Lateral Flow Test using Carbon Black Nano-Strings

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Introduction

Lateral flow test, also called immuno-chromatographic test, is a rapid and sensitive form of immunoassay for analysis in complex media.
The reaction takes place in a thin porous matrix layered on top of an optical clear polyester film, mostly formed as a 5 mm wide and 50 mm long strip (see Fig. 1). The analyte is transported from an application zone along the membrane and binds to an immobilised antibody in a combined capture and detection zone. Another antibody, directed towards a different epitope on the analyte, is labelled with a coloured particle, and then transported through the capture zone where it binds to the analyte in the immuno-complex. The non-bound particles are washed away with the passing liquid flow.
Carbon black particles of the type used in the printing industry have an intensive black colour and can be modified to bind antibodies with preservation of their activity. These particles can be used to construct tests with high sensitivity for visual detection and semi-quantitative determination. In combination with a common scanner the test can be made fully quantitative. MAIIA Diagnostics provides different types of specially selected and designed (modified) carbon black nano-string particles which show much higher sensitivity than commonly used coloured polystyrene or gold particles.
This technique can be used for rapid and extremely sensitive analysis of low concentrations of substances in complex media such as urine and serum. MAIIA Diagnostics' lateral flow test for erythropoietin (EPO) shows a detection limit of 1.2 femtomolar, 0.035 ng EPO/L.

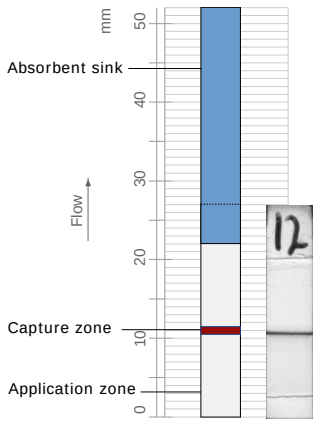

Fig. 1: The lateral flow strip with the application zone and the combined capture and detection zone is shown to the left. The same strip after performing the test using 25 µl of sample with a concentration of 1000 ngEPO/L is shown on the right.

Carbon black nano-strings as a label

About 100 types of carbon black are currently found on the market, each with its own specific profile of characteristics. The particles differ in size, structure and surface groups. The particle consists of chained or cluster like branched aggregates of roughly spherical particles, the primary particles. These primary particles, with sizes ranging from 10-500 nm, are fused together in the production-process, so the final particle may occasionally consist of several hundreds of primary particles in a string.

On the surface of the carbon black particle there exists different functional groups containing oxygen such as quinones which can react with e.g. amino groups in the proteins under formation of covalent bonds. The surface also binds proteins via other interactions. The particles have very large surface area and can bind high amounts of antibodies.

The black intense colour, the high "molecular weight" and chainlike structure makes carbon black nano-strings an excellent label for lateral flow tests. MAIIA Diagnostics provides specially produced carbon black suspensions suitable for labelling with antibodies.

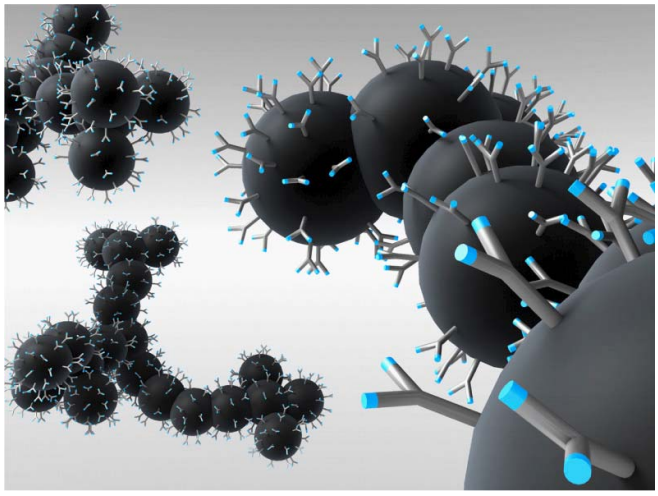

Fig. 2: Carbon black nano-strings covered with antibodies.

