

[0182] This will be referred to in the text as functionalized latex 2.

Assay Procedures

[0183] Assay 1: 1 Step Wet Assay with Manual Wash

[0184] 7 μ l 0.5% functionalized paramagnetic particles (with bound b1H12) is added to an eppendorf and placed on a magnetic separator. The supernatant is removed and the particles resuspended in 49 μ l 30 mg/ml BSA in 0.05% tween-20 in PBS, pH 7.2. To this, 7 μ l 0.25% functionalized latex 1 (with biotinylated 5A6 bound) is added and the solution mixed.

[0185] 8 μ l of this mixture is removed and added to 2 μ l PSA protein in 60 mg/ml BSA in PBS, pH 7.2 (N.B. PSA is at 5 \times required final concentration). This is mixed and incubated for 5 min at room temperature.

[0186] The eppendorf is then added to a magnetic separator and the supernatant carefully removed. 20 μ l 0.05% tween-20 in PBS, pH 7.2 is then added to the pellet, whilst remaining on the magnetic separator. The supernatant is removed and a fresh 20 μ l 0.05% tween-20 in PBS, pH 7.2 used to resuspend the particle complexes, once removed from the magnetic separator.

[0187] This is repeated with several different concentrations of PSA, producing washed wet assay complexes which have been diluted 2 \times from the original reaction.

[0188] These diluted, washed complexes are measured in 3 different ways as follows:

[0189] Washed wet assay measurement 1:

[0190] 2 μ l washed wet assay complexes are added to 38 μ l PBS in a 384 well black Optiplate for fluorescent measurement. The plate is then measured using a Perkin Elmer Victor3 V. The fluorescent signal in the well is measured using the inbuilt program 'Fluorescein (485 nm/535 nm, 0.1 s)', adapted for use in a 384 well format. This program uses excitation at 485 nm and emission at 535 nm with a 0.1 s measurement time.

[0191] Washed wet assay measurement 2:

[0192] 8 μ l washed wet assay complexes are used to fill a 'MST pro strip V1' cartridge (as shown in FIG. 26). The washed wet assay reagents were applied to the cartridge via sample inlet port (226). This allowed the sample (in this case washed wet assay reagents) to fill the 6 channels up to fluidic stop features (229, 228, 239, 237) (which can also act as fill detect electrodes via electrical connection to the reader through connector (230)). This cartridge is inserted into a 'MST pro meter V1' reader (as shown in FIG. 12) and the signal measured by the optics in the reader which scans each channel using a linear movement of the optical head at position (222, 242) In this method, the washed wet assay complexes are spread homogeneously throughout the channel and hence detection area (i.e. they are not concentrated into a band by use of a magnetic field). (It should be noted that measurements are not made in channel (243) in these experiments as this has been set up as an hematocrit correction channel with hematocrit electrodes (237) for whole blood measurements which are not used here. It should also be noted that the hematocrit electrodes, have in this case been made from a hydrophobic material to prevent filling of the entire channel and hence waste sample. For functioning hematocrit electrodes an less hydrophobic or hydrophilic material would be used.

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[0193] Washed wet assay measurement 3:

[0194] The cartridges (MST pro strip V1) filled with washed wet assay complexes, as described in washed wet assay measurement 2 above were then remeasured using the Victor3 V. This was carried out by attaching the cartridge to a 384 well black Optiplate and aligning the cartridge channel to be measured (at position 222, 241) over a specific well. The fluorescent signal of the channel was then measured by measuring the signal of the appropriate corresponding well using the inbuilt program 'Fluorescein (485 nm/535 nm, 0.1 s)', adapted for use in a 384 well format. This program uses excitation at 485 nm and emission at 535 nm with a 0.1 s measurement time. This was repeated for each channel to be measured.

[0195] Results for Assay 1:

[0196] Results for comparisons between the different measurement methods of the washed wet assay complexes are shown in FIGS. 16, 17 and 18.

[0197] FIG. 16 shows the total PSA washed wet assays measured in a MST pro strip V1 in the MST Pro Meter V1 (washed wet assay measurement 2). Total PSA wet assays were performed as per the experimental methodology described above. The total PSA washed wet assay was measured in the MST Pro Meter V1 as shown in FIG. 12. The results clearly show a systematic assay response with an initial linear phase followed by a non linear phase. The clinical cut off for Total PSA assays used in the screening of prostate cancer is 4 ng/ml. The assay is clearly sensitive enough to make accurate measurements above the below the cut off threshold value in a quantitative manor. In this data set the paramagnetic particle-PSA-latex bound complexes are homogeneously distributed throughout the strip channels during the optical measurement phase. The MST Pro Meter has only been utilised to measure Total PSA washed wets assays in a strip.

[0198] FIG. 17 shows the Correlation between the Total PSA washed wet assays measured in strips in the MST Pro meter and the Victor3 V. The same strips (containing the washed Total PSA washed wet assays) that were measured in the MST Pro Meter (see FIG. 16, wet washed assay measurement 2) were then measured in the Victor3 V (washed wet assay measurement 3), with the results are summarised in FIG. 17. A clear correlation between the two measurement methods (MST Pro Meter V1 and the Victor3 V) is observed especially considering the Victor3 V is a conventionally plate reader and is not intended to measure fluorescent signals in laminate strip. This probably explains the greater error associated with the Victor V measurements. The data demonstrates the optical measurement performed by the MST Pro Meter V1 is a highly sensitive accurate measurement especially when compared to a very expensive "gold standard" fluorescent plate reader technology (Victor3 V).

[0199] FIG. 18 shows the correlation between the Total PSA washed wet assays measured in strips in the MST Pro meter and the Victor V. The Total PSA washed wet assays were also measured in a conventional microtiter plate assay using the Victor3 V (washed wet assay measurement 1). These results were then compared to the Total PSA washed wet assays measured in a strip using the MST Pro Meter V1 (washed wet assay measurement 2). The correlation between the two measurement methodologies is shown in FIG. 18. An excellent correlation is observed between the two methodologies demonstrating the ability to make highly sensitive and