

applied at various locations of the device **20**, such as to the conjugate pad **22**, where it may bind to the analyte of interest before reaching the sample channel **14**. Although only one conjugate pad **22** is shown, it should be understood that additional conjugate pads may also be used in the present invention. Besides the conjugate pad **22**, the analyte may be bound to a redox label within the sample channel **14** or any other location of the assay device **20**, or even prior to being applied to the device **20**.

[**0051**] The term “redox label” refers to a compound that has one or more chemical functionalities (i.e., redox centers) that may be oxidized and reduced. Such redox labels are well known in the art and may include, for instance, an enzyme such as alkaline phosphatase (AP), horseradish peroxidase (HRP), glucose oxidase, beta-galactosidase, urease, and so forth. Other organic and inorganic redox compounds are described in U.S. Pat. No. 5,508,171 to Walling, et al.; U.S. Pat. No. 5,534,132 to Vreeke, et al.; U.S. Pat. No. 6,241,863 to Monbouclette; and U.S. Pat. No. 6,281,006 to Heller, et al., which are incorporated herein in their entirety by reference thereto for all purposes. Horseradish peroxidase (HRP), for instance, is an enzyme that is commonly employed in electrochemical affinity assay devices. Two methods are commonly used for the preparation of antibody-coupled horseradish peroxidase (HRP) conjugates, i.e., “glutaraldehyde” and “periodate” oxidation. As is known in the art, the “glutaraldehyde” method involves two steps and results in high molecular weight aggregates. Further, the “periodate” method involves three steps. For instance, as shown in **FIG. 6**, the “periodate” method may reduce interference of HRP active-site amino groups because it is only conjugated through carbohydrate moieties. Specifically, the “periodate” method opens up the carbohydrate moiety of the HRP glycoprotein molecule to form aldehyde groups that will form Schiff bases with antibody amino groups. Thus, although not required, it may be desired to use HRP formed by the “periodate” method to minimize background current.

[**0052**] Besides being directly attached to the analyte, the redox label may also be indirectly attached to the analyte through a specific binding member for the analyte. Specific binding members generally refer to a member of a specific binding pair, i.e., two different molecules where one of the molecules chemically and/or physically binds to the second molecule. For instance, immunoreactive specific binding members may include antigens, haptens, aptamers, antibodies, and complexes thereof, including those formed by recombinant DNA methods or peptide synthesis. An antibody may be a monoclonal or polyclonal antibody, a recombinant protein or a mixture(s) or fragment(s) thereof, as well as a mixture of an antibody and other specific binding members. The details of the preparation of such antibodies and their suitability for use as specific binding members are well known to those skilled in the art. Other common specific binding pairs include but are not limited to, biotin and avidin, biotin and streptavidin, antibody-binding proteins (such as protein A or G) and antibodies, carbohydrates and lectins, complementary nucleotide sequences (including label and capture nucleic acid sequences used in DNA hybridization assays to detect a target nucleic acid sequence), complementary peptide sequences including those formed by recombinant methods, effector and receptor molecules, hormone and hormone binding protein, enzyme cofactors and enzymes, enzyme inhibitors and enzymes, and

so forth. Furthermore, specific binding pairs may include members that are analogs of the original specific binding member. For example, a derivative or fragment of the analyte, i.e., an analyte-analog, may be used so long as it has at least one epitope in common with the analyte.

[**0053**] The redox labels may be used in a variety of ways to form a probe. For example, the redox labels may be used alone to form probes. Alternatively, the redox labels may be used in conjunction with polymers, liposomes, dendrimers, and other micro- or nano-scale structures to form probes. For example, the redox labels may be used in conjunction with particles (sometimes referred to as “beads”) to form the probes. Naturally occurring particles, such as nuclei, mycoplasma, plasmids, plastids, mammalian cells (e.g., erythrocyte ghosts), unicellular microorganisms (e.g., bacteria), polysaccharides (e.g., agarose), and so forth, may be used. Further, synthetic particles may also be utilized. For example, in one embodiment, latex particles are utilized. Although any latex particle may be used in the present invention, the latex particles are typically formed from polystyrene, butadiene styrenes, styreneacrylic-vinyl terpolymer, polymethylmethacrylate, polyethylmethacrylate, styrene-maleic anhydride copolymer, polyvinyl acetate, polyvinylpyridine, polydivinylbenzene, polybutylene-terephthalate, acrylonitrile, vinylchloride-acrylates, and so forth, or an aldehyde, carboxyl, amino, hydroxyl, or hydrazide derivative thereof. Other suitable particles may be described in U.S. Pat. No. 5,670,381 to Jou, et al. and U.S. Pat. No. 5,252,459 to Tarcha, et al., which are incorporated herein in their entirety by reference thereto for all purposes. In addition, inorganic particles, such as colloidal metallic particles (e.g., gold) and non-metallic particles, carbon particles, and so forth, may also be utilized. The mean diameter of the particles may generally vary as desired. For example, in some embodiments, the mean diameter of the particles may range from about 0.01 microns to about 1,000 microns, in some embodiments from about 0.01 microns to about 100 microns, and in some embodiments, from about 0.01 microns to about 10 microns. In one particular embodiment, the particles have a mean diameter of from about 0.01 to about 2 microns. Generally, the particles are substantially spherical in shape, although other shapes including, but not limited to, plates, rods, bars, irregular shapes, etc., are suitable for use in the present invention. As will be appreciated by those skilled in the art, the composition, shape, size, and/or density of the particles may widely vary.

[**0054**] Referring to **FIGS. 3-5**, various embodiments for forming an assay device **20** from the substrates **40** and **80** will now be described in more detail. Although not required, it is generally desired that the substrates **40** and **80** be laminated in such a manner that the detection working electrode **42** is adjacent to and in electrical communication with, but does not contact, the reference and counter electrodes **46** and **48**. Thus, as shown in **FIG. 3**, the surface **27** of the substrate **40** is placed over the surface **29** of the substrate **80** to form the laminate assay device **20**. In the embodiment shown in **FIGS. 3-4**, the substrates **40** and **80** are aligned substantially parallel to each other. In the embodiment shown in **FIG. 5**, the substrates **40** and **80** are aligned substantially perpendicular to each other. It should be understood, however, that the present invention is not limited to any particular alignment or configuration of the laminated substrates.