

the first surface to form one of the negative control sites. Note that the composition for negative control sites also shall not contain molecules that interact with the detection reagent. In an example, at least one of the negative control sites is located at a proximal end of the array that is closest to the inlet port.

**[0062]** In another embodiment of the disclosure, the array on the first surface of the waveguide contains one or more positive control sites, wherein at least one of such positive control site is formed by printing onto the first surface a composition that contains a molecule that detectably binds to one or more analytes in the sample. In one aspect, the composition for the positive control site contains a molecule that consistently binds to one or more analytes in the sample. In another aspect, the composition for the positive control site contains a molecule that binds to the detection reagent. In another aspect, at least one of the positive control sites contains an antibody against human immunoglobulin. In yet another aspect, at least one of the positive control sites contains a human immunoglobulin. In still another aspect, at least one of the positive control sites contains a protein labeled with an excitable tag. In an embodiment, at least one of the positive control sites is located at a distal end of the array farthest from the inlet port.

**[0063]** In another embodiment, the array may have two or more reaction sites and some of the reaction sites may contain identical molecules selected from the plurality of capture molecules for purpose of duplicate reading. In one aspect, each of the reaction sites may contain a different capture molecule selected from the plurality of capture molecules.

**[0064]** In another embodiment, the plurality of capture molecules is a plurality of antigens, wherein the antigens are peptides, polypeptides, or proteins. In one aspect, each of the different molecules on different reaction sites may bind different markers characteristic of different diseases. Thus, the presence or absence of signals from each reaction site may be indicative of whether or not the sample is positive for the particular disease. For example, one reaction site may carry an HIV antigen that binds to anti-HIV antibodies, while another reaction site may carry a HCV antigen that binds to anti-HCV antibodies. Signals from these two reaction sites may be indicative of whether or not the sample contains antibodies against HIV and HCV, respectively.

**[0065]** Alternatively, because one disease, pathogen, or other indication may have more than one marker, different reaction sites may carry capture molecules that bind to these different markers characteristic of the same disease, pathogen, or indication. For instance, glycoprotein 41 ("gp41"), p24, gp31, gp160 and gp36 (for HIV-2) are antigens commonly used in HIV-1/2 antibody assay. It may also be beneficial to have an array of reaction sites with one, some, or all of the HIV-1 antigens that are commonly used in the HIV-1 Western Blot: p17, P24, p31, gp41, p51, p55, p66, gp120, and gp160. Subtype-specific antigens, such as gp41 Type O may also be applicable. Array reaction sites may carry antigens individually, e.g., one site carrying gp41, another site carrying gp120, etc. Alternatively, a reaction site may contain a combination of antigens, such as one site carrying both gp41 and gp160. Signals from reaction sites may be detected and processed to indicate whether or not the sample contains anti-HIV antibodies. Reaction site signals may be further processed to define overall reactivity status for HIV infection.

**[0066]** In another embodiment, reaction site analysis algorithms may be defined within the assay system to define sample status. For example, an analysis algorithm may be

used to render a determination of "reactive" or "positive" for a given disease, pathogen, or indication, if any one of a number of reaction sites yields a signal. Alternatively, the analysis algorithm may use some combination of signals on multiple reaction sites to render a determination of "reactive" or "positive" for a given disease pathogen or indication.

**[0067]** In one aspect, signal from each reaction site may be treated as a binary value, such as positive or negative relative to a pre-defined cutoff value of measured signal. In another aspect, signal from each reaction site may be measured as a quantitative signal value.

**[0068]** In another embodiment, the analysis algorithm for determining sample status for a particular disease, pathogen, or indication may be predefined in the firmware or software associated with the reader instrument. In another embodiment, the analysis algorithm may be configurable according to information carried on a given assay device (e.g., cartridge). For example, a cartridge may carry information (e.g., in a barcode affixed to the cartridge) that defines the specific analysis algorithm to be used for that given cartridge. In another embodiment, a given cartridge may carry a code for selecting a particular analysis algorithm that has been pre-loaded on the reader instrument software.

**[0069]** In another embodiment, the analysis algorithm may be based on a reactivity signature or pattern that has been defined by running multiple known samples on the reaction site array. For example, a statistically significant collection of known samples may be considered a "training set" for defining an analysis algorithm.

**[0070]** In another embodiment, the disclosed system may be used to detect infections by at least one microorganism (e.g., virus, bacteria, fungus, parasite, etc.), wherein the microorganism is the causative agent of at least one disease selected from the group consisting of AIDS, syphilis, hepatitis, tuberculosis and combination thereof. In one aspect, two more antigens from the same microorganism may be immobilized to the first surface of the waveguide to form two or more reaction sites. The immobilized antigen may bind to antibodies produced by the host animal or human against the same antigen. Therefore, signals from the two or more reaction sites may indicate the presence or absence of infection by the one microorganism. In another aspect, two more antigens from different microorganisms may be immobilized to the first surface of the waveguide to form two or more reaction sites. Signals from the two or more reaction sites may indicate the presence or absence of infection by the different microorganisms.

**[0071]** In another embodiment, the first surface may contain two or more reaction sites, and at least one of the reaction sites may contain an immobilized antigen, while at least another one of the reaction sites may contain an immobilized antibody. The presence or absence of detectable interactions between the antigen and analytes in the sample may indicate whether or not the sample contain detectable amount of an antibody against this antigen. In the meantime, presence or absence of detectable interactions between the antibody and analytes in the sample may indicate whether or not the sample contain detectable amount of an antigen that may bind the immobilized antibody. The combination of antigen and antibody in the same array may provide an assay with improved levels of accuracy and confidence. For example, it may be beneficial in HIV-1/2 screening assays to measure both antibody reactivity and the presence or absence of viral antigen, such as p24 antigen. Antibody reactivity may be used to