using reaction volumes of 5 to 100 microliters. These reaction volumes accommodate test specimen volumes sufficient to ensure the detection of scarce assay targets in dilute specimens. Microfluidic systems that reduce reaction volumes relative to those employed in traditional laboratory molecular testing necessarily also reduce the volume of specimen that can be added to the reaction. The result of the smaller reaction volume is a reduction in capacity to accommodate sufficient specimen volume to ensure the presence of detectable amounts of target in dilute specimens or where assay targets are scarce.

SUMMARY OF THE INVENTION

An aspect of the present invention provides a cassette for detecting a target nucleic acid, the cassette comprising a plurality of chambers, a plurality of vent pockets connected to the chambers, and a heat labile material for sealing one or more of the vent pockets, wherein at least one the vent pocket comprises a protrusion. The protrusion preferably comprises a dimple or an asperity.

Another aspect of the present invention provides a cassette for detecting a target nucleic acid, the cassette comprising a plurality of chambers, a plurality of vent pockets connected to the chambers, a heat labile material for sealing one or more of the vent pockets, a heat stable material, and a gasket disposed between the heat labile material and the heat stable material, the gasket comprising an opening encompassing the plurality of vent pockets. The cassette preferably comprises a flexible circuit, the flexible circuit comprising patterned metallic electrical components disposed on the heat stable material. The gasket preferably comprises a second opening, or is limited in dimension, such that the flexible circuit will be in direct contact with fluid in at least one of the chambers. The electrical components preferably comprise resistive heating elements or conductive traces. The resistive heating elements are preferably aligned with the vent pockets and the chambers. The cassette preferably comprises one or more ambient temperature

sensors for adjusting a heating temperature, heating time, and/or heating rate of one or more of the chambers.

Another aspect of the present invention provides a cassette for detecting a target nucleic acid, the cassette comprising a vertically oriented detection chamber, a lateral flow detection strip disposed in the detection chamber oriented such that a sample receiving end of the detection strip is at the bottom end of the detection strip, and a space in the detection chamber below the lateral flow detection strip for receiving fluid comprising amplified target nucleic acids, the space comprising sufficient capacity to accommodate an entire volume of the fluid at a height that enables the fluid to flow up the detection strip by capillary action without flooding or otherwise bypassing regions of the detection strip. The space preferably comprises detection particles such as dye polystyrene microspheres, latex, colloidal gold, colloidal cellulose, nanogold, or semiconductor nanocrystals. The detection particles preferably comprise oligonucleotides complementary to a sequence of the amplified target nucleic acids or ligands, such as biotin, streptavidin, a hapten or an antibody, capable of binding to the amplified target nucleic acids. The detection particles have preferably been dried, lyophilized, or present on at least a portion of the interior surface as a dried mixture of detection particles in a carrier, such as a polysaccharide, a detergent, or a protein, to facilitate resuspension of the detection particles. A capillary pool of the fluid preferably forms in the space, providing improved mixing and dispersion of the detection particles to facilitate comingling of the detection particles with the amplified target nucleic acid. The cassette optionally performs an assay having a volume less than about 200 µL, and preferably less than about 60 µL.

Another aspect of the present invention provides a cassette for detecting a target nucleic acid, the cassette comprising one or more recesses for containing at least one lyophilized or dried reagent, at least one of the recesses comprising one or more structures for directing fluids to facilitate rehydration of the at least one dried or lyophilized reagent, the recesses disposed in one or more channels connected to the chambers or in one or more of the channels. The structures preferably comprise ridges, grooves, dimples, or combinations thereof.

Another aspect of the present invention provides a cassette for detecting a target nucleic acid, the cassette comprising at least one chamber comprising a feature to prevent fluid vertically entering a top of the

chamber from flowing directly into an outlet of the chamber, wherein the feature is triangular or trapezoidal in shape. The feature preferably deflects the fluid to the side of the chamber opposite from the outlet. The resulting flow path of the fluid preferably comprises a horizontal component, thereby sufficiently increasing the effective length of the flow path and sufficiently decreasing the flow velocity of the fluid to restrict the amount of fluid exiting the outlet. The feature preferably creates a swirling of fluid within the chamber, thereby increasing mixing of reagents within the fluid. The outlet is optionally tapered. A channel located downstream of the outlet optionally comprises turns for increasing an effective length of the channel. The feature is preferably located near or at a bottom of the chamber or near a middle of the chamber.

