

What is claimed is:

1. A method of detecting the presence of at least a first target nucleic acid sequence in a sample, comprising:

subjecting the sample to an amplification reaction capable of amplifying the target nucleic acid sequence in the presence of at least a first set of nucleic acid probes, the first set of nucleic acid probes comprising:

a capture probe comprising a fluorophore attached thereto;

a target specific nucleic acid probe complementary to at least a portion of the capture probe and the target nucleic acid sequence, and comprising a quencher attached thereto, such that the quencher quenches fluorescence from the fluorophore when the target specific probe is hybridized to the capture probe; and

detecting fluorescence from the sample following one or more cycles of the polymerase chain reaction, an increase in fluorescence being indicative of the presence of the target nucleic acid sequence.

2. The method of claim 1, wherein the capture probe is attached to a surface of a substrate.

3. The method of claim 1, wherein the amplification reaction comprises a PCR reaction.

4. The method of claim 3, wherein the PCR reaction employs a polymerase enzyme having exonuclease activity.

5. The method of claim 4, wherein the PCR reaction is capable of amplifying a plurality of different target nucleic acid sequences and is carried out in the presence of a plurality of different sets of nucleic acid probes, each different set of probes comprising a different capture probe having a fluorophore attached thereto and a different target specific probe, each target specific probe being complementary to a different capture probe and a different one of the plurality of target nucleic acid

sequences, and having a quencher attached thereto, such that the quencher quenches fluorescence from the fluorophore when each target specific probe is hybridized to a complementary capture probe.

6. The method of claim 5, wherein each of the capture probes in the plurality of different sets of probes is immobilized upon a substrate in a detectably distinct location.

7. The method of claim 1, wherein the at least first target nucleic acid sequence comprises a variola virus sequence.

8. The method of claim 7, wherein the target specific nucleic acid probe comprises one or more of: VAR-1 probe (SEQ ID NO: 19), VAR-2 probe (SEQ ID NO: 22), VAR-3 probe (SEQ ID NO: 25), or VAR-4 probe (SEQ ID NO: 28).

9. The method of claim 1, wherein the target nucleic acid sequence is amplified through use of one or more of: VAR-1 primer 1 (SEQ ID NO: 17), VAR-1 primer 2 (SEQ ID NO: 18), VAR-2 primer 1 (SEQ ID NO: 20), VAR-2 primer 2 (SEQ ID NO: 21); VAR-3 primer 1 (SEQ ID NO: 23), VAR-3 primer 2 (SEQ ID NO: 24), VAR-4 primer 1 (SEQ ID NO: 26), or VAR-4 primer 2 (SEQ ID NO: 27).

10. A reaction mixture, comprising:
a sample containing a target nucleic acid of interest;
amplification reagents for amplifying the target nucleic acid sequence of interest; and
at least a first probe set comprising a capture probe comprising a fluorophore attached thereto, and a target specific nucleic acid probe complementary to at least a portion of the capture probe and the target nucleic acid sequence, and comprising a quencher attached thereto, such that the quencher quenches fluorescence from the fluorophore when the target specific probe is hybridized to the capture probe

11. The mixture of claim 10, wherein the target specific nucleic acid probe comprises one or more of: VAR-1 probe (SEQ ID NO: 19), VAR-2 probe (SEQ ID NO: 22), VAR-3 probe (SEQ ID NO: 25), or VAR-4 probe (SEQ ID NO: 28).

12. The mixture of claim 11, wherein the mixture further comprises one or more amplification primers chosen from the group consisting of: VAR-1 primer 1 (SEQ ID NO: 17), VAR-1 primer 2 (SEQ ID NO: 18), VAR-2 primer 1 (SEQ ID NO: 20), VAR-2 primer 2 (SEQ ID NO: 21); VAR-3 primer 1 (SEQ ID NO: 23), VAR-3 primer 2 (SEQ ID NO: 24), VAR-4 primer 1 (SEQ ID NO: 26), or VAR-4 primer 2 (SEQ ID NO: 27).
13. A reaction chamber, comprising:
a reaction region having disposed therein:
a sample containing a target nucleic acid of interest;
amplification reagents for amplifying the target nucleic acid sequence of interest; and
at least a first probe set comprising a capture probe comprising a fluorophore attached thereto, and a target specific nucleic acid probe complementary to at least a portion of the capture probe and the target nucleic acid sequence, and comprising a quencher attached thereto, such that the quencher quenches fluorescence from the fluorophore when the target specific probe is hybridized to the capture probe.
14. The reaction chamber of claim 13, wherein the capture probe is immobilized upon an interior surface of the reaction chamber.
15. The reaction chamber of claim 13, wherein a plurality of different capture probes are immobilized in an array on an interior surface of the reaction chamber, each different capture probe being immobilized in a discrete location.
16. A method of detecting the presence of at least a first target nucleic acid sequence in a sample, comprising:
subjecting the sample to an amplification reaction capable of amplifying the target nucleic acid sequence in the presence of at least a first set of nucleic acid probes, the first set of nucleic acid probes comprising:
a target specific nucleic acid probe having a fluorescent label associated therewith complementary to at least a portion of the target nucleic acid sequence, a capture probe associated

with a solid support and complementary to at least a portion of the target specific nucleic acid probe; and

detecting fluorescence from the solid support following one or more cycles of the polymerase chain reaction, a decrease in fluorescence being indicative of the presence of the target nucleic acid sequence.

