

[0067] The present invention may be better understood with reference to the following examples.

EXAMPLE 1

[0068] The ability to form laminated assay devices, such as shown in **FIGS. 1-4**, was demonstrated.

[0069] The bottom strips: The bottom strips were formed from a Mylar® plastic substrate available from DuPont. The substrate had a thickness of 0.25 to 0.38 millimeters, a length of 5 centimeters, and a width of 1.3 centimeters. The substrate was initially printed with a silver ink line (5000) obtained from DuPont Biosensor Group of Research Triangle Park, N.C. The line had a width of 0.1 centimeters and a length of 1 centimeter in the area to be adjacent to the conductive lead, and a width of 0.05 centimeters and a length of 1 centimeter in the area to be adjacent to the carbon electrode. Next, a detection working electrode was printed over the silver ink line with carbon ink (7101) obtained from DuPont Biosensor Group of Research Triangle Park, N.C. The detection working electrode had a width of 0.1 centimeters and a length of 0.3 centimeters. The connection between the carbon working electrode and the silver line was accomplished by overlapping the electrode and line.

[0070] An insulation layer and flow channel were then printed simultaneously onto the substrate using a UV-curable dielectric composition available from DuPont under the name "5018G." Printing was performed with a screen printer available from Affiliated Manufacturers, Inc. ("AMI-Presco") of North Branch, N.J. under the name "HC-53." The screen frame utilized had a size of 5×7 inches, a mesh size of 80×0.0037 to 400×0.0007, and a stencil angle of 22 to 45 degrees. The resulting insulation layer had a length of 4 centimeters and a width of 0.5 centimeters, and essentially covered the substrate area not otherwise covered by the electrodes, leads, or flow channels. The resulting flow channel had a length of 4 centimeters and a width of 0.1 centimeters. The height of the flow channel ranged from about 10 to about 150 micrometers, and was measured using a micrometer available from Mitutoyo America Corporation of Aurora, Ill.

[0071] B: The top strips: The top strips were also formed from a Mylar® plastic substrate available from DuPont. The substrate had a thickness ranging from 0.25 to 0.38 millimeters, a length of 4.5 centimeters, and a width of 1.3 centimeters. The substrate was initially printed with a silver ink line (5000) obtained from DuPont Biosensor Group of Research Triangle Park, N.C. The line had a width of 0.1 centimeters and a length of 1 centimeter in the area to be adjacent to the conductive lead, and a width of 0.05 centimeters and a length of 1 centimeter in the area to be adjacent to the silver/silver chloride electrode. Next, a counter/reference electrode was printed over the silver ink line with silver/silver chloride ink (5847) obtained from DuPont Biosensor Group of Research Triangle Park, N.C. The counter/reference electrode had a width of 0.2 centimeters and a length of 0.3 centimeters. An insulation layer was also printed onto the substrate using a UV-curable dielectric composition available from DuPont under the name "5018G", such as described above. The resulting insulation layer had a length of 3.4 centimeters and a width of 1.3 centimeters. Once formed, an area having a width of 0.95 centimeters and a length of 1.3 centimeters was cut from the top strip, such as shown in **FIG. 2**.

[0072] C. Curing and Drying: Once formed, the top and bottom strips were separately cured and dried. Specifically, the dielectric material used to form the strips was cured by placing the substrate under a solar simulator, which is available from Solar Light Co. of Philadelphia, Pa. under the name "LS 1000-4R-UV", for about 5 to 10 seconds. Thereafter, each strip was left at room temperature for 2 hours, and then heated at 37° C. for 2 hours. The temperature was then raised to 60° C. and dried an additional 2 hours. Thereafter, the temperature was again raised to between 120 to 140° C. for 20 minutes. Such stepwise drying helped achieve high uniformity of the electrode surface, while also removing residue solvents of the original ink formulations.

[0073] D. Electrode Surface Treatment: 0.5 microliters of LH- α -HRP monoclonal antibody conjugate (Fitzgerald Industries Int'l of Concord, Mass.) was drop coated onto the surface of the detection working electrode with an Eppendorf microliter pipette. The LH- α -HRP monoclonal antibody conjugate had a concentration of about 5 nanograms per milliliter in a mixture of 80% PBS buffer and 20% isopropanol, and had a pH of 7.4. The resulting electrode strip was then placed at room temperature and allowed to air dry. Thereafter, the coated working electrode was treated with 1 microliter of a protein stabilizing formulation (20 wt. % Stabilcoat® from SurModics, Inc. of Eden Prairie, Minn. and 0.05 wt. % Tween 20 in a PBS buffer, pH of 7.4). The incubation time was 15 minutes. After incubation, the remaining solution was removed by a wicking material, and the electrode strip was dried under an air stream. In addition, the entire detection area, including the working and counter/reference electrodes, was treated with about 100 microliters of a solution containing P-casein (1 wt. %), Tween 20 (0.05 wt. %), and PBS buffer (pH of 7.4), and dried.

[0074] E. Membrane Strips: Membrane strips of a nylon mesh (11 mesh size, commercially available from Millipore Corp. of Billerica, Mass.) were provided that had a length of 15 centimeters and a width ranging from 3.5 to 4.5 centimeters. Two glass fiber pads (sample and conjugate pads) were laminated to the bottom of the strip using tape. The conjugate pad was in direct contact with the membrane, and the sample pad was in direct contact with the conjugate pad. The conjugate pad was treated with 3 microliters of LH- α -HRP monoclonal antibody conjugate (5 micrograms per milliliter in PBS buffer) and dried for 30 minutes. The LH- α -HRP monoclonal antibody conjugate was obtained from Fitzgerald Industries Int'l of Concord, Mass. The membrane strips were placed onto a sampling instrument commercially available from Kinematic Automation of Twain Harte, Calif. under the name "Matrix 2210 (Universal Laminator)." Thereafter, the strips were cut into individual strips having a width ranging from 1 to 10 millimeters using a strip cutter commercially available from Kinematic Automation under the name "Matrix 2360."

[0075] F. Lamination of Electrode Strips: To laminate the top and bottom electrode strips in a face-to-face relationship, double sided tape was first applied to the bottom strip. Specifically, the double sided tape was placed along both sides of the flow channel present on the bottom electrode strip. Thereafter, the membrane strip was placed over the flow channel and the surface of the working electrode. The top strip was then laminated in a face-to-face relationship with the bottom strip, as shown in **FIG. 3**. After lamination, a cellulosic wicking pad (Millipore Co.) having a width of