

(Though it would also be possible to allow the binding phase while the magnet is in place and the magnetic beads are held in place). Alternatively the magnetic particle—analyte complex could be transported upstream in the sample cartridge, to contact the label. Alternatively as previously described all the binding reagents (magnetic particle capture phase and label) could be deposited in the sample channel and the binding reaction would occur in the sample channel. The magnetic accumulation and air/fluid wash by the reader ensures the magnetic particle-analyte-label complexes are quantified in an air/fluid environment.

**[0127]** The flexibility of the present system allows the potential for a very flexible and sensitive measurement. The whole system at this point is set up for high capture efficiency resulting in very sensitive assays as the magnetic particles are effectively preloaded with analyte, therefore collisions with label should result in successful binding events. For clarity a binding scheme will be described, however due to the flexibility of the present platform design nearly any assay architecture conceived can be formatted.

**[0128]** A wash step is generally performed to remove any unbound label. Subsequent optical measurement is then made in a 'clean' environment. In the case of fluorescent detection, this allows the use of fluorophores which would not be usable in some matrices (for example blood) where significant quenching of the specific signal would (without removal of this matrix) otherwise occur.

**[0129]** Further amplification of signal if needed could be delivered if required by resuspending and pumping a tertiary binding reagent into the channel. For example an additional labelled particle (same or different label) with antibodies against a component of the primary labelled particle could be used (e.g. an immunogen coupled to the labelled particle to which good antibodies exist). Once again a magnetic holding and wash step would be performed to remove any unbound secondary label coated labelled particles. The reader would then measure the label in the same manner (if the same label was used) or in a different manner if a different label was used. This additional measurement could be used to increase the range and sensitivity of the measurement.

**[0130]** Many current POC immunoassays that use fluorescent labels measure the labels in a blood or plasma matrix. As previously described there are many components in blood and plasma (human serum albumin, bilirubin, haemoglobin etc) that interfere with either the excitation or emission wavelength used to measure the concentration of the captured fluorophore. As a result the precision within and between bloods of other POC immunoassays can be affected purely by the measurement of the fluorescent label alone (in addition to all the matrix binding problems). As a result fluorophores are used that try to minimise these effects, these are therefore not necessarily the best fluorescent label to use but a necessity of making the fluorescent measurement in a "dirty matrix". The present platform will however, allow an extended choice of fluorescent labels allowing the labels that result in the best assay performance to be chosen. This alone is an advantage; it also means multiplexing can more easily be achieved as discussed.

**[0131]** The present invention makes the possibility of multiplexing (i.e. the detection of more than one analyte using a single sample) within a single channel using magnetic particles as a capture phase possible. For example, if it is not possible to use multiple fluorescent labels due to the optical properties of blood and plasma then multiplexing would not

be possible within a channel because there is no way to provide specificity of measurement as the magnetic particles cannot be spatially distributed into magnetic particles specific for analyte 1, 2 etc. This is why planar capture is used as capture phase in POC platforms that can perform multiplexing. For example, some panel tests use the same fluorescent label (due to the blood and plasma limitations) for the labels with the capture antibodies for each analyte spatially distributed on the strip to allow multiplexing capability. As the magnetic particles are mobile and susceptible to the applied magnetic field, specificity due to spatial distribution of the magnetic particles (specific against different analytes) is difficult to achieve due to the fluorescent label optical limitations in plasma or blood. However, by providing a wash step as in the present invention, multiplexing within a single channel using magnetic particles' and fluorescent labels become a reality. In addition the 6 channel strip design allows multiplexing to be achieved with one fluorophore and thus increase the overall multiplexing capability when within channel multiplexing (multiple fluorophores) is taken into account.

**[0132]** Due to the flexibility offered by the ability to perform one step, multi-step assays and multiple wash steps there is a great opportunity to extend analyte measurement ranges or linearise the dose response curve of an assay. For example, typical immunoassay dose response curves are sigmoidal. This is driven by either reagent saturation (insufficient reagent to maintain linear binding) or saturation of the label/detection method (i.e. the detection methods becomes saturated and can no longer measure the label in a linear fashion). The present platform will however, allow a full linear response across the measurement range, and this can be achieved by several methods. For example, the reader can measure the concentration of the fluorescent particles at each stage of the multi step assay. Therefore after the initial fluorescent particle binding step has occurred, the reader could measure the fluorescence intensity. This could be a range measurement, for example, so if the intensity is over a threshold value (set during calibration) it uses the fluorescence intensity to calculate the analyte concentration (calibration 1) and the test stops at this point. If the fluorescence intensity is however below the threshold value this would indicate a low analyte concentration and the previously described additional amplification steps would occur (calibration 2) and the subsequent fluorescence intensity used to calculate the analyte concentration. This could also be achieved by using the two channels whereby one channel is tuned to make very sensitive measurements whilst the other is tuned (reagent concentration and/or binding time) to make linear measurements across the remaining portion of the analyte measurement range. Optical saturation resulting in non linearity can also be combated by reader. The present platform allows many different ways of achieving linear responses across the measurable ranges, which will allow more accurate calibration resulting in better within and between blood precision resulting in better ATE. It is envisaged that magnetic particle distribution could be used to affect both the measurement of the fluorescent label and the binding reactions. For example, the magnetic particles could be measured as a homogenous/distributed distribution throughout the channel allowing range measurements whilst the same magnetic particles could then be accumulated to increase the fluorescence intensity to drive the sensitivity of the measurement. Likewise the same principle could be used to affect the binding reactions and tune the assays accordingly.