

**DEVICE, ARRAY, AND METHODS FOR DISEASE DETECTION AND ANALYSIS**

## CROSS REFERENCE TO RELATED APPLICATIONS

This application is a continuation of U.S. patent application Ser. No. 12/031,625, filed Feb. 14, 2008 (pending) which claims the benefit of U.S. Provisional Application No. 60/902,147, filed Feb. 15, 2007, the entire disclosure of which is hereby incorporated by reference in its entirety for all purposes.

## STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

The United States Government has rights in this invention pursuant to Contract No. DE-AC52-07NA27344 between the United States Department of Energy and Lawrence Livermore National Security, LLC for the operation of Lawrence Livermore National Laboratory.

## BACKGROUND

## 1. Field of the Invention

The invention relates to the fields of biology and chemistry.

## 2. Description of the Related Art

Research over the past decade has focused on discovering new biomarkers that provide accurate diagnosis of disease, guide therapeutic decision making, and predict the future patterns of disease. Cancer antigen (CA)-125 and Carcinoembryonic antigen (CEA) have both shown promise as biomarkers for ovarian and colorectal cancer, respectively (Fields M M, C. E. Ovarian cancer screening: a look at the evidence. *Clin J Oncol Nurs.* 10, 77-81 (2006); Hasholzner U, S. P., Reiter W, Zimmermann A, Hofmann K, Schalhorn A. CA 242 in comparison with established tumour markers in colorectal, pancreatic and lung cancer. *Anticancer Res.* 19, 2477-2480 (1999)). However, while these biomarkers have shown some potential for possible specific detection of ovarian and colon cancers, no single marker has yet been identified for breast cancer. This can be due to the fact that breast cancer is not a single disease, but a genetically heterogeneous set of diseases, thus suggesting that it can not be possible for breast cancer to be diagnosed with any single marker. The present invention addresses this need by providing multiple, mutually complementary biomarkers that provide a sensitive diagnostic assay for breast cancer.

Point of care (POC) devices and systems can process samples for a number of different types of biomarkers in a variety of settings, such as clinical laboratories, patients' bedside and doctors' offices. Various forms of single biomarker POC technologies are available including Lateral flow assays (LFA) (Panteghini M, P. F. Characterization of a rapid immunochromatographic assay for simultaneous detection of high concentrations of myoglobin and CK-MB in whole blood. *Clin Chem Clin Biochem* 42, 1292-1293. (1996); Millipore Corp A Short Guide: Developing Immuno-chromatographic Test Strips. (1996); Lou S C, P. C., Ching S, Gordon J. One-step competitive immunochromatographic assay for semiquantitative determination of lipoprotein (a) in plasma. *J. Clin Chem* 39 (1993); Lee-Lewandrowski E, L. K. Selected topics in point-of-care testing—Urinalysis, pregnancy testing, microbiology, fecal occult blood, and other tests. *Clin Lab Med* 21, 389 (2001)), Disposable microchips (Pugia M J, B. G., Peters R P, Profit J A, Kadel K, Willms T, Sommer R, Kuo H H, Schulman L S. Microfluidic tool box as technology

platform for hand-held diagnostics. *Clin Chem.* 51, 1923-1932 (2005)), the RAMP™ platform (Donald E. Brooks, D. V. D., Paul C. Harris, Joanne E. Harris, Mark E. Miller, Andrew D. Olal, Linda J. Spiller and Zongen C. Xie RAMP: A Rapid, Quantitative Whole Blood Immunochromatographic Platform for Point-of-Care Testing. *Clinical Chemistry* 45, 1676-1678 (1999)), and the Dual Path Platform (DPP) technology (Carlos Ponce, E. P., Elizabeth Vinelli, Alberto Montoya, Vilma de Aguilar, Antonio Gonzalez, Bianca Zingales, Rafael R. Aldao, Mariano J. Levin, Javan Esfandiari, Eufrosina S. Umezawa, Alejandro O. Luquetti, and José Franco da Silveira. Validation of a rapid reliable test for the diagnosis of Chagas' disease in blood banks and medical emergencies in Central America. *The Journal of Clinical Microbiology.* 5065-5068 (2005)). In addition, multiplexed LFAs have also been developed (Jeong D S, C. E. Simultaneous Quantitative Determination of Multiple Analytes with Fluorescence-Tagged Probes by Immunochromatography. *Korean J Biol Sci* 7, 89-92 (2003)).

Multiplexed LFAs, although sensitive and specific, require elaborate imaging devices for sensitive quantification, thus limiting application at the POC. Another form of multiplexed assay are microfabricated flow channels which pass a sample over an immobilized array (Delehanty J. B. Ligler F. S A microarray immunoassay for simultaneous detection of proteins and bacteria. *Anal. Chem.* 74, 5681-5687 (2002); C. R. Taitt, J. P. G., Y. S. Shubin, L. C. Shriver-Lake, K. E. Sapsford3, A. Rasooly, F. S. Ligler A Portable Array Biosensor for Detecting Multiple Analytes in Complex Samples. *Microbial Ecology* 47, 175-185 (2004); Frances S. Ligler, C. R. T., Lisa C. Shriver-Lake, Kim E, Sapsford, Yura Shubin, Joel P. Golden Array biosensor for detection of toxins. *Anal Bioanal Chem* 377, 469-477 (2003); Mark J. Feldstein, J. P. G., Chris A. Rowe, Brian D. MacCraith, Frances S. Ligler Array Biosensor: Optical and Fluidics Systems. *Journal of Biomedical Microdevices* 1, 138-153 (1999)). Use of these assays requires that the sample, detection antibodies, and wash buffers be sequentially introduced at one end of the chamber and drawn over the microarray surface using a peristaltic pump. These assays demonstrate better multiplexed sensitivities as compared to the LFAs, however, they involve sequential detection along the length of the strip rather than simultaneous detection, which limits the number of biomarkers that can be simultaneously analyzed due to the number of capture zones that can be created along the length of the strip. In addition, the flow channels are made using polydimethylsiloxane (PDMS) as the material which requires elaborate microfabrication facilities to manufacture. Also, the fluid exchange through the channels was achieved using a peristaltic pump, and the assay involved multiple incubation and wash steps, making it challenging to automate and reduce this device to a small, rugged portable form.

The present invention addresses these problems by providing a channel flow device that allows simple, rapid, and sensitive detection of multiple biomarkers.

## SUMMARY

Disclosed herein is a detection device. In one aspect, the detection device includes a solid support including a plurality of distinct capture molecule groups, each distinct capture molecule group including a plurality of capture molecules specific for a biomarker, wherein the plurality of distinct capture molecule groups is specific for a plurality of biomarkers; a cover plate, wherein the cover plate forms an upper surface positioned above the solid support; a vertical support, wherein the vertical support forms a connection between the