

the T3-ALP conjugate competing for antibody binding sites. After approximately 15 minutes, a measured aliquot of the incubated solution was injected into the capillary. The capillary and the buffer reservoirs are filled with the TBE buffer solution containing AttoPhos Substrate. The aliquot of T3-ALP conjugate from the incubation solution was injected at one end of the capillary. Generally, the assay can be performed at constant or zero potential mode. Constant potential assays are performed in a single step. After the enzyme conjugate is injected to the capillary, a constant potential is maintained until both the enzyme conjugate and product passed the detector window. Although product is continuously swept away from the enzyme conjugate, the reaction rate at which product is produced is adequately high relative to the separation time that a small amount of product accumulates. When the electrophoretic mobility of the enzyme conjugate-substrate complex is greater than that of the product, an injection peak will be evident on the electropherogram on the right hand side of the absorbance plateau as seen in FIGS. 5(A), 6(a) and 6(b).

During zero potential mode, the product is allowed to accumulate and then transported to the detector. Zero potential assays are performed in three steps: separation, incubation and product transportation. In the separation step, the injected enzyme conjugate migrates in the buffered substrate-filled capillary for a predetermined period of time under a electric field. Then power is interrupted and the product is allowed to accumulate for a fixed time in the incubation step. The product formed is then transported to the detector by reapplying potential. The profile in this mode is a peak on the top of a plateau as seen in FIG. 6(a).

In continuous potential mode (or constant potential mode), a plateau of the fluorescent product is expected. FIGS. 17(a) and 17(b) show representative electropherograms for high and low applied voltage, respectively, in the continuous mode. As expected, the fluorescent signal increases as the applied voltage decreases. At lower applied voltage, T3-ALP conjugate moved slower in the AttoPhos Substrate-filled capillary so the contact time of the T3-ALP conjugate and the AttoPhos Substrate is increased, producing more fluorescent product as evidenced by a higher plateau (FIG. 17(b)).

In the zero potential mode, the fluorescent product accumulated in a the zone of the capillary where the T3-ALP conjugate stopped. Switching the potential to zero before the T3-ALP conjugate passed the detector allowed an increased incubation time and thus, increased sensitivity. That is, the amount of T3-ALP conjugate detected can be very small since the T3-ALP conjugate generates a greater amount of fluorescent product, i.e., the amount of T3-ALP conjugate is "amplified." Consequently, a very sensitive analysis is achieved. FIG. 18 shows the incubation time versus the fluorescence signal for the T3 electrophoretically mediated micro-analysis enzyme immunoassay. As expected, the greater incubation time, the greater the fluorescent signal. Since the AttoPhos Substrate was in large excess, there was a linear relationship between the incubation time and the fluorescence signal.

Both high and low voltage was tested in the assay. With high voltage (20 or 30 kV), the analysis time was reduced to 3 minutes without a zero potential incubation period. With low voltage (10 kV), a more stable baseline and signal were obtained even after a zero potential incubation period. The combination of high voltage (20 kV) and low voltage (10 kV) was finally determined to produce the best results (FIG. 19). The high voltage was applied before the zero potential incubation period to reduce the analysis time and low

voltage was applied after the zero potential incubation period to stabilize the baseline.

The zero potential assay was carried out for a zero potential pause of 1, 2 and 3 minutes. The fluorescent signal was substantially linear for all times. The T3 electrophoretically mediated micro-analysis assay conditions were optimized so a zero potential incubation period in the capillary amplified the fluorescence signal from the product as much as possible without affecting the linearity of the signal. The optimal electrophoretically mediated micro-analysis of T3-ALP conjugate was determined to have a zero potential incubation period of 3 minutes.

The electropherograms in FIG. 20 are of various known concentrations of T3 serum standards that were subjected to the immunological reaction with T3-ALP conjugate and capillary electrophoresis micro-analysis using the optimal conditions described above. The fluorescence signal of the product produced from the unbound T3-ALP conjugate was measured for each concentration of T3 subjected to the competitive reaction with the T3-ALP conjugate. Under the same experimental conditions, the correlation between the amount of product detected and the amount of T3 in the serum standards permitted a calibration curve to be constructed (FIG. 21). Quantitation was based on determinations of the peak height above the constant potential product plateau in the electropherogram. The peak area above the constant potential product plateau also can be used to determine the amount of product formed.

The amount of unbound T3-ALP conjugate is proportional to the amount of T3 present in a sample. In the T3 electrophoretically mediated micro-analysis enzyme immunoassay, the amount of unbound T3-ALP conjugate quantitatively was determined by measurement of the fluorescent product so the amount of T3 in the original sample could be calculated. A calibration curve was constructed using a known amount of T3 under a given set of experimental conditions to determine the fluorescence signal the results. Using the same relationships, under the same set of experimental conditions, an unknown amount of T3 in a sample can be quantitatively determined using the constructed calibration curve.

The results indicate that the variation of the signals for is not related to the electrophoretically mediated micro-analysis technique. Possible sources for the variation of the signal may be non-specific binding of the free ALP and/or T3-ALP conjugate to the microplate wall, variation in the incubation time of the serum standards and a high free ALP concentration that disrupts the background.

Other embodiments are within the following claims.

What is claimed is:

1. A method for performing an assay comprising:

- (a) introducing a first reactant and a sample containing an analyte into a channel containing an electrophoresis medium, wherein the analyte comprises or is complexed to a competitor, said competitor comprising a biorecognition portion and a second reactant portion;
- (b) applying an electric potential along the length of the channel to cause said first reactant and said second reactant portion of said competitor to form or deplete a detectable product;
- (c) imposing along the length of said channel an electric potential sufficient to cause said detectable product to be separated from said first reactant by differential migration along said channel; and
- (d) detecting said detectable product.