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D-dimer quantification from autologous red blood cells agglutination by a lens-free imaging device

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Abstract

Quantification of biomarkers closest to the patient remains an impressive challenge for the development of personalized medicine. The detection of D-dimer, a fibrin degradation product, indicative of deep vein thrombosis, pulmonary embolism or stroke is a particular biomarker of interest. In this study, we developed a quantitative assay based on the measurement of autologous red blood cells agglutination by a lens-free device. An indicator, obtained from the shape of the gray level histogram of the images, allows us for a linear quantification of D-dimer opening the door for a Point-of-Care device.

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Keywords: Red blood cells agglutination; D-dimer quantification; lens-free detection

D-dimer is the degradation product of crosslinked fibrin. It is frequently used as a biomarker in the clinical diagnostic of deep vein thrombosis, pulmonary embolism or ischemic stroke. Besides traditional ELISA assays, agglutination assays of latex beads or whole blood by bispecific reagents are also considered [1]. In particular, the SimpliRED D-dimer assay, a whole blood test was introduced in 1990 [2]. The test uses a bispecific reagent prepared from two fragments of monoclonal antibodies, one raised against D-dimer while the other against Red Blood Cells (RBC). The mixing of this reagent with patient's blood provokes RBC agglutination in presence of elevated concentration of D-dimer. The SimpliRED D-dimer assay is a manual test whose qualitative result is appreciated by naked eyed observation. Thus, strong inter-operator variability have been reported and this assay was

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not considered as sensitive as classical ELISA tests [3]. In this study, we aim at providing an agglutination indicator independent of the operator observation while providing information on the quantification of D-dimer concentration.

To this end, we measure the absorption of green DEL light ($\lambda = 530$ nm within hemoglobin absorption peak) through microfluidic chambers (6 mm diameter and 150 μm thick) filled by 1.45 μl of patient blood, 3.5 μl of bispecific reagent solution and 3.5 μl of positive control solutions varying by twofold dilutions with the negative control solution of the SimpliRED D-dimer Kit (BBInternational). Solutions are pipetted into the circular chamber, manually stirred and the glass slide is continuously tilted during 2 minutes before the coverslip is added. Then, absorption measurements are performed using a lens-free imaging device. All images are taken with the same parameters for four relative concentrations of the SimpliRED D-dimer positive control (0, 0.25, 0.5 and 1) (Figure 1a). The choice of the green light implies that the reduced intensity of the images is mainly due to adsorption by the RBCs. The agglutination leads to the local increase of RBC concentration corresponding to stronger absorption of light. Thus, dark spots are representative of agglutination clusters. From the lens-free images, histograms of the gray level are determined and present a dark peak at low gray level corresponding to large amount of RBCs or agglutinates. The position of this dark peak is clearly dependent on the concentration of D-dimer present in the sample (Figure 1b). In fact, the inverse of the gray level position of the darkest peak is shown to depend linearly on the D-dimer concentration (Figure 1c). Most importantly, the result for the relative concentration of D-Dimer $R_C = 0.25$ is significantly different from the negative control one (Figure 1c) while the naked eye observation of the top images (Figure 1a) may not discriminate the presence of agglutinates.

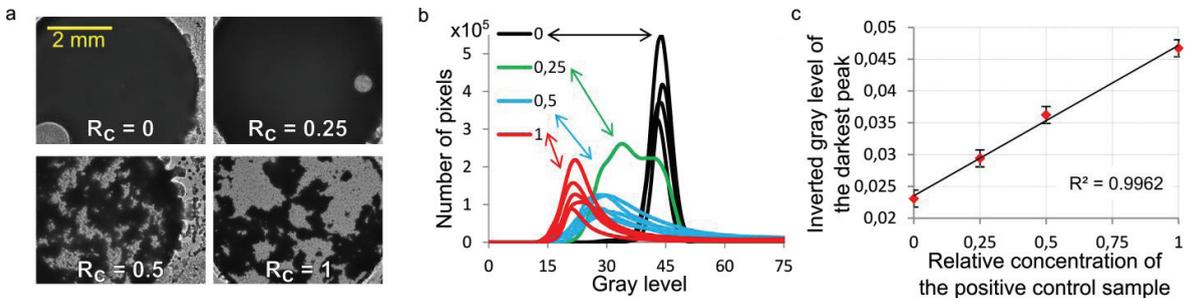


Fig. 1. a) Lens-free images of the 150 μm thick microfluidic chambers, b) gray levels histograms and c) inverse of the darkest peak gray level for various concentrations of D-dimers.

In this study, we demonstrate the interest of lens-free imaging device for the measurement of RBC agglutination providing quantitative information on the presence of the D-Dimer biomarker. Further works concern the integration of the biological protocol within passive biochips and the real-time measurement of the agglutination process from lens-free imaging in order to adapt the assay to the Point-of-Care LabPad® device developed by Avalun.

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