

ing a Z-transition). Extraction buffer chamber **3710** holds a liquid extraction reagent (which may be in a reagent ampoule) for extracting analyte(s) of interest. Such extraction buffers may include buffers that are known in the art to be suitable for extracting the specific analyte(s) of interest that the cartridge is designed to measure and may also include anti-foam agents, including, without limitation, SE-15, Antifoam 204, Antifoam A, Antifoam B, Antifoam C, Antifoam Y-30, and combinations thereof (available from Sigma-Aldrich Corp., St. Louis, Mo., www.sigmaaldrich.com).

[0286] The invention includes cartridges and methods for carrying out assays for detecting influenza infections. In particular, applicants have discovered that the sensitivity of assays for detection of influenza and/or for determining influenza subtype by detection of influenza hemagglutinin proteins can be significantly enhanced by extraction of the samples under acidic conditions (pH 4.0 to 5.2 or 4.5 to 5.0). Suitable extraction reagents may achieve acidic pH through the inclusion of strong acids such as hydrochloric and sulfuric acid. Advantageously, the extraction reagent is a buffered solution at or near the desired pH that includes a buffering agent with buffering capacity in the appropriate pH range (e.g., appropriate buffering agents include, but are not limited to, ones based on carboxylic acids such as acetic acid and lactic acid and, especially, polycarboxylic acids such as citric and glutaric acid and also include quaternary ammonium buffers such as MES). In one embodiment, the concentration of the buffer is between 10 to 500 mM or between 100 and 200 mM or around 117 mM and the pH of the buffer is between 4.0 to 5.2 or 4.5 to 5.0. In a specific embodiment, the buffer includes 30 mM glutaric acid. Alternatively, the buffer may include 15 mM citric acid. In addition, the buffer may include about 0.10 to 0.5 M NaCl, e.g., 0.15 M NaCl. The extraction reagent may also include an anti-foam agent and a surfactant (e.g., a non-ionic surfactant such as Tween 20, Thesit, Triton X-100 or an ionic surfactant such as deoxycholic acid or CHAPSO), preferably at a concentration near to or greater than the CMC. In one embodiment, the extraction reagent includes greater than 0.02% Triton X-100 or greater than 0.05% Triton X-100 or about 0.1% Triton X-100. In one embodiment, the extraction reagent comprises glutarate buffer (or alternatively, citrate buffer) at a concentration of between 10 and 50 mM, a salt (e.g., sodium chloride) at a concentration between 100 and 200 mM, a non-ionic detergent (e.g., Triton X-100) at a concentration between 0.02 and 1% and an anti-foam agent (e.g., SE-15) at a concentration between 0.1 and 1% and has a pH between 4.2 and 5.2.

[0287] In one embodiment, the pH of the extracted sample is at least partially neutralized prior to or during analysis of the extracted sample by immunoassay. The method may therefore include treatment of the extracted sample with a reagent (e.g., a dry reagent pill within the cartridge fluidic network) that comprises a neutralization reagent that brings the pH to pH 6.0 or greater, pH 6.5 or greater or pH 7.0 or greater. The neutralization reagent may be a strong base such as sodium or potassium hydroxide or a buffering agent with buffering capacity in the appropriate pH range (e.g., HEPES, phosphate, Tris, etc.). In one embodiment, the concentration, after reconstitution in the extracted sample, is between 50-1000 mM or between 100 and 400 mM and the pH is between 6.0 to 8.5 or 6.5 to 8.0.

[0288] The sensitivity and specificity of an Influenza A test using the assay cartridge and methods of the present invention as calculated against a viral cell culture result is about 75%

and about 100%, respectively, and in one embodiment, about 80% and about 100%, respectively. In a specific embodiment, the sensitivity of an Influenza A test as calculated against a viral cell culture result is about 82% and the specificity of an Influenza A test is about 99%. The sensitivity and specificity of an Influenza B test using the assay cartridge and methods of the present invention as calculated against a viral cell culture result is about 75% and about 100%, respectively, and in one embodiment, about 80% and about 100%, respectively. In a specific embodiment, the sensitivity of an Influenza B test as calculated against a viral cell culture result is about 81% and the specificity of an Influenza B test is about 100%. The sensitivity and specificity of Influenza A as calculated against viral cell culture and RT-PCR was about 75% and about 100%, respectively, and in one embodiment, about 80% and about 100%, respectively. In a specific embodiment, the sensitivity of Influenza A as calculated against viral cell culture and RT-PCR is about 88% and the specificity of Influenza A as calculated against viral cell culture and RT-PCR is about 100%. The sensitivity and specificity of Influenza B using the assay cartridge and methods of the present invention as calculated against viral cell culture and RT-PCR result was about 75% and about 100%, respectively. In a specific embodiment, the sensitivity of Influenza B as calculated against viral cell culture and RT-PCR is about 79% and the specificity of Influenza B as calculated against viral cell culture and RT-PCR is about 100%. The sensitivity and specificity of Influenza A/subtype H1 as calculated against the viral cell culture and RT-PCR result is about 80% and about 100%, respectively.

[0289] Sample chamber **3720**, however, includes additional features. Firstly, the integrated filter is located near the end of the sample chamber and the connection to the extraction reagent conduit is located 1 to 2 cm (roughly 1.5 cm) from the end of the chamber. The inlet and outlets from the sample chamber are located near the opposite ends of a typical nasal/throat swab head, when the swab head is fully inserted, providing for efficient extraction with the minimal volume of extraction buffer. Secondly, the sample chamber consists of a first region and a second region and these regions are oriented at an angle with respect to each other and that angle is selected to bend the shaft upon insertion of the applicator stick into the sample chamber, thereby promoting fracture of the shaft, and the sample chamber includes two sample collection head retention features: barb **3721a** and shelf **3721b**, both of which may be provided by the injection molded cartridge body. In one embodiment, the first region is proximate to a sample introduction port and the second region is distal to the sample introduction port. Barb **3721a** is located near the end of the sample chamber adjacent to the location of a fully inserted swab head. In one embodiment, the second region of the sample chamber terminates in a sample chamber base and the barb is positioned at or near the sample chamber base. The barb is angled so as to allow for insertion of the swab head, but to also catch the swab head matrix and prevent removal or shifting of the swab head from the end of the chamber upon breaking and removal of the swab shaft. In one embodiment, the sample chamber includes a sample collection head extraction location at or near the terminus of the cavity of the sample chamber and the barb is positioned in the cavity so as to retain the collection head in the extraction location. The extraction location is a position within the sample chamber within which the collection head resides once the shaft is fractured. Shelf **3721b** is located at roughly the location of the broken shaft end of a fully inserted swab