or a nucleotide sequence having at least 90% sequence identity to SEQ ID NO: 29 over its entire length, or a complement thereof;

(8) a *Cryptococcus neoformans* probe comprising a nucleotide sequence of SEQ ID NO: 32 or a nucleotide sequence having at least 90% sequence identity to SEQ ID NO: 32 over its entire length, or a complement thereof;

(9) a *Cryptococcus deneoformans* probe comprising a nucleotide sequence of SEQ ID NO: 33 or a nucleotide sequence having at least 90% sequence identity to SEQ ID NO: 33 over its entire length, or a complement thereof;

(10) a *Cryptococcus gattii* comprising a nucleotide sequence of SEQ ID NO: 34 or a nucleotide sequence having at least 90% sequence identity to SEQ ID NO: 34 over its entire length, or a complement thereof;

(11) a *Fusarium spp.* probe comprising a nucleotide sequence of SEQ ID NO: 35 or a nucleotide sequence having at least 90% sequence identity to SEQ ID NO: 35 over its entire length, or a complement thereof;

(12) a *Trichosporon spp.* probe comprising a nucleotide sequence of SEQ ID NO: 36 or a nucleotide sequence having at least 90% sequence identity to SEQ ID NO: 36 over its entire length, or a complement thereof;

(13) a *Schizosaccharomyces pombe* probe comprising a nucleotide sequence of SEQ ID NO: 37 or a nucleotide sequence having at least 90% sequence identity to SEQ ID NO: 37 over its entire length, or a complement thereof; and

(14) a *Candida spp.* probe comprising a nucleotide sequence of SEQ ID NO: 31 or a nucleotide sequence having at least 90% sequence identity to SEQ ID NO: 31 over its entire length, or a complement thereof.

14. The method according to claim 1, wherein the subject is a human.

15. The method according to claim 14, wherein if one or more of the target nucleic acids are detected in the sample, the subject is diagnosed with fungal sepsis.