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Single-step whole-blood immunoassay for small-drug quantification within paper-based microstructures

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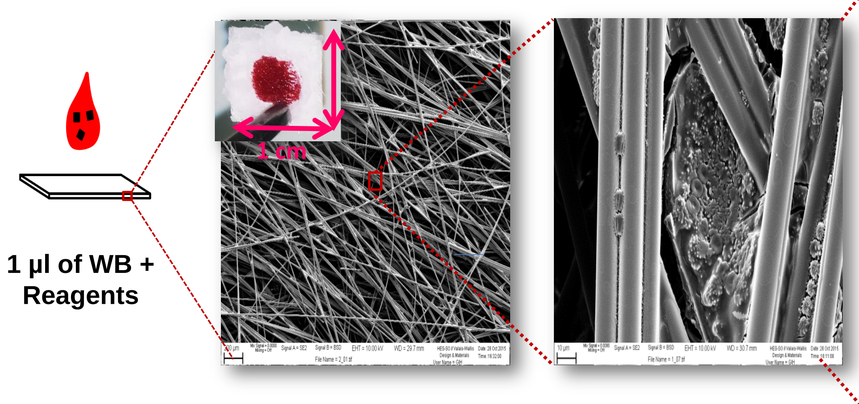
Aim of the study

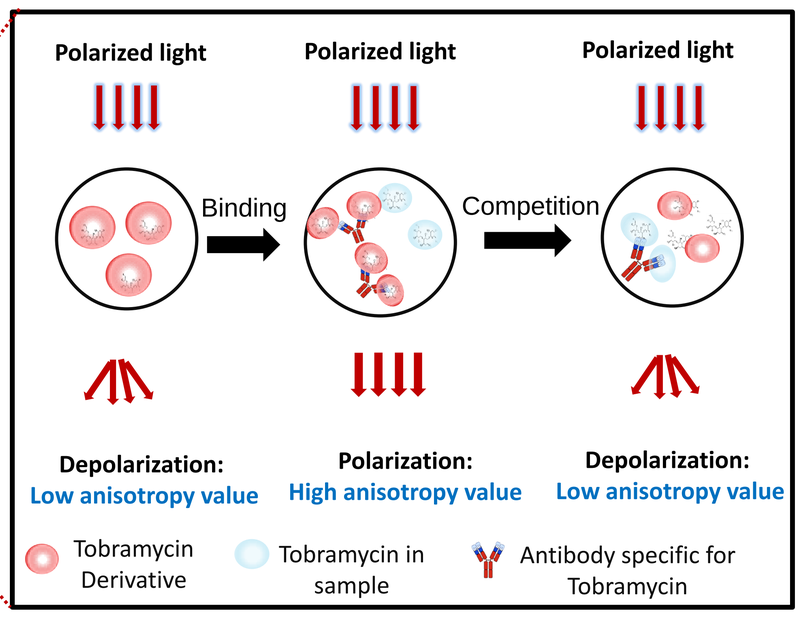
Blood tests for monitoring therapeutic drugs are nowadays exclusively performed in central laboratories, even when therapies demand for immediate decisions. This requires significant amounts of sample while being expensive and introducing significant delays that may affect the clinical outcome. Rapid and accurate whole-blood quantification of critical drugs at the point-of-care would enable fast turnaround time and provide a convenient solution for patients.

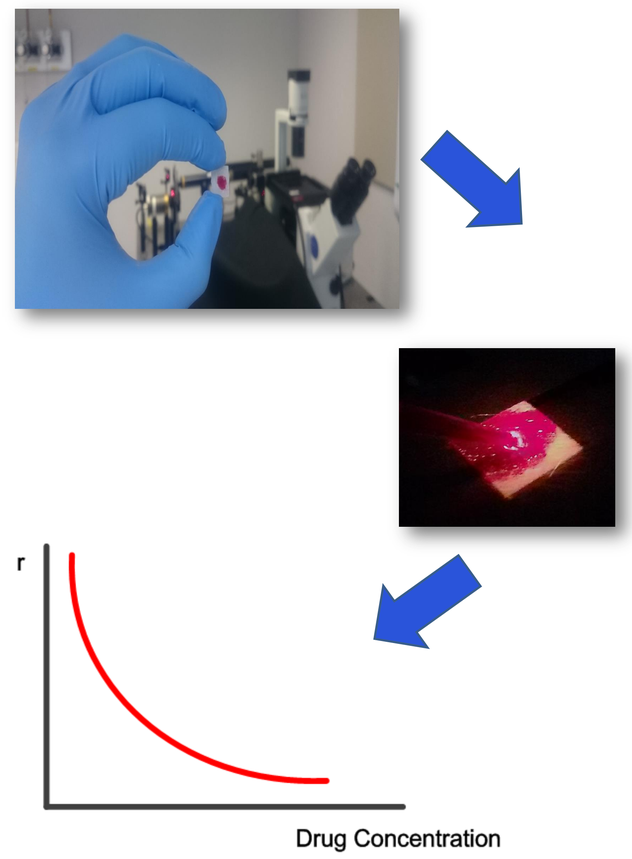
We have established a **quantitative and rapid single-step** approach for small-molecule quantification using minute amounts of whole blood with minimal sample preparation. The developed fluorescence polarization immunoassay (FPIA) for Tobramycin, a 456 Da antibiotic molecule, was miniaturized with significantly reduced blood sample requirements (just 1 µl), reagent consumption and number of steps, without compromising assay reliability. The immunoassay was performed within paper-based micro-chambers as an ideal platform for a Point-Of-Care (POC) device for clinical chemistry due to it is light weight, its biocompatibility, ease of use and disposal as well as its very low cost.[1]

