

incompatible with the materials used to manufacture the cartridge, e.g., silicon, glass or polymeric parts, a variety of coatings may be applied to the surfaces of these parts that contact the reagents. For example, components that have silicon elements may be coated with a silicon nitride layer or a metallic layer of, e.g., gold or nickel, sputtered or plated on the surface to avoid adverse reactions with these reagents.

**[0100]** Similarly, inert polymer coatings, Parylene® coatings, or surface silanation modifications may also be applied to internal surfaces of the cartridge in order to make the overall system more compatible with the reactions being carried out. For example, in the case of nucleic acid analysis, it may be desirable to coat the surfaces with, e.g., a non-stick coating to prevent adhesion of nucleic acids to the surface. Additionally, patterned metal electrical conductors for activating actuators, heaters, sensors, and the like may be used. Such conductors may be coated with insulator coatings in those instances where electrical leads are placed in contact with fluids, to prevent shorting out or gas formation from electrolysis. Such insulators are well known in the art, e.g. screen-printed polymers, epoxies, ceramics and the like.

**[0101]** Although the preferred embodiment incorporates flow controllers, e.g. valves, it is possible for a continuously-flowing fluid stream to be guided, divided and diverted to various regions within the cartridge without the incorporation of valves. In one embodiment, the fluid stream flows down a channel with relatively little flow resistance into a second region, e.g., a waste chamber. The waste chamber may be vented through a port blocked with a hydrophobic porous membrane, such as Goretex®. When the waste chamber is filled, and all the air in the waste chamber is expelled through the membrane vent, the fluid sample cannot pass through the membrane, and a back-pressure is developed.

**[0102]** The back-pressure is sufficiently large to force the remaining fluid stream through a smaller, secondary, capillary channel, pressure sensitive filter, or other flow restrictor located upstream from the first chamber. Once fluid flow is initiated through the small channel, no additional fluid will flow into the first channel and the fluid stream will be completely diverted into the secondary channel. Optionally, the smaller channel may be locally heated to induce diversion of the flowing sample into the smaller channel before the larger region or chamber is full.

**[0103]** In addition, fluid may be prevented from flowing back upstream by fluid diodes. FIG. 4 shows one example of such a fluid diode. Fluid is permitted to flow in a direction from A to B, but prevented from flowing in the opposite direction from B to A. The diode 91 comprises a top portion 43 having a port 45 and an adjoining recess 47; a flex circuit plate 49 having a flap 51; and a bottom portion 53 having a channel 55. When the diode 91 is deactivated, a magnetic disc 57 on the flap 51 is attracted towards the top portion 43 by an external magnetic force provided by, e.g., the external instrument. The flap is biased against the recess 47 in the top portion 43, thus allowing fluid flowing through the port 45 to pass beneath the flap 51 and through the channel 55 of the bottom portion 53.

**[0104]** When the diode 91 is activated, the magnetic force is disabled and the flap 51 returns to a sealing position due to the spring constant of the flap which prevents fluid from passing from the port 45 beneath the flap 51 and through the channel 55. In this manner, fluid present in the bottom portion 53 is prevented from flowing backwards through the port 45 of the top portion.

**[0105]** The cartridge preferably has a venting element to release back pressure of fluids. The vent may include an opening to the external environment (e.g. inlet port or outlet port with or without a hydrophobic vent). Conveniently, the vent may be an internal expandable cavity such as a corrugated membrane or an elastic latex membrane. Release of fluids through the vent may be passive or active, as in the application of a vacuum to a port on the cartridge.

**[0106]** The inclusion of gas permeable fluid barriers, e.g., poorly wetting filter plugs or hydrophobic membranes, in the cartridges also may be used to create a fluid direction and control system. Such filter plugs, when incorporated at the end of a chemically interactive region opposite a fluid inlet, allow air or other gas present in the interactive region to be expelled during introduction of the liquid component into the region. Upon filling of the region, the fluid sample contacts the hydrophobic plug thus stopping net liquid flow. Fluidic resistance may also be employed as a gas permeable barrier to accomplish this same result, e.g., using fluid passages that are sufficiently narrow to provide an excessive resistance, thereby effectively stopping or retarding liquid flow while permitting air or gas flow.

**[0107]** A variety of materials are suitable for use as poorly wetting or gas permeable filter plugs including, e.g., porous hydrophobic polymer materials, such as spun fibers of acrylic, polycarbonate, Teflon®, pressed polypropylene fibers, or any number of commercially available filter plugs. Alternatively, a hydrophobic membrane can be bonded over a through-hole to supply a similar structure. Modified acrylic copolymer membranes are commercially available from, e.g., Gelman Sciences (Ann Arbor, Mich.) and particle-track etched polycarbonate membrane's are available from Poretics, Inc. (Livermore, Calif.). Venting of heated chambers may incorporate barriers to evaporation of the sample, e.g., a reflux chamber. Excessive evaporation of fluid from the sample may be prevented by disposing a mineral oil layer within the chamber and over the top surface of the sample to permit the evolution of gas while controlling evaporation.

**[0108]** Lysing regions within the cartridge can be designed to effect lysing of target cells by physical, chemical or other means, or a combination of such means. Physical means includes the mechanical disruption of the cells, such as by the vibration of glass or plastic beads or other particles or by impacting the target cells or viruses onto sharp microstructures. Thermal energy transfer, such as by heating a virus suspension to 95° C. or by repeated freeze-thawing of activated bacterial spores to disrupt cell walls, may also be used.

**[0109]** Chemical lysing can be employed alone or in combination with physical or ultrasonic lysing. Typical chemical lysing agents fall into several categories, such as enzymes, detergents, and chaotropes. Lysosome is an enzyme that hydrolytically attacks the cell walls of many bacteria; trypsin is a protease enzyme that breaks the cell membrane of most eukaryotic cells. Other proteases with specificity for certain peptide sequences can be employed and are preferred if the target moiety is liable to certain proteases. Proteinase K is often used because it also digests nuclear proteins and host cell enzymes that may interfere with polymerase chain reaction (PCR). For eucaryotic cells, detergents such as Triton X-100 or sodium dodecyl sulfate solubilize the cell membrane and release intracellular contents. Chaotropes such as guanidine isothiocyanate or urea can be used to lyse cells and have the additional benefit of inhibiting RNases that can destroy target RNA.