

distilled or sterile deionized water, to remove common inhibitors of amplification and to rinse away denatured proteins. The filter retaining nucleic acids from the sample can then be removed from the supporting apparatus and directly applied to amplifying reagents, e.g. PCR. This can be done using the whole filter disc, or a portion thereof, depending upon the quantitative requirements for DNA. Where desirable the nucleic acid material may be eluted from the filter preferably using deionized water at a temperature in the range 75 to 950 C. Other eluting reagents include dilute neutral buffers, such as 10 mM Tris at pH 7 and 5 mM to 20 mM sodium or potassium phosphate buffers. Alternatively, a filtering matrix can be incorporated in a disposable nucleic acid testing cartridge, as described below.

**[0095]** The preferred embodiment of the individual extraction device is described as follows: The chemically-impregnated filter is a disc composed of a reproducible thin matrix that is biochemically inert, preferably a commercially available filtering paper. The lytic salts and optionally a detergent are dispensed onto the surface of the filter and then dried within the matrix. As a practical matter, the size of the filter-disc is restricted by the outer-diameter of the filter holder, and must be wider than the channel through which the wash fluid passes. Chemical impregnation is by means of a liquid cocktail containing a chaotropic salt, with or without detergent, a weak basic buffer, and a chelating agent. The cocktail is dispensed onto the filter-disc, dried and then the filter is stored in a sealed environment until use.

**[0096]** In the preferred embodiment, the filter holder device provides rigid support to the filter-disc (optionally with a placement-assisting gasket) with a central small-diameter channel through which the wash fluid may pass from one side of the filter-disc to the other. The device contains both an inlet and an outlet on opposite sides of the filter-disc to allow for the introduction and later removal of the wash fluid. Its construction material should be biochemically inert, preferably a molded plastic. It is designed to be disposable, but it optionally could be reusable if properly cleaned, e.g. autoclaved. The filter base-pad is a subcomponent that assists in the proper placement of the filter-disc in line with the wash fluid channel. Optionally a filter-positioning gasket may be employed for sizes of filter that are smaller than the internal diameter of the device. For example a thin adhesive layer with a central hole that holds the filter-disc onto the filter base-pad over the channel may be used. In this embodiment, a double-sided adhesive tape with a central hole slightly smaller than the outer-diameter of the filter-disc is preferred. Wash fluid is preferably distilled water and is used to remove chemical inhibitors of amplification.

**[0097]** As is well known in the art, conditions of sterility and biochemical inertness are intrinsic to the choice of materials employed for the construction of the device, the handling of fluids and the source of the wash fluid. Samples, e.g. bodily fluids, can be introduced to the filter-disc through the inlet of the filter holder, or onto the filter-disc before assembly into the device, provided care is taken to ensure sterility.

**[0098]** In one embodiment, the filter holder can be a Swinex filter holder, preferably the 13 mm diameter version (Millipore Corp.), which is also provided with a Teflon™ gasket and is constructed of molded polypropylene. In a preferred embodiment, a modification was performed upon the filter holder where additional acrylic pieces are cut to exactly fit the void spaces inside both the top and bottom pieces of the filter holder. These pieces are preferably held in place with adhe-

sive, e.g. Loctite epoxy glue, and have a drilled central channel of a smaller diameter than the standard device. The inlet to the filter holder can also optionally be modified with an end piece from an Eppendorf 100  $\mu$ L pipette tip that is held into position with adhesive.

**[0099]** The filter positioning gasket is preferably a double-sided adhesive tape gasket (iSTAT Canada Ltd.), laser cut to a thickness of about 25  $\mu$ m on a PET film base with about 75  $\mu$ m of a rubber-acrylic hybrid adhesive, sandwiched between two polyester liners for protection. A two-sided adhesive has the advantage of providing a better seal of the filter holder during the washing procedure. Note that the polyester liners are removed during assembly of the device to expose the adhesive.

**[0100]** The filter disc is preferably Whatman 4 Qualitative Grade plain cellulose paper, (Whatman Inc.), with the following manufacturer's specifications; particle retention greater than 20-25  $\mu$ m, coarse porosity, filtration speed ASTM 12 sec., Herzberg 37 sec., and a smooth surface. Other similar filter materials and grades may be used include Whatman 3 MM, Pall GF A/B, Texwipe (cleaning cloth), Whatman 1, Whatman 3, Whatman 4, Whatman 6 and Pall 1660 membranes.

**[0101]** Chemical impregnation of the filter is preferably with a liquid cocktail that contains chaotropic salts, preferably a guanidinium salt such as guanidine isothiocyanate, with or without detergent preferably Triton-XI00™, a weak basic buffer preferably TRIS, and a chelating agent preferably EDTA. Alternative reagents include guanidinium salts (e.g. guanidinium hydrochloride and guanidinium thiocyanate), non-ionic detergents and chelating materials. The cocktail is applied to Whatman 4 paper in solution for minimal loading of approximately 3.75  $\mu$ L/cm<sup>2</sup> of 2M guanidine isothiocyanate, 1% Triton XI00, 10 mM TRIS buffered to pH 8.8 and 2 mM EDTA. The cocktail is then dried under a heat lamp (Philips, Heat-Ray 250 w infrared) about 5 cm below the light surface for 3 minutes, then cooled at room temperature for a minimum of 10 minutes and stored in a sterile centrifuge tube until use. Note that where the intended sample material is blood, it has been found that impregnation with a solution of 200 mM NaOH can be substituted for all the reagents used in the cocktail solution. Other strong basic solutions can also be used e.g. KOH.

**[0102]** By way of demonstration, two different bodily fluids have been used for the extraction of genomic DNA. These are (i) white blood cells within a whole blood sample, that are untreated by either chelating or anticoagulation agents, and (ii) buccal cells obtained from a cheek swab. When utilized as described below, the present device can extract amplifiable DNA from both fluids with a minor variation in the protocol. Based on this disclosure, those skilled in the art will recognize that other types of sample containing nucleic acid may also be extracted by making further minor variations in the protocol.

**[0103]** The component elements of filter holder are shown in FIG. 4(a) in side view, FIG. 4(b) exploded side view, FIG. 4(c) top view and FIG. 4(d) with a void volume insert. The device comprises a filter holder top 20 and bottom 21, an inlet channel 22, void spaces 23 and 24, a filter disc 25 on a filter disc base and an outlet channel 26. In the preferred embodiment, as shown in FIG. 4(d), a lower volume modification employs a void-filling structures (27, 29) and an inlet adaptation element 28 to facilitate better transfer of fluid into the narrower central channel via inlet 22. The lower volume device requires the filter-disc to be positioned with a filter