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UNIVERSAL SAMPLE PREPARATION SYSTEM AND USE IN AN INTEGRATED ANALYSIS SYSTEM

CROSS-REFERENCE

This application is a divisional application of U.S. patent application Ser. No. 12/795,515, filed on Jun. 7, 2010, which claims priority to and the benefit of U.S. Provisional Patent Application No. 61/184,759, filed on Jun. 5, 2009, U.S. Provisional Patent Application No. 61/235,664, filed on Aug. 20, 2009, U.S. Provisional Patent Application No. 61/349,680, filed on May 28, 2010, and International Patent Application No. PCT/US2010/037545, filed on Jun. 4, 2010. All of the aforementioned patent applications are incorporated herein by reference in their entirety for all purposes.

STATEMENT AS TO FEDERALLY SPONSORED RESEARCH

This invention was made with Government support under Contract No. 2004*H838109*000 awarded by the Central Intelligence Agency. The Government may have certain rights in this invention.

The instant application contains a Sequence Listing which has been submitted in ASCII format via EFS-Web and is hereby incorporated by reference in its entirety. Said ASCII copy, created on Apr. 4, 2013, is named 35232-719.401_SL.txt and is 1,110 bytes in size.

BACKGROUND OF THE INVENTION

Sample preparation is a ubiquitous problem in biological analytical systems. The issue of providing sufficiently purified targets from diverse raw sample types to reliably perform downstream analytical assays is pervasive and covers cell biology, genomics, proteomics, metabolomics, food biology, molecular diagnostics, and many other biological and medical assays. While many advances in sample preparation have been made the chief solution has been to develop reagents that are used manually or in robotic systems that use rectilinear stages or multi-axis arms to manipulate samples.

Microfluidics and nanofluidics allow miniaturized sample volumes to be prepared for analysis. Advantages include the nanoscale consumption of reagents to reduce operating costs and full automation to eliminate operator variances. Microfluidic sample preparation can either interface with existing or future detection methods or be part of a completely integrated system. In the present application, methods and apparatuses are disclosed that integrate full volume sample preparation with volumes over 10 mL with microliter and smaller volumes for sample preparation and analysis.

Starting from the sample, the present invention can be applied to concentrate, and pre-separate components for further processing to detect and classify organisms in matrices comprising aerosol samples, water, liquids, blood, stools, nasal, buccal and other swabs, bodily fluids, environmental samples with analysis by ELISA, PCR or other nucleic acid amplification techniques, single molecule detection, protein arrays, mass spectroscopy, and other analytical methods well known to one skilled in the art.

Microfluidic nucleic acid purification can be performed to prepare the sample for nucleic acid assays. For DNA analysis, PCR amplification is one current method. Microarray DNA, RNA and protein analysis also requires extensive sample preparation before the sample can be applied to the microarray for reaction and readout.

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Samples can be obtained by a wide variety of substrates and matrices. The matrix may contain complex mixtures including inhibitory compounds such as hemes, indigo, humic acids, divalent cations, and proteins etc that interfere with DNA-based amplification. Aerosols can contain large amounts of molds, metals, and soils humic and other acids that all interfere with PCR amplification—the gold standard.

Early work showed that as few as three seeded organisms could be detected from diluted samples of soil extracts followed by PCR amplification of two 16S ribosomal gene fragments. Low-melting-temperature agarose has been used to extract DNA from soil samples for 16S and 18S rDNA PCR amplification using universal primers. Spun separation gels in column format can be used, such as Sephadex columns. Multistep purifications such as organic extractions combined with Sephadex columns were developed. Bead beating was found to be an effective way to prepare samples for high numbers of organisms and grinding in liquid nitrogen to detect low numbers of organisms. While these methods are effective they were best suited for research laboratory environments.

Solid phase extractions to columns, beads, and surfaces can be used to purify DNA before DNA analysis. Proteinase K followed by a Qiagen QIA Amp silica-gel membrane columns and IsoCode Stix, an impregnated membrane-based technology, followed by heating, washing and a brief centrifugation were compared for *B. anthracis* Sterne vegetative cells in buffer, serum, and whole blood and spores in buffer and found to work well.

A variety of separations can be performed using the devices and methods of the invention. For example, the devices and methods of the invention can be used to perform chromatography, phase-based or magnetic-based separation, electrophoresis, distillation, extraction, and filtration. For example, a microfluidic channel or a capillary can be used for chromatography or electrophoresis. As well, beads, such as magnetic beads can be used for phase-based separations and magnetic-based separations. The beads, or any other surfaces described herein, can be functionalized with binding moieties that exhibit specific or non-specific binding to a target. The binding can be based on electrostatics, van der Waals interactions, hydrophobicity, hydrophilicity, hydrogen bonding, ionic interactions, as well as partially covalent interactions like those exhibited between gold and sulfur. In preferred embodiments, the devices and methods of the invention utilize immunomagnetic separations.

Immunomagnetic separation (IMS) is a powerful technology that allows targets to be captured and concentrated in a single step using a mechanistically simplified format that employs paramagnetic beads and a magnetic field (see Grodzinski P, Liu R, Yang J, Ward M D. Microfluidic system integration in sample preparation microchip-sets—a summary. Conf Proc IEEE Eng Med Biol Soc. 2004; 4:2615-8, Peoples M C, Karnes H T. Microfluidic immunoaffinity separations for bioanalysis. J Chromatogr B Analyt Technol Biomed Life Sci. 2007 Aug. 30, and Stevens K A, Jaykus L A. Bacterial separation and concentration from complex sample matrices: a review. Crit Rev Microbiol. 2004; 30(1):7-24.). IMS can be used to capture, concentrate, and then purify specific target antigens, proteins, toxins, nucleic acids, cells, and spores. While IMS as originally used referred to using an antibody, we generalize its usage to include other specific affinity interactions including lectins, DNA-DNA, DNA-RNA, biotin-streptavidin, and other affinity interactions that are coupled to a solid phase. IMS works by binding a specific affinity reagent, typically an antibody or DNA, to paramagnetic beads which are only magnetic in the presence of an external magnetic field. The beads can be added to complex