Principle

1. Whole-blood processing/filtering → **2. Assaying within the paper micro-chamber** → **3. Recording and drug quantification**



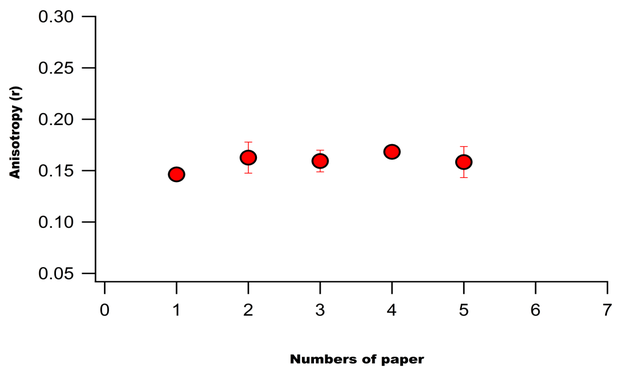


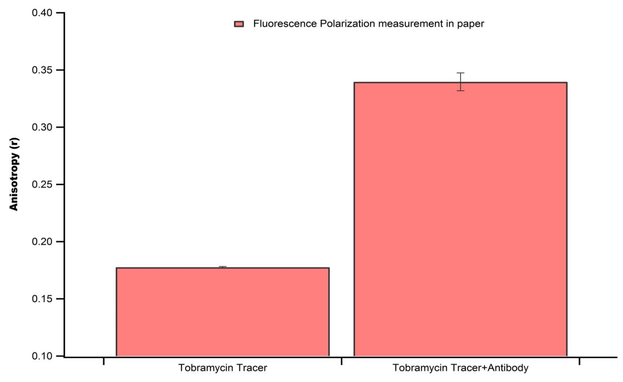


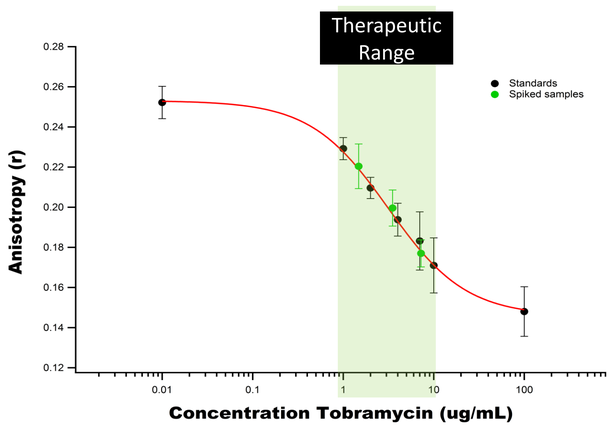
Processing: SEM images depicting the paper microstructure used for drug quantification using FPIA. Blood cells are trapped onto fibers of the paper microchamber enabling whole-blood separation. Cell filtration occurs from top to the bottom allowing measurements at the bottom part of the micro-chamber.

FPIA into microstructures: The FP immunoassay is based on the variations in polarization of the fluorescence signal as a result of the competition between the drug analyte and the fluorescently-labeled drug derivative (tracer) for the specific binding sites of the antibody. The large size of the complex formed between the antibody and the fluorescent tracer results in high anisotropy while free tracer released in presence of drug in the sample results in low anisotropy.

Results







Repeatability: Measurements of a constant concentration of the tracer (in the nM range) within and between paper-based microstructures, showed low variability in terms of coefficients of variation (CV). (replicates=3)

Fluorescence polarization in paper: higher polarization was measured in paper when the drug derivative was bound to the antibody due to the increased size of the complex. (replicates=3)

Dose-response curve for Tobramycin quantification performed in paper: the calibration curve was assessed using seven standards while the analytical performance using spiked samples (at **low**, **medium** and **high** concentrations) obtaining recoveries above 90%.

Conclusions

- ✓ Fluorescence polarization immunoassays can be performed within paper using minute amounts of blood while preserving the analytical performance.
- ✓ Our results demonstrated that paper micro-chambers can assume simultaneously two major functions:
  1) Being a filter to separate red blood cells and extract serum;
  2) Being a measurement chamber to enable accurate quantification of small drug molecules such as Tobramycin using fluorescence polarization immunoassays.

References:

1. Connelly, Rolland, and Whitesides, “Paper Machine’ for Molecular Diagnostics.” *Anal. Chem.*, 2015, *87* (15), pp 7595–7601

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