

MOLECULAR DIAGNOSTICS AMPLIFICATION SYSTEM AND METHODS

[0001] This application claims priority under 35 U.S.C. § 119(e) to U.S. Provisional Application No. 60/754,266, filed on Dec. 29, 2005, the entire contents of which are hereby incorporated by reference herein.

BACKGROUND

[0002] 1. Field of the Invention

[0003] The present invention relates to an integrated nucleic acid test cartridge capable of performing amplification based on temperature cycling and isothermal methods. Furthermore, it relates to devices and methods for receiving a sample suspected of containing a nucleic acid target, performing amplification and transferring an amplicon for detection. The amplification cartridge can be equipped with a sensing means including at least optical and electrochemical sensors. The cartridge can perform various methods of amplification including, but not limited to, polymerase chain reaction, rolling circle amplification and strand displacement amplification. The amplification device also has the ability to function with a portable power supply or means therefor.

[0004] 2. Background Information

[0005] Applications of nucleic acid testing are broad. The majority of conventional commercial testing relates to infectious diseases including Chlamydia, gonorrhea, hepatitis and human immunodeficiency virus (HIV) viral load; genetic diseases including cystic fibrosis; coagulation and hematology factors including hemochromatosis; and cancer including genes for breast cancer. Other areas of interest include forensics and paternity testing, cardiovascular diseases and drug resistance screening, termed pharmacogenomics. The majority of testing currently occurs in centralized laboratories using non-portable and operationally complex instruments. Conventionally, tests generally require highly skilled individuals to perform the assays. As a result, the time taken between obtaining a sample suspected of containing a specific nucleic acid fragment and determining its presence or absence is often several hours and even days. However, as with other kinds of blood tests, physicians and scientists often require results more quickly and that are obtainable in a convenient user-friendly format. Consequently, there is a need for a portable analysis system capable of performing nucleic acid testing quickly and conveniently.

[0006] Methods of extracting nucleic acids from cells are well known to those skilled in the art. A cell wall can be weakened by a variety of methods, permitting the nucleic acids to extrude from the cell and permitting its further purification and analysis. The specific method of nucleic acid extraction is dependent on the type of nucleic acid to be isolated, the type of cell, and the specific application used to analyze the nucleic acid. Many methods of isolating DNA are known to those skilled in the art, as described in, for example, the general reference Sambrook and Russell, 2001, "Molecular Cloning: A Laboratory Manual," pages 5.40-5.48, 8.1-8.24, A1.17-A1.19, and A1.25-A1.27. For example, conventional techniques can include chemically-impregnated and dehydrated solid-substrates for the extraction and isolation of DNA from bodily fluids that employ lytic salts and detergents and that contain additional reagents for long-term storage of DNA samples, as described in, for example, U.S. Pat. No. 5,807,527 (detailing FTA paper), and

U.S. Pat. No. 6,168,922 (detailing Isocard Paper). Conventional techniques can also include particle separation methods, such as those described in, for example, U.S. Reissue Patent No. RE37,891.

[0007] Several methods and apparatuses for amplification of nucleic acid are known to those of ordinary skill in the art. It is known that Polymerase Chain Reaction (PCR) is inhibited by a number of proteins and other contaminants that follow through during the standard methods of purification of genomic DNA from a number of types of tissue samples. It is known that additional steps of organic extraction with phenol, chloroform and ether or column chromatography or gradient CsCl ultracentrifugation can be performed to remove PCR inhibitors in genomic DNA samples from blood. However, these steps add time, complexity and cost. Such complexity has limited development of a simple disposable cartridge useful for nucleic acid analysis. Therefore, the development of new, simple methods to overcome inhibitors found in nucleic acid samples used for nucleic acid amplification processes is desirable.

[0008] Nucleic acid hybridization is used to detect discernible characteristics about target nucleic acid molecules. Techniques like the "Southern analysis" are well known to those skilled in the art. Target nucleic acids are electrophoretically separated, then bound to a membrane. Labeled probe molecules are then permitted to hybridize to the nucleic acids bound to the membrane using techniques well known in the art. This method is limited, however, because the sensitivity of detection is dependent on the amount of target material and the specific activity of the probe, and, in the example of a radioactively labeled probe, the time of exposure of the signal to the detection device can be increased. Alternatively, as the probe's specific activity may be fixed, to improve the sensitivity of these assays, methods of amplifying nucleic acids are employed. Two basic strategies are employed for nucleic acid amplification techniques; either the number of target copies is amplified, which in turn increases the sensitivity of detection, or the presence of the nucleic acid is used to increase a signal generated for detection. Examples of the first approach include polymerase chain reaction (PCR), rolling circle (as described in, for example, U.S. Pat. No. 5,854,033), and nucleic acid system based amplification (NASBA). Examples of the second include cycling probe reaction, termed CPR (as described in, for example, U.S. Pat. Nos. 4,876,187 and 5,660,988) and SNPase assays, e.g., the Mismatch Identification DNA Analysis System (as described in, for example, U.S. Pat. Nos. 5,656,430 and 5,763,178). More recently, a strategy for performing the polymerase chain reaction isothermally has been described by Vincent et al., 2004, EMBO Reports, vol 5(8), and is described in, for example, U.S. Application Publication No. 2004/0058378. A DNA helicase enzyme is used to overcome the limitations of heating a sample to perform PCR DNA amplification.

[0009] The PCR reaction is well known to those skilled in the art and was originally described in U.S. Pat. No. 4,683,195. The process involves denaturing nucleic acid, a hybridization step and an extension step in repeated cycles, and is performed by varying the temperature of the nucleic acid sample and reagents. This process of subjecting the samples to different temperatures can be effected by placing tubes into different temperature water baths, or by using Peltier-based devices capable of generating heating and cooling,