

**[0198]** The reagent chambers are chambers adapted to hold liquid reagents used during the course of assays carried out in a cartridge. The reagent chamber design considerations for preferred embodiments of a cartridge depend, in part, upon the particular assay(s) to be performed by the cartridge. For example, a cartridge may have one, two or more reagent chambers depending on the number of reagents required by the assay format. Liquid reagents that may be held in a reagent chamber include buffers, assay diluents, solutions containing binding reagents (e.g., proteins, receptors, ligands, haptens, antibodies, antigens, nucleic acids and the like), solutions containing enzymes and/or enzyme substrates, solutions containing control reagents, ECL read buffers containing ECL coreactants (e.g., tertiary amines such as piperazine-N,N'-bis(2-ethanesulfonic acid) and tripropylamine), wash solutions, anti-foam agents, extraction reagents (e.g., solutions containing detergents, acids, bases, nitrous acid, nitrate salts, etc.) and the like. A cartridge may have one, two or more reagent chambers depending, e.g., on the number of reagents required by the assay format. The reagent chamber design considerations for preferred embodiments of a cartridge depend, in part, upon the particular assay(s) to be performed by the cartridge. The reagent chamber is connected to a reagent conduit for transferring reagent from the chamber to other fluidic components in the cartridge. The reagent chamber is, preferably, also connected to a reagent vent port (optionally, through a reagent vent conduit). The arrangement of the conduit connections to the chamber falls under similar design considerations as those described for the sample chamber, sample conduit and sample port; preferably, the reagent conduit intersects the chamber at or near the bottom and the reagent vent/vent conduit intersects the chamber at or near the top (relative to the orientation of the cartridge during use). Optionally, a filter element is placed before or in the reagent conduit, e.g., if the reagent solution is expected to contain particles that may clog the cartridge fluidics or otherwise negatively affect assay performance.

**[0199]** In one embodiment of the invention, a cartridge has one or more reagent compartments that are empty or contain only dried reagents. Prior to conducting an assay, the user or cartridge reader dispenses liquid reagents into these chambers (e.g., through reagent vent ports or through reagent introduction ports similar to the sample introduction port described above) which, optionally, reconstitute any dried reagent present in the chambers; the reagents are thus prepared for use in the assay. Sealable closures may be used to prevent leakage of the reagents after their addition.

**[0200]** Preferably, where an assay requires the use of liquid reagents, some or all of these liquid reagents are stored in liquid form in reagent chambers so as to minimize the number and complexity of the operations that must be carried out by a user or cartridge reader. In one preferred embodiment the reagent chamber(s) can be filled with the requisite assay reagent(s) at the time of cartridge manufacture and subsequently sealed. When used to store liquid reagents, the reagent chambers should be designed so as to prevent leakage and/or evaporative loss of the reagents from the chambers during storage. In a particularly preferred embodiment the assay reagents are incorporated into assay reagent modules that can be assembled into the cartridge's assay reagent chambers during manufacture. By designing the assay modules to have desired properties such as resistance to leakage and evaporative loss, the design and manufacture of the rest of the cartridge is greatly simplified. In such a preferred embodi-

ment, an assay reagent release mechanism would preferably be incorporated within the cartridge reader for releasing the assay reagent from the reagent module. The assay reagent release mechanism is preferably adapted and configured to engage the reagent module and release/recover its contents.

**[0201]** The reagent module is a container such as an ampoule (e.g., glass, plastic, or the like), a pouch (e.g., plastic, metal foil, plastic/metal foil laminates, rubber, or the like), a blister pack, a syringe, or the like, or any other container that can be filled with fluid, sealed and dropped into the cartridge for subsequent fluid delivery. Preferred materials include glass, plastics with good water vapor barrier properties (e.g., cyclic olefin copolymers such as copolymers of ethylene and norbornene, nylon 6, polyethylene naphthalate, polyvinylidene chloride and polychlorotrifluoroethylene) and metal foil/plastic laminates because of their chemical inertness and their resistance to evaporative losses, other suitable materials will be apparent to the skilled practitioner. Ampoules, preferably, comprise a material that can be made to shatter or break on impact such as glass or hard plastic. Embodiments incorporating breakable ampoules preferably also include filters to ensure that substantially all of the fragments that may result upon rupturing the ampoules are not permitted to enter the fluidic network and possibly obstruct/block fluid flow. FIG. 19 depicts a cutaway top view of a cartridge showing filters 1515, 1516 at 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.

**[0202]** 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 ampoule (not: 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 releases 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.

**[0203]** The ampoules may be broken serially (one at a time) or in parallel (simultaneously or substantially simultaneously). In one embodiment, an assay cartridge contains two ampoules with two different reagents (for example, a sample extraction buffer and a wash buffer). A cartridge reader for