17. The method of claim 16, wherein the at least first target nucleic acid sequence comprises a variola virus sequence.

18. The method of claim 17, wherein the target specific nucleic acid probe comprises one or more of: VAR-1 probe (SEQ ID NO: 19), VAR-2 probe (SEQ ID NO: 22), VAR-3 probe (SEQ ID NO: 25), or VAR-4 probe (SEQ ID NO: 28).

19. The method of claim 16, wherein the target nucleic acid sequence is amplified through use of one or more of: VAR-1 primer 1 (SEQ ID NO: 17), VAR-1 primer 2 (SEQ ID NO: 18), VAR-2 primer 1 (SEQ ID NO: 20), VAR-2 primer 2 (SEQ ID NO: 21); VAR-3 primer 1 (SEQ ID NO: 23), VAR-3 primer 2 (SEQ ID NO: 24), VAR-4 primer 1 (SEQ ID NO: 26), or VAR-4 primer 2 (SEQ ID NO: 27).

CLAIMS

1. A method of detecting a plurality of target nucleic acid sequences in a sample, comprising:
 - subjecting the sample to an amplification reaction capable of amplifying a plurality of different target nucleic acid sequence in the presence of a plurality of different set of nucleic acid probes, each different set of nucleic acid probes comprising:
 - a different capture probe attached to a surface of a substrate, and comprising a fluorophore attached thereto;
 - a different target specific nucleic acid probe complementary to a different capture probe and a different one of the plurality of target nucleic acid sequence, and comprising a quencher attached thereto, such that the quencher quenches fluorescence from the fluorophore when each target specific probe is hybridized to a complementary capture probe; and
 - detecting fluorescence from the sample following one or more cycles of polymerase chain reaction, an increase in fluorescence being indicative of the presence of at least one target nucleic acid sequence.
2. The method of claim 1, wherein the amplification reaction comprises a PCR reaction.
3. The method of claim 2, wherein the PCR reaction employs a polymerase enzyme having exonuclease activity.
4. The method of claim 1, wherein each of the capture probes in the plurality of different sets of probes is immobilized upon a substrate in a detectably distinct location.
5. The method of claim 1, wherein the at least one target nucleic acid sequence comprises a variola virus sequence.
6. The method of claim 5, wherein the target specific nucleic acid probe comprises one or more of: VAR-1 probe (SEQ ID NO: 19), VAR-2 probe (SEQ ID NO: 22), VAR-3 probe (SEQ ID NO: 25), or VAR-4 probe (SEQ ID NO:28).

7. The method of claim 1, wherein the target nucleic acid sequences are amplified through use of two or more of: VAR-1 primer 1 (SEQ ID NO: 17), VAR-1 primer 2 (SEQ ID NO: 18), VAR-2 primer 1 (SEQ ID NO: 20), VAR-2 primer 2 (SEQ ID NO: 21); VAR-3 primer 1 (SEQ ID NO: 23), VAR-3 primer 2 (SEQ ID NO: 24), VAR-4 primer 1 (SEQ ID NO: 26), or VAR-4 primer 2 (SEQ ID NO: 27).

8. A reaction mixture, comprising:
a sample containing a plurality of target nucleic acid sequences of interest;
amplification reagents for amplifying the target nucleic acid sequences of interest; and
a plurality of different sets of nucleic acid probes, each different set of probes comprising a different capture probe having a fluorophore attached thereto and a different target specific probe, each target specific probe being complementary to a different capture probe and a different one of the plurality of target nucleic acid sequences, and having a quencher attached thereto, such that the quencher quenches fluorescence from the fluorophore when each target specific probe is hybridized to a complementary capture probe, wherein the target specific nucleic acid probe comprises two or more of: VAR-1 probe (SEQ ID NO: 19), VAR-2 probe (SEQ ID NO: 22), VAR-3 probe (SEQ ID NO: 25), or VAR-4 probe (SEQ ID NO: 28).

9. The mixture of claim 8, wherein the mixture further comprises two or more amplification primers chosen from the group consisting of: VAR-1 primer 1 (SEQ ID NO: 17), VAR-1 primer 2 (SEQ ID NO: 18), VAR-2 primer 1 (SEQ ID NO: 20), VAR-2 primer 2 (SEQ ID NO: 21); VAR-3 primer 1 (SEQ ID NO: 23), VAR-3 primer 2 (SEQ ID NO: 24), VAR-4 primer 1 (SEQ ID NO: 26), or VAR-4 primer 2 (SEQ ID NO: 27).

10. A reaction chamber, comprising:
a reaction region having disposed therein:
a sample containing a plurality of target nucleic acid sequences of interest;
amplification reagents for amplifying the target nucleic acid sequences of interest; and
a plurality of different sets of nucleic acid probes, each different set of probes comprising a different capture probe having a fluorophore attached thereto and a different target specific probe, each target specific probe being complementary to a different capture

probe and a different one of the plurality of target nucleic acid sequences, and having a quencher attached thereto, such that the quencher quenches fluorescence from the fluorophore when each target specific probe is hybridized to a complementary capture probe, wherein each of the capture probes in the plurality of different sets of probes is immobilized upon an interior surface of the reaction chamber.

11. The reaction chamber of claim 10, wherein a plurality of different capture probes are immobilized in an array on an interior surface of the reaction chamber, each different capture probe being immobilized in a discrete location.