

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 nucleic acid, the cassette comprising at least one reaction chamber; wherein, when said cassette is oriented vertically, a top of said reaction chamber comprises an inlet and a projection extending downward into said reaction chamber to minimize or prevent capillary fluid flow across said top of said reaction chamber, and wherein a first side of said projection extends substantially vertically adjacent to said inlet, and a second side of said projection extends upward toward said top of said reaction chamber at an angle less than approximately 60 degrees from vertical.

~~The present invention is~~ There is also disclosed 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 pockets comprises a protrusion. The protrusion preferably comprises a dimple or an asperity and preferably sufficiently prevents molten heat labile material from attaching to a heat stable material disposed adjacent to the heat labile material to prevent resealing of the vent pocket after the heat labile material is ruptured.

~~The present invention~~ There is also disclosed 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 gasket is preferably sufficiently thick to provide a sufficient air volume to equilibrate pressures and ensure free air movement between open 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.

~~The present invention~~There is also disclosed 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 .

~~The present invention~~There is also disclosed 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 detection chambers or one or more channels connected to the detection chambers. The structures preferably comprise ridges, grooves, dimples, or combinations thereof.

~~The present invention~~There is also disclosed 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. 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 feature is preferably triangular or trapezoidal in shape. 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.

~~The present invention~~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.

~~The present invention~~There is also disclosed a cassette for detecting a nucleic acid, the cassette comprising at least one reaction chamber, wherein, when the cassette is oriented vertically, a top of the reaction chamber comprises an inlet and a projection extending downward into the reaction chamber to minimize or prevent capillary fluid flow across said top of the reaction chamber. The projection is preferably generally triangular in shape. A first side of the projection preferably extends substantially

CLAIMS

~~What is claimed is:~~

1. A cassette for detecting a nucleic acid, the cassette comprising at least one reaction chamber;

wherein, when said cassette is oriented vertically, a top of said reaction chamber comprises an inlet and a projection extending downward into said reaction chamber to minimize or prevent capillary fluid flow across said top of said reaction chamber, and

wherein a first side of said projection extends substantially vertically adjacent to said inlet, and a second side of said projection extends upward toward said top of said reaction chamber at an angle less than approximately 60 degrees from vertical.

2. The cassette of claim 1, wherein said projection is generally triangular in shape.

~~3. The cassette of claim 1 wherein a first side of said projection extends substantially vertically adjacent to said inlet.~~

~~4. The cassette of claim 3 wherein a second side of said projection extends upward toward said top of said reaction chamber at an angle less than approximately 60 degrees from vertical.~~

~~5~~3. The cassette of claim ~~4~~1, wherein said angle is less than approximately 45 degrees from vertical.

~~6~~4. The cassette of claim ~~5~~3, wherein said angle is less than approximately 30 degrees from vertical.

~~7. The cassette of claim 6 wherein said second side of said projection extends vertically toward said top of said reaction chamber.~~

~~85.~~ The cassette of claim 1, further comprising a recess for containing at least one lyophilized or dried reagent, said recess disposed in a channel connected to said inlet of said reaction chamber.

~~96.~~ The cassette of claim ~~8-5,~~ wherein said projection reduces or prevents sequestration of newly resuspended reagent from the bulk of the reaction solution volume.

~~107.~~ The cassette of claim ~~8-5,~~ wherein said recess comprises one or more structures for directing fluids to facilitate rehydration of the at least one dried or lyophilized reagent.

~~118.~~ The cassette of claim ~~10-7,~~ wherein said structures comprise ridges, grooves, dimples, or combinations thereof.

~~9. The cassette of claim 5, wherein said recess is located upstream of said projection.~~

~~1210.~~ The cassette of claim 1, wherein said reaction chamber comprises a recess for containing at least one lyophilized or dried reagent.

~~11. The cassette of any one of the preceding claims, comprising a plurality of chambers including said at least one reaction chamber.~~

12. The cassette of claim 11, further comprising a plurality of vent pockets connected to said plurality of chambers.

13. The cassette of claim 12, further comprising a gasket comprising an opening encompassing said plurality of vent pockets.

14. The cassette of claim 13, further comprising a flexible circuit which comprises patterned metallic electrical components disposed on a heat stable material adjacent to said gasket.

15. The cassette of claim 14, wherein said gasket has a second opening, or is limited in dimension, such that said flexible circuit is in direct contact with fluid in at least one of said plurality of chambers.

16. The cassette of any one of claims 11 to 15, further comprising one or more ambient temperature sensors for adjusting a heating temperature, heating time, and/or heating rate of one or more of said plurality of chambers.

17. The cassette of any one of claims 11 to 16, wherein said plurality of chambers comprises a detection chamber for detecting said nucleic acid.

18. The cassette of claim 17, further comprising a detection strip disposed in said detection chamber and oriented such that a sample receiving end of said detection strip is at the bottom end of said detection strip.

19. The cassette of claim 18, wherein said detection strip is a lateral flow detection strip.

20. The cassette of any one of claims 17 to 19, wherein said detection strip is spaced apart from the bottom of said detection chamber.

21. The cassette of claim 20, wherein the space between said detection strip and the bottom of said detection chamber comprise detection particles and/or is configured to retain a volume of fluid that comprises amplified target nucleic acids.

22. The cassette of claim 21, wherein said detection particles comprise dye polystyrene microspheres, latex, colloidal gold, colloidal cellulose, nanogold, or semiconductor nanocrystals.

23. The cassette of claim 21 or 22, wherein said detection particles comprise oligonucleotides complementary to a sequence of said amplified target nucleic acids, or ligands capable of binding to said amplified target nucleic acids.

24. The cassette of any one of claims 21 to 23, wherein said detection particles are dried, lyophilized, or present on at least a portion of an interior surface as a dried mixture of detection particles in a carrier.