

the bottom of chambers **1510** and **1511**. These filters may be integrally molded/machined, etched/etc. into the corresponding chambers. Alternatively, as illustrated in **FIG. 20** depicting a bottom view of a cartridge body, the filters **2020,2021** may be separate components that are incorporated into the corresponding chambers during the manufacturing/assembly process; e.g., filter inserts that can be inserted/snapped into a receptacle within the chamber that is arranged and configured to engagingly receive the filter insert.

**[0198]** The assay reagent release mechanism for releasing the contents of a breakable ampoule may be a simple mechanical device that is actuated to exert a force onto the ampoule; e.g., deliver a sharp blow to the ampoule thereby rupturing it and releasing its contents into the assay reagent chamber. **FIG. 21** depicts one preferred embodiment of a reagent chamber employing assay reagent ampoules **2120, 2121**. Preferably, a cover layer (not shown), most preferably made from a flexible material, is sealed to the top of the cartridge body so that liquid does not leak from the cartridge after the ampoules are ruptured (see, e.g., cover layer **1401** in **FIG. 14**). **FIG. 21** also shows assay release mechanism **2110** (preferably, a component of a cartridge reader) which can be actuated so that hammer element **2115** strikes an ampoule, preferably by striking a flexible cover layer that then transfers the impact force to the ampoule (while, preferably, remaining intact so that it confines the released liquid to the reagent chamber). It has been observed that striking the ampoule quickly with an adequate impulsive force produces a more complete rupturing of the ampoule and thereby more effectively releasing the assay reagent. Whereas a slowly applied force increasing in magnitude until ultimately the ampoule fractures results in less complete rupture and less effective assay reagent release.

**[0199]** In an alternative embodiment, a pierceable container such as a pouch or blister pack may be employed. Preferably, the pierceable container has a pierceable wall made from a plastic film, a metal foil, or most preferably, a metal foil/plastic film laminate. In such an embodiment the assay reagent release mechanism could employ a piercing scheme. **FIG. 22** shows an exploded view of one preferred embodiment of a reagent chamber for holding a pierceable container. Reagent chamber **2210** has piercing tip **2212** located at the bottom of the chamber. Chamber **2210** is connected to reagent conduit **2216** and, optionally, a vent conduit (not shown). Reagent module **2220** comprises module body **2230**, preferably made of injected molded plastic, that defines the walls of a fluid compartment, having a first opening **2232** and a second opening **2234**. Fluid is sealed in the compartment by first opening cover **2242** and second opening cover **2244**, the covers preferably made of a plastic-metal laminate (most preferably and aluminum coated mylar film) Module **2220** also, preferably, has tongue **2250** that fits in chamber groove **2214** so as to properly align module **2220** in chamber **2210** and hold module in an elevated position above piercing element **2212**. Chamber **2210** also, preferably, has a chamber cover layer that prevents leakage of reagent from the chamber after rupture of module **2220**. On application of a threshold downward force to module **2220**, preferably through a flexible chamber cover layer, module **2220** is pushed against tip **2212**, piercing first opening cover **2242** and releasing the reagent into the chamber. Module **2220** also, preferably, comprises a second piercing tip **2236** that is attached to the module walls via a cantilever (the

second piercing element and cantilever are preferably integral to the module body; such a component is readily manufacturable, e.g., by injection molding). When piercing tip **2212** pierces first opening cover **2242** in a module with a second tip element **2236**, piercing tip **2212** pushes second piercing tip **2236** until it pierces second opening cover **2234** making a second opening in module **2220** and facilitating extraction of the fluid from the pouch; i.e., venting the pouch itself.

**[0200]** In another alternate embodiment, liquid reagents are stored in a syringe comprising a syringe chamber and a plunger. The chamber may be an integral component of the cartridge, a module that is inserted into the cartridge or a separate component that is attached (e.g., via a luer lock connection) to the cartridge prior to use. Actuation of the plunger may be used to release the contents of the syringe into a reagent chamber or, alternately, to transfer the contents directly into other fluidic components of the cartridge.

**[0201]** An important consideration for cartridge based assay systems relates to long term storage of the cartridge prior to use; i.e., "shelf life" of the cartridge. Certain assay reagents (especially biological reagents and/or binding reagents such as enzymes, enzyme substrates, antibodies, proteins, receptors, ligands, haptens, antigens, nucleic acids and the like), when dissolved in a liquid medium require special handling and storage in order to improve their shelf life. In certain instances, even if the assay reagents dissolved in liquid media are handled and stored in strict compliance with the special handling and storage requirements their shelf life is impracticably short. Furthermore, the need to observe special handling and storage requirements adds to the complexity and cost of the cartridge based system employing such reagents. The special handling and storage requirements can be substantially reduced, if not eliminated, and the complexity and cost of the system can be minimized by using more stable dry, or dehydrated, forms of the assay reagents. The use of dry reagents can also simplify mixing operations and reduce the volume and weight of a cartridge. Reagents that may be included in dry form include biological reagents, binding reagents, pH buffers, detergents, anti-foam agents, extraction reagents, blocking agents, and the like. The dry reagent may also include excipients used to stabilize the dry reagents such as sugars (e.g., sucrose or trehalose). For assays may encounter acidic or basic samples (e.g., samples that are inherently acidic/basic and/or samples that are extracted or otherwise treated with an acidic/basic reagent), a dry reagent may include a neutralizing reagent (e.g., an acid, base of a pH buffer). In especially preferred embodiment that involve extraction of samples with nitrous acid, the extracted sample is passed over a dry reagent comprising a base or, more preferably, the base form of a buffering agent (e.g., Tris, Hepes, phosphate, PIPES, etc.). A sufficient amount of the base or buffering agent is included to bring the pH of the extracted sample to a value that is compatible with subsequent assay reactions carried out on the sample (e.g., binding reactions with binding reagents).

**[0202]** Dry reagents may be employed in a cartridge based assay system in a number of ways. As described above, dry reagents may be stored in a reagent chamber that is filled prior to use by a user or by a cartridge reader apparatus. Similarly, dry reagents may be stored in other fluidic components such as within fluidic conduits or chambers, most preferably within a fluidic conduit connecting the sample