There is also disclosed a method of controlling vertical flow of a fluid through a chamber in a cassette for detecting a target nucleic acid, the method comprising deflecting a flow of fluid entering a top of the chamber, thereby preventing the fluid from flowing directly into an outlet of the chamber. The method preferably comprises reducing a flow velocity of the fluid, thereby reducing a distance the fluid flows down a channel connected to the outlet before the fluid stops. The method preferably comprises dividing a flow of the fluid into the chamber into a first fluid flow that contacts a wall of the chamber and is directed upward, and a second fluid flow that enters the outlet. The first fluid flow preferably swirls in the chamber, thereby increasing mixing of reagents within the fluid. The second fluid flow preferably forms a meniscus and travels through a channel connected to the outlet, the meniscus increasing pressure in closed air space in the channel downstream of the fluid until the pressure stops the flow of fluid in the channel. The outlet is optionally tapered, thereby increasing compressible air volume at the entrance to the outlet. The method optionally comprises providing turns in a channel connected to the outlet, thereby increasing an effective path length of the channel and reducing a flow velocity of fluid in the channel.

Objects, advantages and novel features, and further scope of applicability of the present invention will be set forth in part in the detailed description to follow, taken in conjunction with the accompanying drawings, and in part will become apparent to those skilled in the art upon examination of the following, or may be learned by practice of the invention. The objects and advantages of the

CLAIMS

1. A cassette for detecting a target nucleic acid, the cassette comprising:

a plurality of chambers;

a plurality of vent pockets connected to said chambers; and

a heat labile material for sealing one or more of said vent pockets;

wherein at least one said vent pocket comprises a protrusion.

2. The cassette of claim 1 wherein said protrusion comprises a dimple or an asperity.

3. A cassette for detecting a target nucleic acid, the cassette comprising:

a plurality of chambers;

a plurality of vent pockets connected to said chambers;

a heat labile material for sealing one or more of said vent pockets;

a heat stable material; and

a gasket disposed between said heat labile material and said heat stable material, said gasket comprising an opening encompassing said plurality of vent pockets.

4. The cassette of claim 3 further comprising a flexible circuit, said flexible circuit comprising patterned metallic electrical components disposed on said heat stable material.

5. The cassette of claim 4 wherein said electrical components comprise resistive heating elements or conductive traces.

6. The cassette of claim 5 wherein said resistive heating elements are aligned with said vent pockets and said chambers.

7. The cassette of claim 3 comprising one or more ambient temperature sensors for adjusting a heating temperature, heating time, and/or heating rate of one or more of said chambers.

8. A cassette for detecting a target nucleic acid, the cassette comprising:

a vertically oriented detection chamber;

a lateral flow detection strip disposed in said detection chamber oriented such that a sample receiving end of said detection strip is at the bottom end of said detection strip; and

a space in said detection chamber below said lateral flow detection strip for receiving fluid comprising amplified target nucleic acids;

said space comprising sufficient capacity to accommodate an entire volume of the fluid at a height that enables the fluid to flow up the detection strip by capillary action without flooding or otherwise bypassing regions of the detection strip.

9. The cassette of claim 8 wherein said space comprises detection particles.

10. The cassette of claim 9 wherein said detection particles are selected from the group consisting of dye polystyrene microspheres, latex, colloidal gold, colloidal cellulose, nanogold, and semiconductor nanocrystals.

11. The cassette of claim 9 wherein said detection particles comprise oligonucleotides complementary to a sequence of the amplified target nucleic acids or ligands capable of binding to the amplified target nucleic acids.

12. The cassette of claim 9 wherein the detection particles have been dried, lyophilized, or present on at least a portion of the interior surface as a dried mixture of detection particles in a carrier to facilitate resuspension of the detection particles.

13. The cassette of claim 9 wherein a capillary pool of the fluid forms in the space, providing improved mixing and dispersion of the detection particles to facilitate comingling of the detection particles with the amplified target nucleic acid.

14. The cassette of claim 8 wherein the cassette has a volume less than about 200 µL.

15. The cassette of claim 14 wherein the cassette has a volume less than about 60 µL.

16. A cassette for detecting a target nucleic acid, the cassette comprising one or more recesses for containing at least one lyophilized or dried reagent, at least one of said recesses comprising one or more structures for directing fluids to facilitate rehydration of the at least one dried or lyophilized reagent, said recesses disposed in one or more channels connected to said chambers or in one or more of said channels.

17. The cassette of claim 16 wherein said structures comprise ridges, grooves, dimples, or combinations thereof.

18. A cassette for detecting a target nucleic acid, the cassette comprising at least one chamber comprising a feature to prevent fluid vertically entering a top of said chamber from flowing directly into an outlet of said chamber, wherein the feature is triangular or trapezoidal in shape.

19. The cassette of claim 18 wherein said feature deflects the fluid to the side of said chamber opposite from said outlet.

20. The cassette of claim 18 wherein said feature creates a swirling of fluid within said chamber, thereby increasing mixing of reagents within the fluid.

21. The cassette of claim 18 wherein said outlet is tapered.

22. The cassette of claim 18 wherein said feature is located near or at a bottom of said chamber or near a middle of said chamber.