Detection with scanner

Scanners for image processing have during the past few years rapidly become very affordable and it is now possible to buy high quality scanners for less than $500.

The technical specifications of interest in the scanners are the optical and greyscale resolutions as well as the quality of the CCD detectors. An optical resolution of 4,800 ppi will show 189 pixels/mm or 35,721 pixels/mm^2. A greyscale resolution of 16-bits allows each pixel to be represented by 65,536 different intensity levels.

MAIIA Diagnostics has developed a special software which locates the capture zone and calculates the intensity of the captured anti-EPO carbon black nano-string.

With an Epson Expression 1680 it is possible to quickly scan surfaces and analyze the signal intensity with very good precision. It is in fact possible to detect 0.02 attomol carbon black/mm^2 by the use of a scanner. This corresponds to 22 carbon black nano-strings molecules per 42 µm pixel.

Fig. 3: An ordinary image scanner can be used as a powerful detection instrument.

Software for quantification of signal intensity

Software developed for quantification of the signal on the lateral flow test is available from MAIIA Diagnostics (see Fig. 4). The program transfers the information from each pixel to blackness intensity values (between 0 and 65,535 for 16-bit resolution). It searches within the capture zone for the peak value as well as outside for a suitable base value and gives the signal intensity in delta blackness per pixel. It also displays the image of each strip together with its profile to making it easy to verify the correctness of the automatic search as well as detecting any anomalies. The signal intensities of the bands from hundreds of strips can be quantified in minutes.

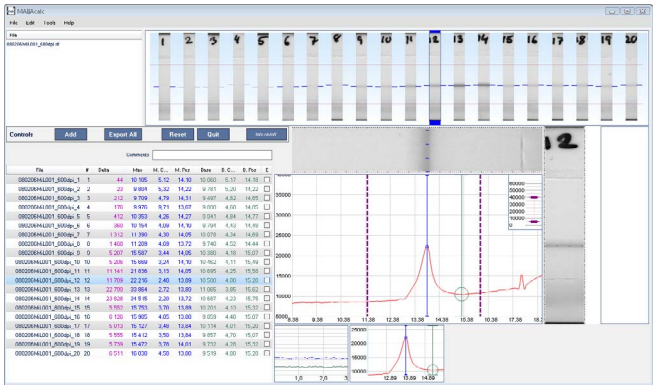

Fig. 4: The software quantifies the signal intensity for each strip and displays its image and zoomable profile. Also shown is the overview of all strips in the scan-serie to correlate location of found peak values. Each strip is added to the list and has a peak, base and delta value as well as real location and precision of the values, all easily transferred to Microsoft Excel for further calculations.

Dilution curve for EPO in buffer

The results when analyzing 25 µl samples of a dilution serie of 3-1000 ng/L of EPO in buffer is shown in Fig. 5. With MAIIA's technique for lateral flow test it takes less then 20 min. to simultaneously process up to 60 strips.

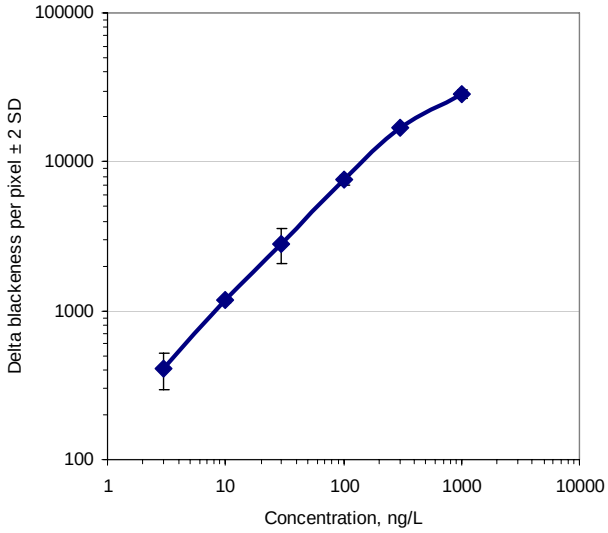

Fig. 5: Results from measuring samples of a dilution serie of 25 µl EPO in buffer, using the 15 min. lateral flow test procedure and scanner detection.