

extracting analyte from milliliter quantities of fluid sample and eluting the analyte into microliter quantity eluates. In the preferred embodiment, the sample volume forced to flow through the cartridge is in the range of 1 to 100 mL, enabling concentration factors of 100 or greater. For example, the analyte from 1 mL of fluid sample may be captured in the device and concentrated into 10  $\mu$ L or less of elution fluid.

**[0077]** A fluid sample may be introduced into the cartridge by a variety of means, manual or automated. For manual addition, a measured volume of material may be placed into a receiving area of the cartridge through an input port and a cap is then placed over the port. Alternatively, a greater amount of sample material than required for the analysis can be added to the cartridge and mechanisms within the cartridge can effect the precise measuring and aliquoting of the sample needed for the specified protocol.

**[0078]** It may be desirable to place certain samples, such as tissue biopsy material, soil, feces, exudates, and other complex material into another device or accessory and then place the secondary device or accessory into the cartridge causing a mechanical action which effects a function such as mixing, dividing, or extraction. For example, a piece of tissue may be placed into the lumen of a secondary device that serves as the input port cap. When the cap is pressed into the port, the tissue is forced through a mesh that slices or otherwise divides the tissue.

**[0079]** For automated sample introduction, additional cartridge design features are employed and, in many cases, impart specimen accession functionality directly into the cartridge. With certain samples, such as those presenting a risk of hazard to the operator or the environment, such as human retrovirus pathogens, the transfer of the sample to the cartridge may pose a risk. Thus, in one embodiment, a syringe may be integrated into a device to provide a means for moving external fluidic samples directly into the cartridge. Alternatively, a venous puncture needle and an evacuated blood tube can be attached to the cartridge forming an assembly that can be used to acquire a sample of blood. After collection, the tube and needle are removed and discarded, and the cartridge is then placed in an instrument to effect processing. The advantage of such an approach is that the operator or the environment is not exposed to pathogens.

**[0080]** The input port can be designed with a consideration of appropriate human factors as a function of the nature of the intended specimen. For example, respiratory specimens may be acquired from the lower respiratory tract as expectorants from coughing, or as swab or brush samples from the back of the throat or the nares. In the former case, the input port can be designed to allow the patient to cough directly into the cartridge or to otherwise facilitate spitting of the expectorated sample into the cartridge. For brush or swab specimens, the specimen is placed into the input port where features of the port and closure facilitate the breaking off and retaining of the end of the swab or brush in the cartridge receiving area.

**[0081]** In another embodiment, the cartridge includes input and output tubes that may be positioned in a sample pool of very large volume, such as a flowing stream of water, so that the sample material flows through the cartridge. Alternatively, a hydrophilic wicking material can serve as an interactive region so that the entire cartridge can be immersed directly into the specimen, and a sufficient amount of specimen is absorbed into the wicking material. The cartridge is then removed, and can be transported to the laboratory or analyzed directly using a portable instrument. In another

embodiment, tubing can be utilized so that one end of the tube is in direct communication with the cartridge to provide a fluidic interface with at least one interactive region and the other end is accessible to the external environment to serve as a receiver for sample. The tube can then be placed into a specimen and serve as a sipper.

**[0082]** The cartridge itself may also serve as the actual specimen collection device, thereby reducing handling and inconvenience. In the case of specimens involved in legal disputes or criminal investigations, the direct accessing of the test material into the fluidic cartridge is advantageous because the chain of custody is conveniently and reliably preserved.

**[0083]** In general applications of the cartridge, chemical interactions of the fluid sample with one or more reagents may be required, so it is desirable to include interactive regions that provide for chemical reagents, the number and type depending on the specific analytical protocol to be facilitated. Multiple interactive regions, each containing different reagents, can be arranged in series to enable the sequential processing of the sample.

**[0084]** Reagents may be exogenously introduced into the cartridge before use, e.g., through sealable openings in each region of the cartridge. Alternatively, the reagents may be placed in the cartridge during manufacture. The reagents may be disposed within the interactive regions that perform the operations for which the reagents will be used, or within regions leading to a particular interactive region. Alternatively, the reagents may be disposed within storage chambers in fluid communication with interactive regions.

**[0085]** The type of reagent utilized at an interactive region depends, inter alia, on the fluid characteristics and size of the sample, the nature and concentration of the target constituents, and the desired processing protocol. In the case of solution phase interactions, the reagents may be aqueous solutions or dried reagents requiring reconstitution. The particular format is selected based on a variety of parameters, including whether the interaction is solution-phase or solid-phase, the inherent thermal stability of the reagent, speed of reconstitution, and reaction kinetics.

**[0086]** Liquid reagents may include, but are not limited to, buffer solutions such as saline, TRIS, acids, bases, detergent solutions, and chaotropic solutions, which are commonly used for DNA and RNA purification and washing. Dried reagents can be employed as precursor materials for reconstitution and solution-phase interaction or as solid-phase reagents, including pH indicators; redox indicators; enzymes such as horseradish peroxidase, alkaline phosphatase, reverse transcriptase, DNA polymerase, and restriction enzymes; enzyme substrates; enzyme-antibody or enzyme-antigen conjugates; DNA primers and probes; buffer salts; and detergents. Furthermore, solid-phase reagent coatings such as serum albumin, streptavidin, and a variety of cross-linkable proteins such as polysaccharides may be employed at the interactive region.

**[0087]** Dried reagents may also be contained within a membrane material that can be employed as an interactive region by physical incorporation of the material into a region communication with fluidic channels. Cellulose, nitrocellulose, polycarbonate, nylon, and other materials commonly used as membrane materials can be made to contain reagents. Such membranes are designed to capture target cells, effect lysis of host cells, release target nucleic acids, and separate contaminants that may interfere with the polymerase chain reaction or other analytical events. These papers may be positioned