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Development of an optical method for analysing quantitative dipstick immunoassays

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Introduction

In the last years immunochromatographical rapid dipstick tests became a serious analytical method within medical analysis. Lots of analytes can now be detected and one can get reliable and reproducible results with these easy-to-use and low-priced test devices.

Heart of the dipstick tests is a polymer membrane (normally cellulose nitrate is used), which incorporates one or more reagents (mostly specific antibodies) in lines or dots. A liquid sample is put onto a pad of non-woven material at the bottom of the test strip. The analyte in the sample reacts with a labeled antibody (latex or gold patricles are convenient for labelling) while moving up the membrane towards the "absorbent pad" at the top of the strip. Only the labelled antigen-antibody-complexes are hold back at the line

Depending on the properties of the analyte one of the following test formats is used:

In the "Sandwich format" first an antibody-antigen-complex is formed. A second epitope of the analyte enables the docking of this complex to a second antibody, immobilized within the test line. A "Sandwich"-complex of the labelled antibody, the antigen and the immobilized antibody is bound to the test line resulting in a test signal. The intensity of the signal increases with the concentration of analyte in the sample.

In the competitive format first an antibody-antigen-complex is formed as well, but here chemicals providing the same epitope like the analyte are immobilized within the test line. To these molecules only unoccupied, labelled antibodies can bind forming a test signal. The intensity of the test signal decreases with the concentration of analyte in the sample.

In both formats the intensity of the test line correlates with the concentration of the analyte in the sample. In spite of this correlation the devices which have been established on the markets are mostly qualitative or semi-quantitative.

Target of this work is to use the correlation between the intensity of the test lines and the concentration of the analyte for a quantitative analysis and to develop a suitable optical method for generating data.

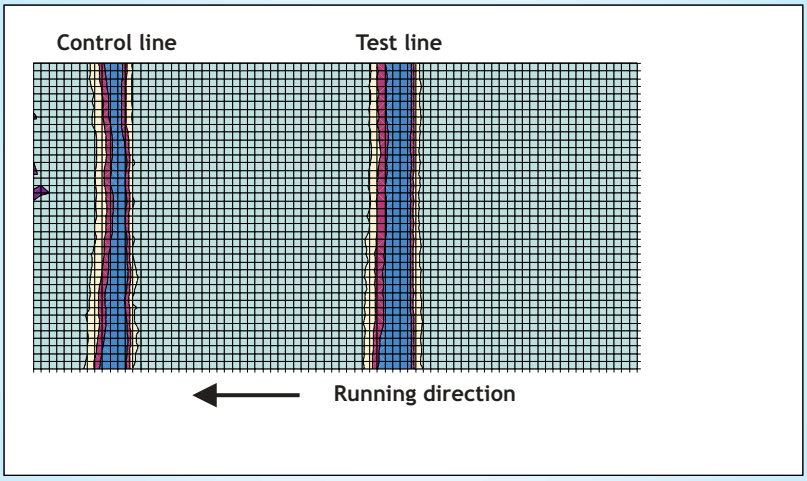

Figure 2: Miscoloured plot of an analysed dipstick test. The darker the colour the more intensive are the lines. It is obvious that the test line is much thicker than the control line. Both lines are inhomogeneous coloured.

Conclusion

The developed analysing system contains individual components which provide a flexible use. The system is applicable for the quantitative analysis of different types of dipstick immunoassays. For a reliable analysis it is necessary to get more information about potential sources of error - referring to the test device as well as to the analysis.

Nevertheless the collected data offer valuable clues to the properties of the test strips. For example, inconsistent colorations of the protein lines are detectable und presentable (Fig. 2) . Thus it is possible to study what effects the changing of different test parameters has to the test signals.

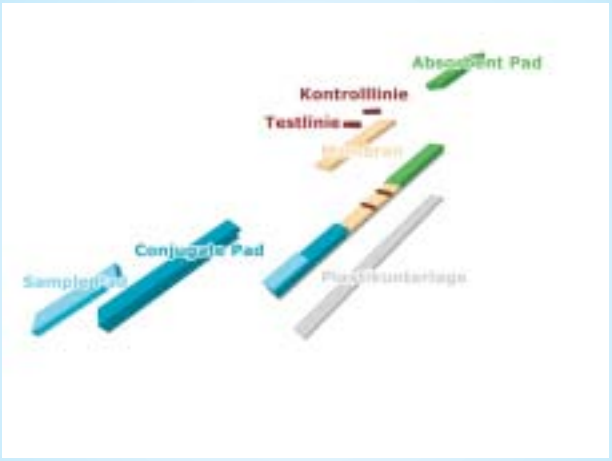

Figure 1: Exploded view of a lateral-flow dipstick test.

Results

A competitive dipstick test device for detecting estrogens in aqueous solutions was established for developing the analysing method. The connection between analyte concentration and signal intensity could be demonstrated. According to the competitive format an increasing of the estrogen concentration resulted in a decreasing of the intensity of the test lines (Fig. 3). This result was visible to the naked eye.

For collecting objective of the line intensities the used test strips were digitally photographed with an 8-bit greyscales camera in an illuminated housing under constant light conditions. The digital images were analysed with a self-made analysing routine of the image processing program Optimas 6.5. The results were represented graphically (Fig. 3).

With the collected data the intensity gradient in a concentration range was graphically representable. The curve confirms the visual impressions (Fig. 2). Furthermore interesting results can be extracted from the digital images and graphically represented such as the equability of the intensity of the test lines (Fig. 2).

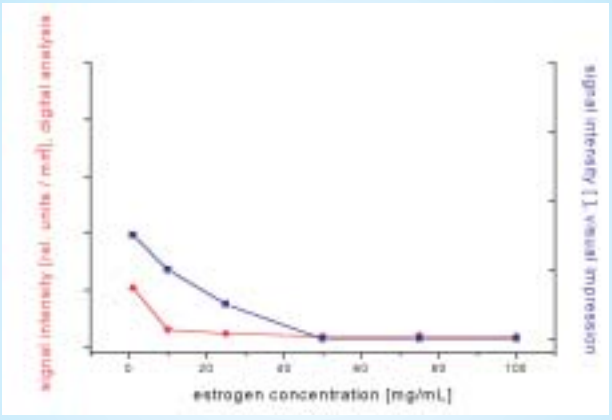

Figure 3: Visual impression and digital analysis of signal intensities collected from a concentration range.