[0013] **FIG. 4** is a schematic illustration of the assay device formed according to **FIG. 3**;

[0014] **FIG. 5** is a schematic illustration of another embodiment of an assay device formed according to the present invention; and

[0015] **FIG. 6** illustrates the "periodate" method of forming a horseradish peroxidase (HRP) conjugate for use in one embodiment of the present invention.

[0016] Repeat use of reference characters in the present specification and drawings is intended to represent same or analogous features or elements of the invention.

DETAILED DESCRIPTION OF REPRESENTATIVE EMBODIMENTS

Definitions

[0017] As used herein, the term "analyte" generally refers to a substance to be detected. For instance, analytes may include antigenic substances, haptens, antibodies, and combinations thereof. Analytes include, but are not limited to, toxins, organic compounds, proteins, peptides, microorganisms, amino acids, nucleic acids, hormones, steroids, vitamins, drugs (including those administered for therapeutic purposes as well as those administered for illicit purposes), drug intermediaries or byproducts, bacteria, virus particles and metabolites of or antibodies to any of the above substances. Specific examples of some analytes include ferritin; creatinine kinase MB (CK-MB); digoxin; phenytoin; phenobarbitol; carbamazepine; vancomycin; gentamycin; theophylline; valproic acid; quinidine; luteinizing hormone (LH); follicle stimulating hormone (FSH); estradiol, progesterone; C-reactive protein; lipocalins; IgE antibodies; cytokines; vitamin B2 micro-globulin; glycated hemoglobin (Gly. Hb); cortisol; digitoxin; N-acetylprocainamide (NAPA); procainamide; antibodies to rubella, such as rubella-IgG and rubella IgM; antibodies to toxoplasmosis, such as toxoplasmosis IgG (Toxo-IgG) and toxoplasmosis IgM (Toxo-IgM); testosterone; salicylates; acetaminophen; hepatitis B virus surface antigen (HBsAg); antibodies to hepatitis B core antigen, such as anti-hepatitis B core antigen IgG and IgM (Anti-HBC); human immune deficiency virus 1 and 2 (HIV 1 and 2); human T-cell leukemia virus 1 and 2 (HTLV); hepatitis B e antigen (HBeAg); antibodies to hepatitis B e antigen (Anti-HBe); influenza virus; thyroid stimulating hormone (TSH); thyroxine (T4); total triiodothyronine (Total T3); free triiodothyronine (Free T3); carcinoembryoic antigen (CEA); lipoproteins, cholesterol, and triglycerides; and alpha fetoprotein (AFP). Drugs of abuse and controlled substances include, but are not intended to be limited to, amphetamine; methamphetamine; barbiturates, such as amobarbital, secobarbital, pentobarbital, phenobarbital, and barbital; benzodiazepines, such as librium and valium; cannabinoids, such as hashish and marijuana; cocaine; fentanyl; LSD; methaqualone; opiates, such as heroin, morphine, codeine, hydromorphone, hydrocodone, methadone, oxycodone, oxymorphone and opium; phencyclidine; and propoxyhene. Other potential analytes may be described in U.S. Pat. No. 6,436,651 to Everhart, et al. and U.S. Pat. No. 4,366,241 to Tom et al.

[0018] As used herein, the term "test sample" generally refers to a material suspected of containing the analyte. The test sample may, for instance, include materials obtained directly from a source, as well as materials pretreated using techniques, such as, but not limited to, filtration, precipitation, dilution, distillation, mixing, concentration, inactivation of interfering components, the addition of reagents, and so forth. The test sample may be derived from a biological source, such as a physiological fluid, including, blood, interstitial fluid, saliva, ocular lens fluid, cerebral spinal fluid, sweat, urine, milk, ascites fluid, mucous, synovial fluid, peritoneal fluid, vaginal fluid, amniotic fluid or the like. Besides physiological fluids, other liquid samples may be used, such as water, food products, and so forth. In addition, a solid material suspected of containing the analyte may also be used as the test sample.

Detailed Description

[0019] Reference now will be made in detail to various embodiments of the invention, one or more examples of which are set forth below. Each example is provided by way of explanation of the invention, not limitation of the invention. In fact, it will be apparent to those skilled in the art that various modifications and variations may be made in the present invention without departing from the scope or spirit of the invention. For instance, features illustrated or described as part of one embodiment, may be used on another embodiment to yield a still further embodiment. Thus, it is intended that the present invention covers such modifications and variations as come within the scope of the appended claims and their equivalents.

[0020] In general, the present invention is directed to an electrochemical-based assay device capable of detecting the presence or quantity of an analyte of interest that is accurate, reliable, and easy-to-use. The device contains two or more substrates, one containing a detection working electrode and another containing an auxiliary electrode, such as a counter/reference electrode(s). The substrates are positioned in a face-to-face relationship so that the electrodes are placed in electrical communication with each other during performance of the assay.

[0021] Referring to **FIG. 1**, for instance, one embodiment of a first substrate **80** that may be used in an assay device of the present invention is illustrated. Although not required, the first substrate **80** may be formed from an insulative material, such as silicon, fused silicon dioxide, silicate glass, alumina, aluminosilicate ceramic, an epoxy, an epoxy composite such as glass fiber reinforced epoxy, polyester, polyimide, polyamide, polycarbonate, etc. A detection working electrode **42** and an optional calibration working electrode **44** are disposed on a surface **29** of the substrate **80**. It should be understood that other detection and/or calibration working electrodes may also be utilized. The detection working electrode **42** is typically formed from a thin film of conductive material disposed on the substrate **80**. Generally speaking, a variety of conductive materials may be used to form the detection working electrode **42**. Suitable materials include, for example, carbon, metals (platinum, palladium, gold, tungsten, titanium, etc.), metal-based compounds (e.g., oxides, chlorides, etc.), metal alloys, conductive polymers, combinations thereof, and so forth. Particular examples of carbon electrodes include glassy carbon, graphite, mesoporous carbon, nanocarbon tubes, fullerenes, etc. Thin films of these materials may be formed by a variety of methods including, for example, sputtering, reactive sputtering, physical vapor deposition, plasma deposition, chemical