

WHAT IS CLAIMED IS:

1. A kit for multiplex detection of Chikungunya virus and Zika virus, comprising a first primer set specific to Chikungunya virus and a second primer set specific to Zika virus,

wherein the first primer set ~~is selected from the group consisting of:~~ comprises

(a) a forward primer consisting of the nucleotide sequence of SEQ ID NO: 1 and a reverse primer consisting of the nucleotide sequence of SEQ ID NO: 2; ~~and~~

~~(b) a forward primer consisting of a nucleotide sequence having at least 90% identity to SEQ ID NO: 1 and a reverse primer consisting of a nucleotide sequence having at least 90% identity to SEQ ID NO: 2,~~

wherein the second primer set is selected from the group consisting of:

(a) a forward primer consisting of the nucleotide sequence of SEQ ID NO: 4 and a reverse primer consisting of the nucleotide sequence of SEQ ID NO: 6;

(b) a forward primer consisting of the nucleotide sequence of SEQ ID NO: 5 and a reverse primer consisting of the nucleotide sequence of SEQ ID NO: 6; and

(c) a first forward primer consisting of the nucleotide sequence of SEQ ID NO: 4, a second forward primer consisting of the nucleotide sequence of SEQ ID NO: 5, and a reverse primer consisting of the nucleotide sequence of SEQ ID NO: 6; and

~~(d) at least one forward primer consisting of a nucleotide sequence having at least 90% identity to the sequence selected from the group consisting of SEQ ID NO: 4 and SEQ ID NO: 5, and a reverse primer consisting of a nucleotide sequence having at least 90% identity to SEQ ID NO: 6.~~

2. The kit according to claim 1 further comprising a probe specific to Chikungunya virus, wherein the probe ~~comprises a nucleotide sequence selected from the group consisting of:~~ consists of

(a) the nucleotide sequence of SEQ ID NO: 3; or
(b) the nucleotide sequence complementary to SEQ ID NO: 3; ~~and~~
(c) ~~a nucleotide sequence having at least 90% identity to SEQ ID NO: 3 or the~~
~~nucleotide sequence complementary to SEQ ID NO: 3.~~

3. The kit according to claim 2, wherein the probe is labeled with a 5'-reporter dye and a 3'-quencher.

4. The kit according to claim 1 further comprising a probe specific to Zika virus, wherein the probe ~~comprises a nucleotide sequence selected from the group~~
~~consisting of:~~ consists of

(a) the nucleotide sequence of SEQ ID NO: 7; or
(b) the nucleotide sequence complementary to SEQ ID NO: 7; ~~and~~
(c) ~~a nucleotide sequence having at least 90% identity to SEQ ID NO: 7 or the~~
~~nucleotide sequence complementary to SEQ ID NO: 7.~~

5. The kit according to claim 4, wherein the probe is labeled with a 5'-reporter dye and a 3'-quencher.

6. The kit according to claim 1 further comprising an internal control template, which is in vitro transcribed RNA of proA gene from *Pectobacterium carotovorum*.

7. The kit according to claim 6 further comprising a control primer set specific to the proA gene, wherein the control primer set ~~is selected from the group~~
~~consisting of:~~ comprises

(a) a forward primer consisting of the nucleotide sequence of SEQ ID NO: 8 and a reverse primer consisting of the nucleotide sequence of SEQ ID NO: 9; ~~and~~

(b) ~~a forward primer consisting of a nucleotide sequence having at least 90% identity to SEQ ID NO: 8 and a reverse primer consisting of a nucleotide sequence~~
~~having at least 90% identity to SEQ ID NO: 9.~~

8. The kit according to claim 7 further comprising a probe specific to the proA gene, wherein the probe ~~comprises a nucleotide sequence selected from the group consisting of:~~ consists of

- (a) the nucleotide sequence of SEQ ID NO: 10; or
- (b) the nucleotide sequence complementary to SEQ ID NO: 10; and
- (c) ~~a nucleotide sequence having at least 90% identity to SEQ ID NO: 10 or the nucleotide sequence complementary to SEQ ID NO: 10.~~

9. The kit according to claim 8, wherein the probe is labeled with a 5'-reporter dye and a 3'-quencher.

10. A method for multiplex detection of Chikungunya virus and Zika virus, comprising the steps of:

- (a) contacting a biological sample with the kit of claim 1;
- (b) amplifying nucleic acids of Chikungunya virus and Zika virus, if present, from the biological sample; and
- (c) detecting a presence of amplified nucleic acids of Chikungunya virus and Zika virus.

