O5 SB4

MINIATURIZED ALL-INCLUSIVE BIOSENSOR BASED ON CHEMILUMINESCENT LATERAL FLOW IMMUNOASSAY FOR FECAL HEMOGLOBIN DETECTION: A HOME-MADE TEST FOR COLORECTAL CANCER SCREENING

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Colorectal cancer is the second leading cause of malignant death and the participation rate to screening programs based on invasive endoscopic diagnostic tests is very low. As an alternative, non-invasive stool testing based on the detection of fecal occult blood represents a valid approach for a rapid screening. However, the widespread guaiac-based test (GFOBT) is affected by a variety of interferences, thus frequently yielding false-negative and false-positive results and requiring the patient to follow a specific pre-test diet. The use of an immunoassay for detecting hemoglobin in stools could overcome these limitations. Lateral Flow Immunoassay (LFIA) is a technology currently widely applied in resource-poor or non-laboratory environments (point-of-care, POC) that is based on ready-to-use strips of cellulose-based materials containing dry reagents that are activated upon fluid sample application. Using enzymes as tracers, coupled with chemiluminescence (CL) detection, it is possible to obtain quantitative information and reach high detectability.
Herein, we report the development of a simple, rapid and accurate biosensor based on a CL-LFIA method applied for quantitative detection of hemoglobin in stool samples, using the smartphone BSI-CMOS photocamera as a light detector [1,2]. The biosensor is based on a competitive immunoassay using peroxidase (HRP)-labeled anti-hemoglobin antibody, which is detected, upon adding the luminol/enhancer/hydrogen peroxide-based CL substrate, by means of a smartphone camera for digital imaging and a specific application for data handling. Using a 3D printer, simple accessories were developed to turn the smartphone into a biosensing device. Since CL system employs labile enzyme that makes it hard to routinely use them for on-site applications, it is proposed to entrap the HRP-labeled anti-hemoglobin antibody into a pullulan-based tablet, which allows to enhance the long-term stability of the enzyme and also to simplify the assay procedure. Indeed, these tablets dissolve rapidly upon addition of the samples, making the test very easy to be performed on site. Moreover, new geometries and smaller dimensions of the LFIA membrane are evaluated, in order to find a good compromise between the execution time, the compactness of the device and the analytical performances of the immunoassay. The developed method is simple and fast (15-min total assay time) (Figure 1) and it allows to detect even small traces of hemoglobin in fecal samples, down to 4 pmol (Figure 2). When compared with the conventional GFOBT the

assay is able to detect lower concentration of blood allowing an early diagnosis. This biosensor could be very useful for frequent self-screening providing a very effective tool for colorectal cancer prevention.

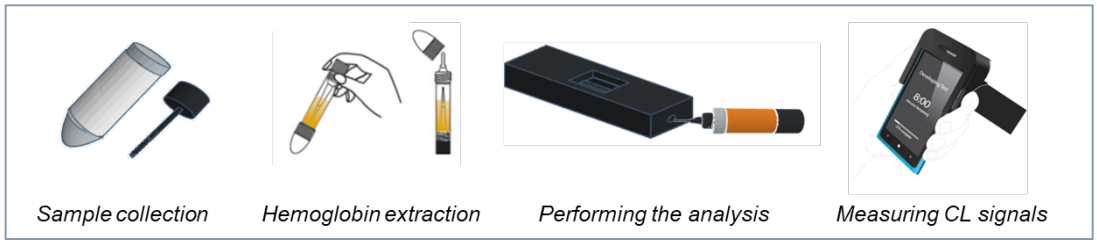

Figure 1. Schematic procedure for the CL-LFIA based method for hemoglobin quantification in feces sample

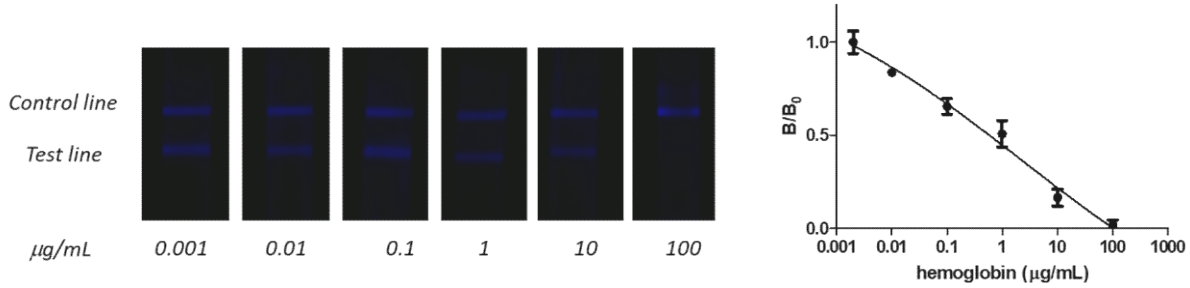

Figure 2. Chemiluminescent images obtained for different haemoglobin concentration and the relative calibration curve

References

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