

fluorescence signal at the immobilized compound location is indicative of the presence of the target analyte within the biological sample. As an example, collection and filtering optics 2645 may be used to capture the fluorescence signal from assay surface 2620. A signal corresponding to the fluorescence so captured may then be directed to an imaging device 2650, such as a CCD or CMOS sensor.

[0139] In a further embodiment, assay system 2600 may be used for rapid, simple detection of multiple target antibodies in a single biological sample. Multiple different antigens may be immobilized at reaction sites on the assay surface, such as in stripes or spots in an array format using printing technology, thereby creating a spatially-localized set of parallel assay locations. The combination of a biological sample, labeled antibody against human IgG, and immobilized antigens on assay surface 2620 may lead to the formation of multiple physically separated antigen-antibody complexes on the assay surface. Illumination of assay surface 2620 results in spatially-localized fluorescence signal that may be read with a detection system 2660 including collection and filtering optics 2645, imaging device 2650, and computer 2670. Computer 2670 may be integrated into the detection system instrument (e.g., single board computer). Alternatively, computer 2670 could be an external device, such as peripheral device 1930 of FIG. 19.

Example 8

Multiplexed HIV/Syphilis/HCV Panel Assay

[0140] Unless otherwise specified in this disclosure, components, reagents, protocol, and other methods used in the system and the assays are as described in the Materials and Methods Section of the Example, and are for the purpose of illustration only.

[0141] This example demonstrates an HIV-1 Ab assay with 100% agreement with known seroreactivity on a collection of 82 HIV Ab-positive and 142 HIV Ab-negative samples, including multiple samples with HCV and syphilis co-infection. It also demonstrates a treponemal-specific syphilis antibody assay that correctly identifies 67 of 68 *T. pallidum* Ab positive and 100 of 102 *T. pallidum* Ab negative samples. The HCV assay correctly identifies 59 of 60 HCV Ab-positive and 120 of 121 HCV Ab-negative samples. Multiplexed assay performance on whole blood samples is also demonstrated.

Materials and Methods

[0142] Biological Reagents. Modern serological assays for infectious diseases are typically based on recombinant proteins, multiple-epitope fusion proteins, and antigenic peptides. Selection, screening, and optimization of immobilized antigens are an important aspect of assay development. For the purposes of this disclosure, commercially available and commonly used proteins were used. However, it is to be recognized that other commercial or customized antigens or antibodies may also be used.

[0143] The HIV-1 assay demonstration utilized two commercially-sourced, purified recombinant proteins: envelope gp41, and capsid antigen p24. For the syphilis treponemal assay, commercially-sourced, recombinant proteins Tp47 and Tp17 were used. Tp47 and Tp17 are commonly used in treponemal-specific syphilis immunoassays. Note that treponemal-specific assays do not readily distinguish between active, latent, and treated syphilis infection. The platform

described here, however, is amenable to the addition of a non-treponemal antibody detection component, as has been described in the literature.

[0144] Due to the high level of genomic and antigenic variability associated with HCV, anti-HCV antibody screening typically depends on multiple antigenic targets. For instance, a number of FDA-approved enzyme immunoassays rely on combinations of recombinant proteins and peptides. Consistent with the need for HCV antigen multiplexing, four commercially-available HCV recombinant proteins were used in this demonstration, including recombinant core protein (nucleocapsid, p22 fusion protein); full length NS3 (c33c); a mosaic recombinant including NS4 immunodominant regions; and a recombinant which contains HCV nucleocapsid, NS3, NS4, and NS5 immunodominant regions, which is also referred to as the multiple epitope antigen in this disclosure.

[0145] Assay Reagents. Other biological reagents include purified human IgG (Sigma, St. Louis, Mo.), goat anti-human IgG (Thermo Scientific, Rockford, Ill.), and goat anti-human IgG conjugated with fluorescent dye (DyLight649, KPL, Inc.). Assay reagents include bovine serum albumin ("BSA", Sigma Life Science, St. Louis, Mo.), phosphate buffered saline ("PBS", Fisher Scientific, Rockford, Ill.), Blocker Casein in PBS (Thermo Scientific, Rockford, Ill.), and Tween20 (Thermo Scientific, Rockford, Ill.).

[0146] Clinical Samples. Five sets of clinical samples were used to characterize the system. A total of 251 different clinical samples were processed in this example.

[0147] Commercial Controls. Well-characterized human plasma and serum samples with known antibody reactivity for each of the three pathogens were commercially-sourced. These samples included four with known HIV-1 antibody reactivity, four with known *T. pallidum* antibody reactivity, and four with known HCV antibody reactivity.

[0148] HIV-1 Antibody Reactive Samples. A total of 25 human serum samples with known HIV-1 Western Blot reactivity were sourced under an Institutional Review Board ("IRB")-approved protocol by the University of California, San Diego Medical Center (UCSD). Co-infection status was not known for the majority of these samples at the time of the assay described herein.

[0149] Syphilis Samples. A collection of 30 de-identified sera known positive for syphilis infection were sourced from the Colorado Department of Public Health and Environment (CDPHE, Denver, Colo.). Syphilis reactivity was determined at the CDPHE Laboratory using rapid plasma regain ("RPR") and *T. pallidum* particle agglutination ("TPPA"). The HIV serostatus of each of these samples was not known upon receipt from the CDPHE. All 30 sera were also characterized with an FDA-approved HIV-1/2 RDT (Trinity Uni-Gold™ Recombigen® HIV).

[0150] Co-Infection Samples. A collection of de-identified clinical samples from existing sample archives were coordinated with IRB approval by UCSD. Samples were selected for likely HIV, HCV, hepatitis B virus, and/or syphilis infection. UCSD clinical samples were all characterized for HIV and HCV infection on a Siemens Centaur™ clinical analyzer. Syphilis samples were tested by RPR with confirmation by TPPA. The UCSD Co-Infection Collection includes a large number of highly complex pathogen antibody and antigen reactivities, as commonly encountered in HIV and HCV infected individuals. In addition to HIV, HCV, HBV, and/or