11. The method according to claim 10 further comprising a step of extracting nucleic acids from the biological sample before the step (a).

12. The method according to claim 10, wherein the step (b) comprises sub-steps of:

- (b1) converting viral genomic RNA to complementary DNA by reverse transcription; and
- (b2) amplifying the complementary DNA by real-time polymerase chain reaction.

13. The method according to claim 10, wherein the kit further comprises a first probe specific to Chikungunya virus and a second probe specific to Zika virus, wherein the first probe ~~is selected from the group consisting of:~~ consists of

- (a) the nucleotide sequence of SEQ ID NO: 3; or
- (b) the nucleotide sequence complementary to SEQ ID NO: 3; ~~and~~
- (c) ~~a nucleotide sequence having at least 90% identity to SEQ ID NO: 3 or the nucleotide sequence complementary to SEQ ID NO: 3,~~

wherein the second probe ~~is selected from the group consisting of:~~ consists of

- (a) the nucleotide sequence of SEQ ID NO: 7; or
- (b) the nucleotide sequence complementary to SEQ ID NO: 7; ~~and~~
- (c) ~~a nucleotide sequence having at least 90% identity to SEQ ID NO: 7 or the nucleotide sequence complementary to SEQ ID NO: 7.~~

14. The method according to claim 13, wherein each of the first probe and the second probe is labeled with a 5'-reporter dye and a 3'-quencher.

15. The method according to claim 14, wherein the first probe and the second probe are labeled with different 5'-reporter dyes.

16. The method according to claim 10, wherein the kit further comprises an internal control template, which is in vitro transcribed RNA of proA gene from *Pectobacterium carotovorum*.

17. The method according to claim 16, wherein the kit further comprises a control primer set specific to the proA gene, which ~~is selected from the group consisting of:~~ comprises

- (a) a forward primer consisting of the nucleotide sequence of SEQ ID NO: 8 and a reverse primer consisting of the nucleotide sequence of SEQ ID NO: 9; ~~and~~

~~(b) a forward primer consisting of a nucleotide sequence having at least 90% identity to SEQ ID NO: 8 and a reverse primer consisting of a nucleotide sequence having at least 90% identity to SEQ ID NO: 9.~~

18. The method according to claim 17, wherein the kit further comprises a probe specific to the proA gene, which ~~comprises a nucleotide sequence selected from the group consisting of:~~ consists of

- ~~(a) the nucleotide sequence of SEQ ID NO: 10; or~~
- ~~(b) the nucleotide sequence complementary to SEQ ID NO: 10; and~~
- ~~(c) a nucleotide sequence having at least 90% identity to SEQ ID NO: 10 or the nucleotide sequence complementary to SEQ ID NO: 10.~~

19. The method according to claim 18, wherein the probe is labeled with a 5'-reporter dye and a 3'-quencher.

20. A kit for detection of Chikungunya virus comprising a primer set ~~selected from the group consisting of:~~ wherein the primer set comprises

- ~~(a) a forward primer consisting of the nucleotide sequence of SEQ ID NO: 1 and a reverse primer consisting of the nucleotide sequence of SEQ ID NO: 2; and~~
- ~~(b) a forward primer consisting of a nucleotide sequence having at least 90% identity to SEQ ID NO: 1 and a reverse primer consisting of a nucleotide sequence having at least 90% identity to SEQ ID NO: 2.~~

21. A kit for detection of Zika virus comprising a primer set selected from the group consisting of:

- (a) a forward primer consisting of the nucleotide sequence of SEQ ID NO: 4 and a reverse primer consisting of the nucleotide sequence of SEQ ID NO: 6;
- (b) a forward primer consisting of the nucleotide sequence of SEQ ID NO: 5 and a reverse primer consisting of the nucleotide sequence of SEQ ID NO: 6; and

(c) a first forward primer consisting of the nucleotide sequence of SEQ ID NO: 4, a second forward primer consisting of the nucleotide sequence of SEQ ID NO: 5, and a reverse primer consisting of the nucleotide sequence of SEQ ID NO: 6; and

~~(d) at least one forward primer consisting of a nucleotide sequence having at least 90% identity to the sequence selected from the group consisting of SEQ ID NO: 4 and SEQ ID NO: 5, and a reverse primer consisting of a nucleotide sequence having at least 90% identity to SEQ ID NO: 6.~~