

gasket attached to adaptation element 29. As a practical matter, the device is prepared in a sterile working environment and tools to prevent cross-contamination of nucleic acids and enzymes are used.

[0104] When using a 13 mm filter-disc 25, about 3-10 μL of bodily fluid can be applied to the chemically-impregnated filter surface, whereas the lower-volume modified device, with a 4.8 mm filter disc functions well with 1-3 μL of fluid. Sample application can be achieved with the assembled device through the inlet port, or directly onto the filter prior to assembly. Where a buccal swab is acquired with a cotton swab, it can be wiped onto the filter disc or washed onto the filter disc through the inlet port. It has been found that another method for isolating buccal cells is by using a commercial mouthwash, e.g. Scope brand. A few microliters of used mouthwash can then be applied into the device.

[0105] Regarding removal of interferents, it was found that sterile water at ambient temperature performs satisfactorily as a wash fluid as it is capable of flushing interferents through the filter-disc without removing nucleic acids from within the matrix of the disc. When water is pumped from a dispensing tip positioned for a tight seal at the inlet to the filter holder, it flushes through the filter-disc washing the sample and passing through to the outlet. For buccal cell samples, a single flush of 20 μL of sterile water per μL of sample is sufficient. For blood samples, 20 μL of sterile water per μL sample is preferably flushed through the filter and repeated three times. Alternatively a single volume passed forward and backwards thrice is sufficient. As an alternative to sterile water the following sterile buffer solutions may be used, 10 mM Tris at pH 7 and 5 mM to 20 mM sodium or potassium phosphate.

[0106] After the washing procedure the filter-disc retains an amplifiable quantity of DNA. It can then be removed from the filter holder and employed in an amplification reaction. It has been found that a 4.76 mm diameter disc can be employed in a 100 μL PCR amplification directly, whereas a 13 mm disc is optimally cut into smaller portions. In an alternative embodiment the nucleic acid material can be eluted from the filter by using hot deionized water or various buffer solutions and then introduced into an amplification device. In another embodiment the filter process is integrated into a disposable device for nucleic acid testing, as described below. FIG. 5 demonstrates the effectiveness of the method and filter holder device, showing PCR amplification of a buccal swab sample. After the extraction process, the filter was removed from the device and placed into a 100 μL PCR reaction chamber using two primers specific for the hemochromatosis gene (Hfe). Once the amplification process was completed, material was applied to lane 1 of a 10% acrylamide 1 \times TBE electrophoresis gel. As expected this generated a 390 by (base pair) fragment indicated by the arrow. Note that control lane 2 contained a 100 by ladder and lane 3 contained water as a negative control.

[0107] It is understood that the manual procedure described above can form the basis for the design of an extraction module included and integrated within a disposable device for performing genetic analyses, or be a separate module that delivers an extract to a disposable device. Delivery can be for example by pipette transfer or by mating features 500, 520 and 521 on each that facilitate transfer (see FIGS. 19 and 20). Such devices are described in detail in the section addressing an integrated single-use device for nucleic acid testing.

Detailed Description of Amplification Methods

[0108] In the present invention, where electrochemical detection is preferred, the main objective of the nucleic acid

amplification step is to generate about a 0.01 picomolar concentration of detectable nucleic acid from the target molecule, as it has been found that this is in the range of the lower detection limit of a sandwich assay with enzymatic amplification and electrochemical detection. The desired one picomolar concentration of fragment is based on Avogadro's number (1 mole= 6×10^{23} molecules), where 1 μmol equals $6\times 10^{23}\times 10^{-12}$, or about 10^{12} molecules. If, as is known, one microliter of blood contains about 5×10^3 molecules of DNA, then one milliliter, which is a reasonably accessible sample volume, contains 5×10^6 molecules, or roughly about 10^7 molecules. To go from the amount of DNA in 1 ml of blood to 0.01 pmol of DNA requires an amplification of about 10^3 fold. This is certainly achievable using several well-known amplification techniques. Performing a similar calculation, for a different sample types and sample volumes, to determine the degree of amplification will be apparent to those skilled in the art.

[0109] In alternative embodiments of a single-use cartridge where optical detection is used, again the objective of the nucleic acid amplification step is to generate a given molar concentration of detectable nucleic acid from the target molecule so as to be in the range of the lower detection limit of the given optical methods. Such calculations will be familiar to those skilled in the art. It is well known in the art that the ability to determine the concentration of a sample via optical detection is dependent on the background level of noise, the extinction coefficient of the optical compound to be detected, the optical system's electronic gain, the volume of the sample and other parameters. A simple relationship between the compound concentration and the absorbance of the sample can be expressed using the Beer-Lambert law ($A=\epsilon cl$), where A is the absorbance, ϵ is the extinction coefficient, c is the molar concentration of the sample, and l is the path length of the sample. Typically the length is 1 cm by definition, (though in the devices described below about 0.02 to about 0.4 cm is more typical). This makes the absorbance related to the concentration using the constant of the extinction coefficient and usually permits detection limits within the pM range.

Polymerase Chain Reaction Amplification

[0110] The polymerase chain reaction (PCR) is well known for its ability to specifically amplify regions of target DNA based on the primer sequences chosen for the PCR reaction. A difficulty with processing this material is in trying to detect the signal based on hybridization homogeneously. By definition, the PCR reaction generates blunt ended double stranded products. However, certain thermostable DNA polymerases possess polyA polymerase activity, which can be used to add an additional A nucleotide. While this has been used commercially for cloning purposes, the single nucleotide overhang is inefficient for hybridization. As another approach to attempt to use the PCR reaction for hybridization, recognition sequences for restriction endonuclease enzymes have been designed into the PCR primers. However, this is limiting, because it requires additional enzymes which typically only generate short overhangs. As with mostly double stranded species, the PCR product is not amenable to hybridization in homogenous reactions. To overcome this limitation, a strategy which uses a limiting amount of one primer over the other has been devised. An alternative is to have promoter regions for bacteriophage RNA polymerases (e.g. SP6). Limiting one of the primers has drawbacks in that the efficiency of the amplification is reduced. Generating RNA with bacterioph-