

receiving receptacle **8** (not shown) causes the one way snap-fit barbs **6** to compress and then snap back in such a manner that the sample collection tube is then irreversibly and tightly captured as part of the microfluidics cartridge assembly **300**. Such methods of providing a one-way snap-fit using flexible plastic retaining elements are well known to those skilled in the art.

[0099] Further insertion of the sample tube assembly **200** into the bridging manifold element **8** causes a small shielded needle or chevron (not shown) within manifold element **8** to puncture the integral plastic membrane element **5** at the outlet end of sample tube **4**. The manifold element **8** has an internal fluid passage (not shown) which thereby fluidly interconnects the pierced sample collection tube **4** with the microfluidic cartridge **7** in a leak-tight manner which does not compromise bio-safety. The sample tube assembly **200** is thus coupled to the manifold **8** in a bio-safe and non-releasable manner which enables the sample contents with the sample tube assembly **200** to be analyzed within the microfluidic cartridge **7**.

[0100] FIG. **5** shows the microfluidics cartridge assembly **300** inserted into an instrument or reader **8** which is capable of performing a number of pre-determined assay steps on the microfluidic cartridge assembly **300**. The instrument **8** is controlled by an internal microprocessor, with a user interface displayed on a liquid crystal display (LCD) device **9**, and with various parameters on a menu accessible via a four way toggle button **10** and with select button **11**. In addition to providing the fluid transport means for the microfluidics cartridge assembly **300**, the instrument **8** also contains a reagent pack (not shown) which is capable of dispensing various reagents and buffers to the microfluidics cartridge assembly **300** in accordance with a pre-determined assay protocol stored in the memory of instrument **8**, and running under the control of the instrument's microprocessor.

[0101] FIG. **15** shows the key elements of the instrument and reagent pack in block diagram format using the example of a reagent pack for a test for H5 avian influenza. The purpose of these various elements shown in this block diagram will become apparent in subsequent description.

[0102] FIG. **6** shows the key elements of the "inner workings" of the microfluidics cartridge assembly **300**. Fluid transport around microfluidics cartridge assembly **300** is accommodated by the layout of various microfluidic channels embedded inside the cartridge, such as microfluidic channel **28**. The logic for the control of fluid transport around the cartridge is accommodated by the use of various valves embedded in the cartridge, here valves **12**, **13**, **14**, **24**, and **25**. These valves are shown in FIG. **6** as 3-way valves, however the 3-way valve logic could also be replaced by an increased number of embedded simpler and cheaper 2-way elastomeric valves, which are well known to those skilled in the art of microfluidics design.

[0103] Also shown in FIG. **6** are a number of ports **19**, **21**, **23**, **26** and **27** of the externally ported hydraulic control interface of the microfluidic cartridge. These ports each enable a fluid tight connection between microfluidic cartridge **7** when assembled in the control platform instrument **8**. The ports and hydraulic control interface enables various reagents to be delivered from the reagent pack stored in instrument **8** to the microfluidics cartridge assembly **300**. Some of the ports only enable an air volume to be aspirated or dispensed in order to allow the biohazardous sample material to be transported only within the microfluidics cartridge assembly **300** without

ever breaching any of the ports. This ensures that biohazardous infectious material is always contained solely within the cartridge assembly **300**.

[0104] Further shown in FIG. **6** are inner workings comprising a solid phase extraction chamber **15**, a waste containment chamber **18**, a test amplification and detection chamber **20**, a positive control amplification and detection chamber **17**, and micro-magnetic stirrer bar elements **16** and **22** and waste disposal chamber **18**. The purpose of these elements will become apparent in subsequent description.

[0105] FIG. **7** shows the first step of the pre-programmed assay controlled by instrument **8**, which is the introduction of a lysis buffer with for example guanidinium thiocyanate in combination with detergents (shown cross-hatched) from a reagent pack (not shown) through port **27** via valve **12**, and via bridging manifold **8** back into sample collection tube **4**. This step causes the lysis buffer to mix with the mucosal sample, thereby lysing the cells contained therein, and causing the nucleic acids within the cellular material to be released.

[0106] FIG. **8** shows the second step of the pre-programmed assay controlled by instrument **8**, which is the aspiration of the lysed sample (shown cross-hatched) from the sample collection tube **4** via bridging manifold **8**, and via valves **12**, **13**, and **14** into the RNA isolation chamber **15**. Aspiration is applied by way of port **23** and valve **25**. The RNA isolation chamber **15** is filled at fabrication with solid phase material such as silica particles which have a surface treatment which will bind only the sample RNA to the surface of the solid phase material. Such solid phase materials are well known to those skilled in the art, and such solid phase materials are available from a range of different manufacturers.

[0107] FIG. **9** shows the third step of the pre-programmed assay controlled by instrument **8**, which is the elution of waste material from the sample (that is, everything except for the sample RNA) via valve **14** to the waste disposal chamber **18**. The eluted waste material is shown cross-hatched, while the remaining captured RNA inside isolation chamber **15** is shown in a dotted pattern.

[0108] FIG. **10** shows the fourth step of the pre-programmed assay controlled by instrument **8**, which is the elution of the sample RNA from the RNA isolation chamber to the test sample amplification and detection chamber **20** via valves **25** and **24**. This elution step is performed with the aid of an elution buffer introduced via port **26** and via valves **13** and **14**. This elution buffer is of a type which is able to release the RNA from the surface of the solid phase material in RNA isolation chamber **15**.

[0109] FIG. **11** shows the fifth step of the pre-programmed assay controlled by instrument **8**, which is the dispensing of primer master mix for the target nucleic acid sequence into the test sample amplification and detection chamber **20** via port **21**. Mixing of the primer master mix with the sample RNA is then performed by micro-magnetic stirrer bar **22**. Further, a positive control, with control primers and template, for the target nucleic acid master mix is optionally dispensed into the positive control amplification and detection chamber **17** via port **19**. Alternatively, a negative control may be run. Continued mixing of the positive control is then performed by micro-magnetic stirrer bar **16**. Not shown in FIG. **11** is also an optional third negative control amplification and detection chamber which would be suitable for an FDA CLIA waived diagnostic device. In the negative control chamber de-ionised water would be introduced and mixed with the sample RNA,