

which can, for example, be used to produce a simple qualitative report regarding the presence or absence of the target analyte, or a fully quantitative report on the concentration of the target analyte within the fluid sample. In certain embodiments, multivariate methods could be applied to analysis of the fluorescence signal data to achieve quantification of different target analytes and to provide better distinction of false positives. While such an approach is well suited for the resolution of complex systems (eg, when multiple analytes are detected in a single channel), the use of a dedicated channel for each target analyte assay enables the use of simple chemometric principles.

**[0071]** In situations where the target analyte is present in concentrations that are significantly greater than the detection threshold concentration, a measurement result can then be reached rapidly, providing a useful early warning. For example, during a fluorescence-based competitive assay for a particular target analyte within a particular channel **42** of the fluidic chip **26**, the rate of decrease of the fluorescence signal from the sensor pads can be readily measured by the reader **13**. In various embodiments, for example when the kinetics of competitive, sandwich or other immunoassays may be difficult to control in a reproducible fashion, (eg. because they depend upon such factors as assay temperature, analyte and reagent concentrations, the degree of mixing of these species, etc.) each assay reaction may be allowed to proceed to equilibrium before undertaking subsequent signal acquisition and data processing steps. Other examples of labels which can also be optically read in alternate configurations include gold or silver nanoparticles, which may be detected by, for example, light scattering or colorimetric means. These examples are in no way exhaustive, but simply illustrate a number of labelling strategies that can be applied in various embodiments of the current inventions.

**[0072]** Referring again to FIGS. **4a** and **4b**, in various embodiments, following spreading of the conditioned, retrieved fluid sample across all of the channel inlet ports **41** the fluid subsequently wets and flows along the one or more fluidic channels **42** of the chip **26** by capillary action. Without wishing to be limited to any particular theory, each of the inlet ports **41** provides a large surface area for ease of fluid access and wetting and for initiation of laminar capillary flow of fluid along the channels **42**. By selecting an inlet surface area to channel cross-section ratio of between about 5 and about 20, consistent induction of fluid flow into the channels **42** can be routinely achieved.

**[0073]** In various embodiments, the sensor pad may comprise two or more separate or adjacent pads.

**[0074]** In various embodiments, the analyte-specific receptors and/or procedural control receptors may include a MIP receptor. In various embodiments, an antibody or antigen may be used as one or both of the receptors.

**[0075]** In various embodiments, an absorbent pad **46** may not be present, for example when the wicking action of pulling fluid towards the bottom of the channel is not required.

**[0076]** In various embodiments, the length of each diffusion zone **44** may be a determinant of the concentration profile of the conjugate within each fluid sample as it reaches its corresponding sensor pad **45** and the resulting analytical performance of each assay. In various embodiments, to ensure levels of assay performance and reproducibility suitable for detection of drugs of abuse in oral fluids, the length of the diffusion zone in each channel **42** is set to a value between about 0.5 mm and about 5 mm. Without wishing to be limited

to any particular theory, combined with the rapid release of conjugate from each reagent pad **43** followed by equally rapid mixing with each fluid sample, the use of a short diffusion zone **44** can ensure that a tight, plug- or band-type, homogeneous concentration profile of conjugate within the conjugate/fluid sample solution arrives at each sensor pad **45**. In various embodiments, this may facilitate increasing assay sensitivity and reproducibility.

**[0077]** Referring to FIG. **4c**, a cross-sectional diagram of the fluidic chip **26** in FIG. **4a** is shown. In this embodiment another aspect of the channels **42** is that there may be a gap **402** between the top surface of the reagent pad **43** and the upper channel wall as well as a gap **403** between the top surface of the sensor pad **45** and the upper channel wall. The upper channel wall is defined by a clear top layer **401** that covers the fluidic channel. Both gaps **402** and **403** have dimensions ranging from 0.05 mm to 0.5 mm. Within these dimensions a quantity of sample fluid is allowed to flow over each respective pad. While this quantity does not contribute to the sensing, it does assist flow along the channel by providing continuity along the channel and thus assists in capillary action. The gaps also provide a regulation of the sample fluid's flow rate. The exact dimensions of both gaps are selected against the viscosity of the intended sample fluids. To give an example, for a sample fluid with a viscosity of 5 the gap **402** above the reagent pad may be 0.05 mm, while the gap **403** above the sensor pad may be 0.25 mm. For the purposes of clarity the gaps **402** and **403** are exaggerated in FIG. **4c**.

**[0078]** In various embodiments, the rate of flow of each conjugate/fluid sample solution is a determinant of the efficiency with which a target analyte and/or its respective conjugate binds to the receptors on its corresponding sensor pad **45**, and the resulting analytical performance of each assay. In this regard, while increased fluid flow rates may decrease the levels of non-specific binding (background) subsequently measured at sensor pads **45**, decreased fluid flow rates may increase the levels of measured signals at the expense of also increasing the levels of background.

**[0079]** To optimize the rate of flow of each conjugate/fluid sample solution across each corresponding sensor pad **45**, and to thereby facilitate optimisation of the performance of each assay, fluid flow may be regulated during conjugate release by controlling the relative dimensions and porosity of the reagent pads **43**. In various embodiments, the dimensions of each of the reagent pads **43** are selected such that the pads fill the complete width of each channel **42** with pad lengths of between about 2 mm and about 20 mm and height of pads between about 0.05 mm and 1 mm. In various embodiments, the mean size of the pores within the reagent pads **43** may be set at about 20  $\mu\text{m}$ . In various embodiments, the mean size of the pores may range between about 5  $\mu\text{m}$  and about 100  $\mu\text{m}$ .

**[0080]** In various embodiments, the rate of fluid flow during the target analyte/conjugate binding stage may be also regulated by varying the relative dimensions and the porosity of the absorbent pads **46**. In various embodiments, the dimensions of each of the absorbent pads **43** may be selected such that the absorbent pads fill the complete cross-section of each channel **42** with pad lengths of between about 3 mm and about 25 mm. In various embodiments, the mean size of the pores may range between about 1  $\mu\text{m}$  and about 100  $\mu\text{m}$ .

**[0081]** In various embodiments, the sensor pads **45** are integrated into the channels **42** in such a way that fluid flow between reagent pads **43** and absorbent pads **46** is not substantially impeded. For example, each of the sensor pads **45**