

centration in FIG. 30 (E-H). Data points for each curve represent the average intensities of eight replicates (background subtracted) obtained using quadruplicate spots in two replicate arrays. A linear relationship was observed between the concentration and fluorescent intensities for all four biomarkers.

FIG. 31 (A-D) shows the comparison between the CCD and PMT based imaging systems for the quantification of dose response fluorescence. Data from the two methods showed a linear relationship with a correlation coefficient ( $r^2$ ) of greater than 0.98, indicating that both methods produce similar results. This result is further supported by the results obtained from the multiplexed assays. The channels with arrays incubated with pooled patient samples from 10 metastatic and 10 control populations were imaged using the CCD system and compared the results to those obtained using the PMT. A ratio of the median fluorescence intensities obtained for metastatic populations to the median fluorescence intensities obtained for control populations is plotted in FIG. 31 (E) for the four biomarkers using both the CCD and PMT based imaging systems. The results from the CCD system are very similar to the ones obtained by using the PMT. A system was thus developed in which the high sensitivities of PMTs used in the large microarray scanners is matched by a miniature CCD camera by controlling the excitation light intensity and the integration time of the camera sensor.

While the invention has been particularly shown and described with reference to a preferred embodiment and various alternate embodiments, it will be understood by persons skilled in the relevant art that various changes in form and details can be made therein without departing from the spirit and scope of the invention.

All references, issued patents and patent applications cited within the body of the instant specification are hereby incorporated by reference in their entirety, for all purposes.

The invention claimed is:

1. A multiplex immunoassay detection device for simultaneous measurement of a plurality of distinct breast cancer markers in a patient serum sample, comprising:

a glass solid support consisting of an array of antibody Clone 191924 for HER2, antibody Clone 36006.211 for MMP-2, antibody Clone M8071022 for CA-15-3 and antibody Clone 190312 for osteopontin;

a glass cover plate, wherein the glass cover plate forms an upper surface positioned above the solid support;

a vertical support comprising adhesive silicone, wherein the vertical support forms a connection between the solid support and the cover plate, the connection forming at least one channel surrounding the array of antibody Clone 191924 for HER2, antibody Clone 36006.211 for MMP-2, antibody Clone M8071022 for CA-15-3 and antibody Clone 190312 for osteopontin, and wherein the at least one channel comprises a first end and a second end and wherein the first end of the at least one channel comprises an opening;

an absorbent material comprising a nitrocellulose membrane connected to the second end;

and a set of biotinylated detection antibodies for detection of breast cancer markers bound to antibody Clone 191924 for HER2, antibody Clone 36006.211 for MMP-2, antibody Clone M8071022 for CA-15-3 and antibody Clone 190312 for osteopontin, the detection antibodies consisting of antibody clone AF-1129 for HER2, anti-

body clone AF-902 for MMP-2, antibody Clone M8071021 for CA 15-3 and antibody Clone AF-1433 for osteopontin.

2. A multiplex immunoassay method for detection of breast cancer markers HER2, MMP-2, CA 15-3, and osteopontin in a patient sample, comprising:

providing a multiplex immunoassay detection device for simultaneous measurement of a plurality of distinct breast cancer markers in a patient serum sample, comprising:

a multiplex immunoassay detection device for simultaneous measurement of a plurality of distinct breast cancer markers in a patient serum sample, comprising:

a glass solid support consisting of an array of antibody Clone 191924 for HER2, antibody Clone 36006.211 for MMP-2, antibody Clone M8071022 for CA-15-3 and antibody Clone 190312 for osteopontin;

a glass cover plate, wherein the glass cover plate forms an upper surface positioned above the solid support;

a vertical support comprising adhesive silicone, wherein the vertical support forms a connection between the solid support and the cover plate, the connection forming at least one channel surrounding the array of antibody Clone 191924 for HER2, antibody Clone 36006.211 for MMP-2, antibody Clone M8071022 for CA-15-3 and antibody Clone 190312 for osteopontin, and wherein the at least one channel comprises a first end and a second end and wherein the first end of the at least one channel comprises an opening; and

an absorbent material comprising a nitrocellulose membrane connected to the second end;

obtaining a first solution comprising the sample;

incubating the first solution with a second solution comprising a set of biotinylated detection antibodies and streptavidin Alexa 546 under conditions to allow binding of the detection antibodies to the breast cancer markers, the set consisting of antibody AF-1129 for HER2, Antibody AF-902 for MMP-2, Antibody Clone M8071021 for CA 15-3 and antibody AF-1433 for osteopontin;

applying the mixture of the first and second solutions to the open first end of the at least one channel of the multiplex immunoassay detection device;

flowing the mixture through the at least one channel over the glass solid support under conditions to allow binding of all breast cancer markers to antibody Clone 191924 for HER2, antibody Clone 36006.211 for MMP-2, antibody Clone M8071022 for CA-15-3 and antibody Clone 190312 for osteopontin;

washing the glass solid support to remove unbound sample, detection antibodies, and streptavidin Alexa 546; and

detecting, with an optical reader, at least one fluorescent signal on the glass solid support, the fluorescent signal indicating the presence of at least one breast cancer marker in the sample.

3. The method of claim 2, wherein the patient sample comprises human blood serum.

4. The method of claim 2, further comprising comparing the detected fluorescent signal to standard curve fluorescent signal and quantifying the breast cancer marker present in the sample.

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