

containing a reactive component. In one example, the ampoule contains an acid such as acetic acid and the dry reagent is a nitrate salt so that rupture of the ampoule results in the preparation of nitrous acid.

**[0214]** A pill zone in which dried reagents are deposited may be prescribed by a boundary which confines the volume of a deposited solution (and, therefore, the dried reagent left after allowing the solution to dry) to a specific region of a substrate. According to one preferred embodiment of the invention, a cartridge comprises a pill zone that is bounded by a boundary surface, the boundary surface being raised or lowered (preferably, raised) and/or of different hydrophobicity (preferably, more hydrophobic) than the pill zone. Preferably, the boundary surface is higher, relative to the substrate surface within the pill zone, by 0.5-200 micrometers, or more preferably by 2-30 micrometers, or most preferably by 8-12 micrometers. Even more preferably, the boundary surface has a sharply defined edge (i.e., providing a steep boundary wall and/or a sharp angle at the interface between the pill zone and the boundary). Preferably, the pill zone surface has a contact angle for water 10 degrees less than the boundary surface, preferably 15 degrees less, more preferably 20 degrees less, more preferably 30 degrees less, even more preferably 40 degrees less, and most preferred 50 degrees less.

**[0215]** In one preferred embodiment the pill zone is defined by a depression cut or molded into the substrate. In another embodiment, the boundary surface around a pill zone is defined by a boundary material applied on the substrate. In one example, the pill zone is defined by a cutout in a film or gasket applied to the substrate, preferably a cutout in a film of adhesive tape. In another preferred embodiment the boundary can be physically defined by applying a coating in a manner which defines the boundary of the pill zone using, e.g., established techniques for forming patterned coatings such as photolithography, patterned deposition, screen printing, etc. In one example, a patterned dielectric coating can be screen-printed onto the surface of a substrate material, the pattern including apertures, the boundaries of which define the pill zone. The reagent can then be dispensed onto the substrate within the pill zone boundary and thereafter dried to form the dried reagent pill.

**[0216]** The waste chambers are chambers adapted to hold excess or waste liquid. In certain embodiments, the detection chamber may also act as a waste chamber. In certain embodiments, however, it is beneficial to have a separate waste chamber, e.g., when carrying out assay formats that involve passing samples through the detection chamber having a volume greater than the volume of the detection chamber or when carrying out assay formats that involve wash steps to remove sample from the detection chamber. Sizing of the waste chambers is preferably done in accordance to the anticipated volumes of sample and liquid reagents that will be used in the assay. Another sizing related factor for the waste chambers that is preferably taken into account relates to the potential for waste fluids, as they enter the waste chamber to foam or bubble. In such instances, where foaming or bubbling is anticipated, the waste chamber volume could be increased sufficiently to avoid any issues that can arise from such foaming or bubbling.

**[0217]** Waste chambers are linked to a waste chamber conduit and, preferably, to a vent port (e.g., through a vent conduit). The waste chamber is configured to allow liquid waste to be delivered to the waste chamber through the waste chamber conduit and, preferably, for air that is included in the

waste stream to escape through a waste chamber vent port. Optionally, the waste chambers contain a water absorbing material, such as a sponge, that retains waste fluid and prevents leakage of the waste fluid on disposal of a cartridge. A factor that is preferably considered when designing the configuration and arrangement of the waste chambers relates to eliminating or substantially reducing the possibility that fluid from the waste chamber can flow back ("back-flow") into the cartridge's fluidic network. In particularly preferred embodiments, as illustrated in FIG. 10, the waste chamber conduits are arranged/routed such that they are fluidically connected to the waste chambers at points **1040**, **1041** that are above the anticipated fill levels/lines (i.e., the fill level/line is defined by the volume of waste fluid that resides within the waste chamber at the conclusion of the assay). This preferred configuration substantially reduces or eliminates the possibility that fluid from the waste chamber can flow back ("back-flow") into the cartridge's fluid network.

**[0218]** The issue of back-flow may also arise in the context of bubbling/foaming of the waste fluids. The vent port is preferably linked via a conduit with a large enough volume to allow a small amount of liquid to enter the conduit (e.g., because of foam in the waste chamber) without this liquid reaching the vent port (as described for above for the sample chamber). Furthermore, aerosol-prevention plugs or gas-selective membranes (i.e., materials that selectively allow the passage of gas but prevent the passage of liquids) may be included into the waste chamber vent conduits or vent ports to prevent release of liquid through these passages. Aerosol-prevention plugs are commonly used in pipette tips to prevent contamination of pipettors and include materials that allow the passage of air when dry but swell and seal up the passage when they come in contact with liquid (e.g., filter materials impregnated or coated with cellulose gum).

**[0219]** An additional measure for eliminating or substantially reducing foaming/bubbling of waste fluids as they are introduced into the waste chamber can be employed in particularly preferred embodiments. Such an additional anti-foaming/bubbling measure may include arranging/routing the waste chamber conduit such that it enters the waste chamber at a position that is located above the fill line and that intersects a vertical wall of the waste chamber, as illustrated by conduit segments **910** and **911** entering waste chambers **930** and **931** in the embodiment depicted in FIGS. **9** and **10**. Such a configuration allows the waste fluid to be introduced into the waste chamber in a manner so as to allow the fluid to run along a vertical wall of the waste chamber. Advantageously, this substantially reduces or eliminates foaming/bubbling of the waste fluid as it is routed into the waste chamber.

**[0220]** Yet another anti-foaming/bubbling measure that may be employed in certain preferred embodiments comprises a vertical web, or partial wall, that can be included in the upper portion of the waste chamber. A particularly suitable embodiment for inclusion of such an anti-foaming/bubbling measure is the two-piece cartridge body design depicted in FIG. **16**. The anti-foaming web/wall is preferably included in the upper portions of the waste chambers **1610**, **1611** located in the upper cartridge component **1500**. Preferably the anti-foaming web is arranged between the waste chamber vent and the waste chamber input. The height of the anti-foaming web preferably extends the full depth of the upper portion of the waste chamber but may be less than the full depth as well. Alternatively, the anti-foaming web can extend