Development of a Quantum Dot-Lateral Flow Assay

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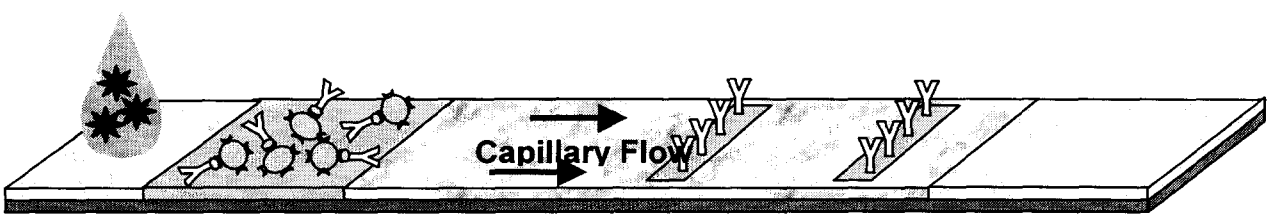
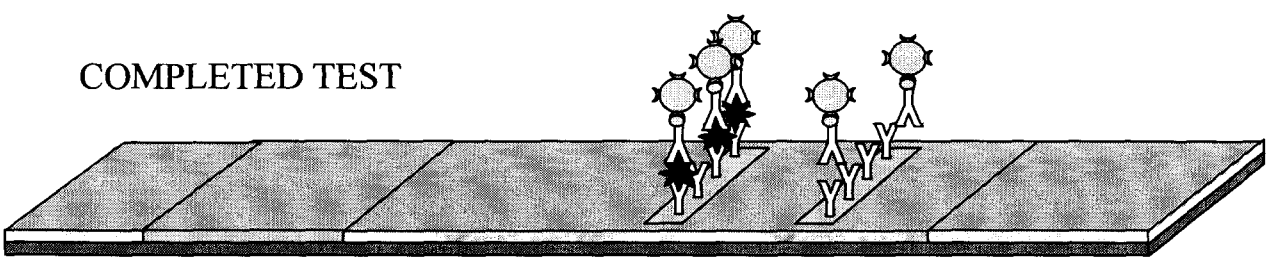
The most expanding area in clinical diagnostics are "rapid tests" based on the lateral flow assay, providing single-use, one-step tests and results within minutes. Lateral flow assays (LFA) are also known as immunochromatography assays, designed for ease of use and clarity of results. The most common example is the home pregnancy test, available from drug stores and used by the general public. LFAs also exist for many other clinical, agricultural and environmental applications, including detection of pathogens, allergens, and drugs-of-abuse, to name a few. Commercial LFA kits utilize gold or latex nanoparticles resulting in a visible qualitative test result. Our method combines the traditional LFA design with fluorescent Quantum dots (QD) supplied by *Quantum Dot Corporation*; these novel semiconductor nanocrystals have been modified to be water-soluble and can be conjugated to antibodies for use in any immunoassay. Furthermore, QDs are recently available over a spectral emission range, using UV/blue light excitation. The QD-LFA offers advantages of increased sensitivity, quantitative testing, plus multiplexing capability.

The diagram shows a typical LFA test strip.

START OF TEST

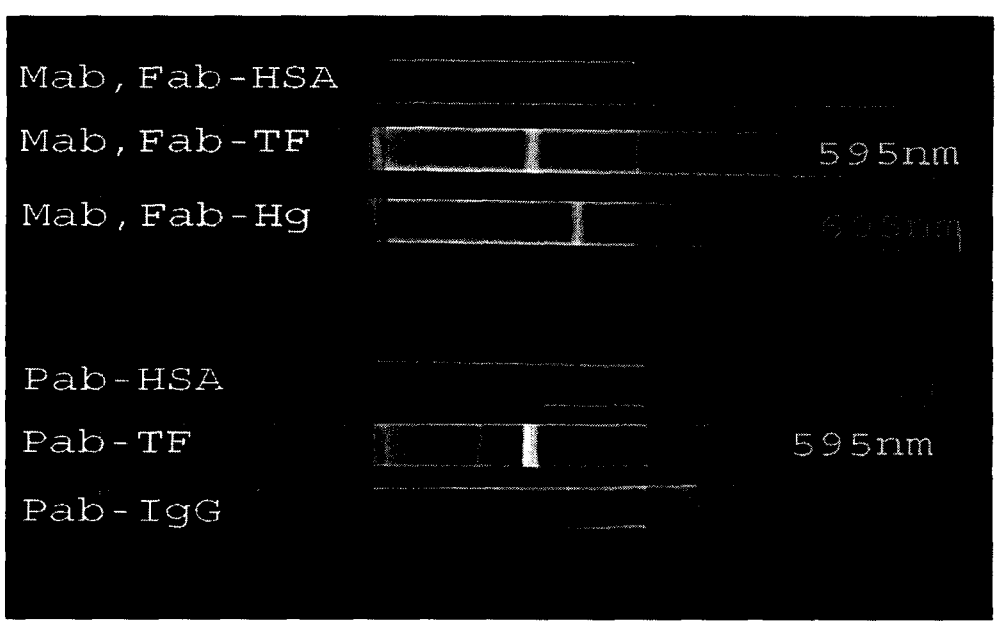


COMPLETED TEST



Sample is added to the sample pad, where it rehydrates and mixes with the label-conjugated antibodies that have been dried on but not bound to the conjugate pad. The sample-antibody conjugate mixture flows through the pores of the nitrocellulose

membrane, passing over the capture and control zones where capture and control antibodies have been permanently immobilized onto the membrane. As the sample flow passes over the capture zone, antibody sandwiches are formed as shown, creating a visible line due to the accumulation of the label-conjugated antibodies. The remaining labeled antibody flows on to the control zone, creating a positive control to confirm the test is functional and complete.

The gold standard LFA is the pregnancy test, which detects "hCG", human chorionic gonadotropin as a marker of pregnancy in a woman's urine. This is the most well understood chemistry among LFAs and is therefore used by us and others as a foundation for comparing novel approaches to the commercial test. We constructed and tested a QD-LFA for hCG, using streptavidin-coated quantum dots conjugated to biotinylated anti-hCG antibodies. The signal was bright, resistant to photobleaching and stable following months of storage. The success of this first assay shows the feasibility of using Sav-QD605 quantum dot conjugates for detection in LFAs.
(EXAMPLES OF POSITIVE AND NEGATIVE TESTS ARE AVAILABLE FOR DEMONSTRATION).

The next generation of QD-LFAs we have developed have been spectrally multiplexed assays. This has not previously been possible with conventional detection schemes, for example, gold/latex conjugates provide a single color result, for qualitative interpretation. Using QDs, each antigen is distinguished by a different color QD-antibody conjugate, and several antigens can be tested on one strip. This is made possible because the many colors of quantum dots are excited by the same wavelength (unlike organic fluorescent dyes). We have been successful in multiplex detection of four plasma proteins (human serum albumin, transferrin, haptoglobin, IgG). Shown below is a comparison of a spectrally multiplexed LFA for plasma proteins, comparing monoclonal-Fab-QD and polyclonal-QD conjugate schemes. With further research and development, the future for QD-LFA looks bright.