

ersburg, Md.). Control spots in the array included human IgG (detect reagent control) and print buffer spots (non-specific binding control).

**[0207]** Printed waveguide arrays were rinsed and then blocked with a protein-based blocker and then coated with a sugar-based stabilizer. Processed waveguides were then assembled into disposable cartridges described previously.

**[0208]** Human serum control samples certified as negative for HIV, hepatitis C virus and RPR (syphilis) were sourced commercially (Valley Biomedical, Winchester, Va.). Serum samples from HIV-positive individuals were from a sample archive at the Antiviral Research Center, San Diego, Calif., provided under an Institutional Review Board approved protocol. Three categories of HIV positive samples were provided: (1) RNA positive, antibody negative samples (acute, or window-phase samples. These samples have been tested negative for HIV-1/2 antibody by Enzyme Immunoassay (EIA); EIA is considered the most sensitive screening test in this protocol and Western Blots were not run once the negative EIA results was generated); (2) Western Blot indeterminate samples; and (3) weak positive Western Blot samples. The HIV-positive collection therefore represents individuals in the early stages of HIV infection.

**[0209]** Samples were assayed on the cartridges at ambient temperature (about 20 to 25° C.). The p24 antigen detection cartridge array contained spatially-arrayed anti-p24 antibody, printed print buffer blanks, and fluorescently-labeled BSA positioning marker features. At the completion of the assay procedure, the cartridges were inserted into the reader instrument for fluorescence imaging. This workflow allows batch processing of the disposable sample cartridges.

**[0210]** Detection of HIV antigen was performed using the following protocol. A 19-microliter aliquot of serum was combined with an 8-microliter volume biotin-labeled detection antibody in sample dilution buffer (PBS, 0.1% Tween-20, non-specific binding blocking reagents) and mixed by aspiration. Immunoassay blocking buffer components may include bovine serum albumin (BSA), polymerized BSA, fetal calf serum or normal serum from other animal species, non-fat dry milk or casein, alkaline-hydrolyzed casein, acid-hydrolyzed casein, fish gelatin, polyethylene glycol (PEG), polyvinyl alcohol (PVA), polyvinylpyrrolidone (PVP), thiol-reactive compounds such as glutathione and L-cysteine, and immunoglobulins, including IgG from specific animal sources (e.g., mouse IgG), polymerized IgG, and species-specific fragments of IgG [Fc, Fab, F(ab')<sub>2</sub>], to block nonspecific binding. Immunoassay blocking buffer may also contain non-ionic detergents such as Tween-20 and Triton X-100. 25 microliters of this diluted sample mixture was loaded into the cartridge input port by transfer pipette. The applied sample passively flowed through the cartridge, covering the printed array. Static incubation was for 20 minutes without user intervention. Following the sample incubation period, a 100-microliter volume of 8 nanomolar streptavidin-SureLight P3 in conjugate dilution buffer (PBS, 0.2% Tween-20, non-specific binding blocking reagents) was added to the cartridge input port and allowed to passively flow through the cartridge. Following a 20-minute incubation with streptavidin-SureLight P3, two 150-microliter volumes of wash buffer (PBS, 0.2% Tween-20, non-specific binding blocking reagents including 10 mg/ml bovine serum albumin (Roche; as above) and 0.5% v/v fetal bovine serum (Atlas Biologicals, Fort Collins, Colo.)) were serially added to the input port, and each wash was allowed to flow through the cartridge for 5 to 6

minutes. The cartridge was then inserted into the reader instrument for fluorescence data collection. Read time and data processing in the instrument is approximately 30 seconds per cartridge. After results acquisition, the cartridge was removed from the reader instrument and disposed as biohazard waste.

**[0211]** Detection of anti-HIV antibody was performed using the following protocol. A 6 microliter aliquot of serum or plasma was diluted in 194 microliters of sample dilution buffer (PBS, 0.5% casein, 0.05% Tween-20). 175 microliters of this diluted sample mixture was then loaded into the cartridge input port by transfer pipette and were allowed to flow passively through the cartridge during a 15-minute incubation period without user intervention. 175 microliters of wash buffer (PBS, 0.1% Tween-20) was then added to the input port and allowed to flow through the cartridge for 3 minutes, followed by the addition of 175 microliters of dye-conjugated anti-human IgG in a second diluent (PBS, 1 mg/ml BSA, 0.05% Tween-20) and allowed to incubate for 10 minutes. The cartridge was then inserted into the reader instrument for fluorescence data collection. Read time and data processing in the instrument was approximately 30 seconds per cartridge. After acquisition of the results, the cartridge was removed from the reader instrument and disposed as biohazard waste.

**[0212]** FIG. 50 shows the results of the HIV-1 p24 antigen and antibody combination assays. Antigen and antibody reactivities were recorded as normalized fluorescence signal intensity after background signal had been subtracted. Background signal was the average of the signal on printed buffer blank spots (negative reference spots) located adjacent to the printed antibody or antigen spot of interest. For the HIV-1 p24 antigen assay, the intensity of the printed fluorescently-labeled BSA positioning spot signals was utilized for normalization; for the HIV-1 antibody assay arrays, the intensity of the printed human IgG spot signal was used for normalization. For both assay formats, spot intensity cutoff values ("co") are based on the mean on-spot signal generated from a panel of HIV-negative samples. For this example, cutoff for a given printed spot was defined as the mean intensity plus three standard deviations for the collection of negatives. Antigen detection data are presented in FIG. 50 as sample signal to cutoff ratios ("s/co"). Antibody detection data are reported as "positive" for  $s/co > 1.15$ , "indeterminate" for  $1.15 \geq s/co \geq 0.85$ , and "negative" for  $s/co < 0.85$ .

**[0213]** As shown in FIG. 50, two of five window-phase sera (RNA-positive, antibody-negative) had detectable p24 antigen in this assay (See results for Sample IDs 3 and 4). These HIV antigen positive samples would not be detected with an antibody-only test. None of the Western Blot indeterminate or weak positive samples gave p24 antigen signal above the cutoff threshold. Of particular note, however, are samples 9 and 13. These are negative in the MBio antibody assay, but show S/CO at 0.6 on the MBio p24 antigen assay. We believe this antigen signal is real, and that these early seroconversion samples have detectable p24 antigen. Further optimization of the cutoffs for the antigen assay will possibly result in these samples achieving positive status on the p24 antigen assay. The lack of p24 antigen signal in most of the antibody-positive samples is expected. As a person generates antibodies against the virus, circulating p24 antigen becomes bound in antigen-antibody complex. In the absence of decomplexation through heat or chemical treatment, which results in denaturation of antibodies and release of their bound antigens, com-