

a process flow may be envisioned in which whole blood from a fingerstick is applied directly to a labeled antigen assay cartridge, wherein a single addition of assay reagents completes the assay procedure. To demonstrate the feasibility of such a process flow using the labeled antigen assay on the assay system described above, whole blood samples from HIV positive donors were drawn into EDTA blood tubes to inhibit coagulation. A portion of each sample was withdrawn and centrifuged to obtain plasma by removal of red blood cells. Then, the above labeled antigen assay procedures were performed using concentrations of 90% whole blood, 50% whole blood, and 50% plasma. As may be seen from FIGS. 43-44, the signal-to-noise ratio results clearly demonstrate efficacy of the method using either whole blood or plasma. Further, the results were comparable when using 50% or 90% whole blood. Cellular and other components in the whole blood do not appear to interfere with the assay.

**[0198]** In another embodiment, fluidic sample chamber in cartridge 2602 may be specifically designed to improve assay performance by controlling fluid flow rates over the assay surface. Static incubations in small fluidic channels generally have limits of detection set by mass transport limitations (e.g., diffusion) in the system. By engineering fluidic sample chamber geometry (i.e., length, width, height, shape) and surface energies, sample flow rate over the assay surface can be optimized for improved assay performance.

#### Example 14

##### HIV Antigen-Antibody Combination Assay

**[0199]** In another embodiment, the disclosed system and method are used to detect both antiviral antibodies and viral antigen(s) in the same sample in a single combination assay. In the context of HIV infection, detection of antibodies against the virus is a well established diagnostic tool. Serological testing, however, only detects infection after the individual has developed an immune response, leaving a “window phase” where viral replication is occurring but no detectable antibodies are present in the host. By adding direct detection of HIV viral antigen such as p24 capsid protein, infection may be detected earlier during the window phase. This so-called antigen-antibody combination assay (sometimes referred to as the 4<sup>th</sup> generation of HIV blood diagnostics assays) may provide more timely and sensitive test results as compared to antibody-only tests.

**[0200]** In this example, p24 antigen is used to illustrate the HIV antigen-antibody combination detection assay in a sandwich immunoassay using two purified monoclonal antibodies (mAbs) to detect the p24 antigen. It is to be understood that polyclonal antibodies can also be used as either capture or detect antibodies. For the purpose of this example, a mAb-mAb pair was used. It is to be understood that other HIV antigens may be used as target proteins. Mouse monoclonal antibodies against HIV-1 p24 antigen were obtained from commercial vendors, such as ImmunoDiagnostics, Inc. (Catalog#1103), Meridian Life science, Inc. (cat#C65690M), Santa Cruz Biotechnology, Inc. (Catalog#sc-57827), United States Biological (Catalog#H6003-33D, H6003-30A, H6003-27J, H6004-72, H6004-73, and H6004-74), Thermo Fisher Scientific, Inc. (Catalog#MA1-83231), PerkinElmer, Inc. (Catalog#NEA-9306001), Maine Biotechnology Services (Catalog#MAB739P), and NIH AIDS Research and Reference Reagents Program (Catalog#3537, 4121, 6457, and 6458). Alternatively, polyclonal anti-p 24 antibodies may

be commercially sourced from vendors such as United States Biological (Catalog#H6005 and H6003-27A), Thermo Fisher Scientific, Inc. (Catalog#PA1-85555), and Maine Biotechnology Services (Catalog#PAB7103P).

**[0201]** An appropriate mAb pair was selected based on pairwise screen as commonly performed in sandwich immunoassay development. One mAb was printed to the activated waveguide surface using a robotic arrayer (Bio-Dot, Inc.) and is referred to as the “capture antibody.” The second antibody in the pair, also called the “detect antibody,” was conjugated to biotin in a standard NHS-ester crosslinking reaction consisting of the antibody and NHS-PEG12-Biotin (Pierce Biotechnology; Rockford, Ill.), then purified by size-exclusion chromatography.

**[0202]** It is to be noted that antibodies against different epitopes of the antigens may be used. In one aspect, antibodies against the same antigen may be combined and tested in all possible permutations in order to identify the best pair with the highest specificity and sensitivity. For example, three different antibodies (Abs) A, B and C may be tested as antibody pairs A-B, B-A, A-C, C-A, B-C, and C-B, with the first antibody being the capture antibody and the second antibody being the detect antibody. Samples with known antigen composition or samples tested using established methods may be used to select the pair having the highest specificity and sensitivity.

**[0203]** In another aspect, either the detect antibody or the capture antibody may contain more than one antibody. In another aspect, the detect antibody is different from the capture antibody. In some cases, the capture antibody and the detect antibody may be interchangeable, or in other words, the capture antibody may be used as the detect antibody while the detect antibody is used as the capture antibody. In other cases, the capture antibody and the detect antibody are unique and are not interchangeable. In another aspect, the detect antibodies shall not significantly bind to the capture antibodies. Typically, the detect antibodies and the capture antibody bind to different epitopes on the antigen. The detect antibody and the capture antibody may be either monoclonal or polyclonal antibodies.

**[0204]** During an infection, the host may produce antibodies against the foreign antigens. Antigen bound to these host antibodies may not be detectable by antibody sandwich assays. These antibody-antigen complexes may be disrupted with heat, low pH (followed by pH neutralization), salt, or combination thereof. These disruption methods may help denature the antibodies which are incapable of re-binding the released antigen. Such disruption process may be referred to as decomplexation.

**[0205]** In this example, the final detect reagent is streptavidin conjugated with a fluorescent dye (SureLight P3, Columbia Biosciences; Columbia, Md.). If p24 antigen is present in the sample, an antibody-antigen-antibody sandwich is created on the waveguide surface. The streptavidin-dye binds to the biotinylated detect antibody and fluorescent signal is detected

**[0206]** The HIV antibody detection assay is similar to that described in Example 8. Recombinant proteins representing HIV-1 envelope glycoprotein 41 (gp41) and capsid antigen p24 were printed to the waveguide surface using the Bio-Dot arrayer. The detect reagent was goat anti-human IgG conjugated to the fluorescent dye DyLight649 (KPL, Inc., Gaith-